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T H È S E

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"Extraction et étude de la réactivité chimique du mesquitol, composé extractible issu de Prosopis juliflora en vue de valorisations potentielles"

Extraction and Study of the Chemical Reactivity of Mesquitol from *Prosopis juliflora* in View of Potential Valorizations

Spécialité: Sciences du Bois et des Fibres

Par

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Without laboratories, men of science are soldiers without arms… Louis Pasteur

DEDICATION

This thesis is dedicated to my family, Nyithi NyaTeso, and my wife, Emily Kimani. They have offered encouragement, prayers and love all through this research work. May God bless them all!

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LIST OF ABBREVIATIONS

^{13}C NMR	: Carbon-13 nuclear magnetic resonance
^{1}H NMR	: Proton nuclear magnetic resonance
a.m.u	: Atomic mass unit
$ABTS^{+}$	: 2,2'-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation
ACN	: Acetonitrile
AI	: Antifungal index
ATP	: Adenosine triphosphate
ATR	: Attenuated total reflectance
BnBr	: Benzyl bromide
BOP	: Benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate
CALB	: Candida antarctica lipase B
CC	: Column chromatography
CD	: Circular dichroism
CSI	: Comité de Suivi Individuel du doctorant
CTLC	: Centrifugal thin layer chromatography
DCCI	: N,N'-dicyclohexylcarbodiimide
DCM	: Dichloromethane
DCU	: Dicyclohexylurea
DEPT	: Distortionless enhancement by polarization transfer
DMAP	: 4-Dimethylaminopyridine
DMSO	: Dimethyl sulfoxide
DNA	: Deoxyribonucleic acid
DPPH	: 2,2-diphenyl-1-picrylhydrazyl
EDC	: 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide
FTIR	: Fourier Transform Infrared Spectroscopy
HMBC	: Heteronuclear multiple bond connectivity
HMPT	: Hexamethylphosphorous triamide
HMQC	: Heteronuclear multiple quantum coherence
HOBT	: Hydroxybenzotriazole
HPLC	: High-performance liquid chromatography
HSCCC	: High speed counter current chromatography

HSQC : Heteronuclear single quantum coherence
IR : Infrared
IC_{50} : Half-maximal inhibitory concentration
LB : Luria-Bertani or Lysogeny broth
LERMaB : Laboratoire d'Etude et de Recherche sur le Matériau Bois
LMWH : Low molecular weight hydrogelator
M/z : Mass-to-charge ratio
MAE : Microwave-assisted extraction
MP : Melting point
MPLC : Medium-pressure liquid chromatography
MS : Mass spectroscopy
NGOs : Non-governmental organizations
NOESY : Nuclear overhauser effect spectroscopy
Rf : Retention factor
RNA : Ribonucleic Acid.
ROESY : Rotative-frame overhauser effect spectroscopy
ROS : Reactive oxidant species
RSA : Radical scavenging activity
TBAI : Tetra-n-butylammonium iodide
TEA : Triethylamine
TLC : Thin-layer chromatography
TMS : Tetramethylsilane
TOCSY : Total correlated spectroscopy
UAE : Ultrasonic-assisted extraction
UV : Ultraviolet

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INTRODUCTION

1 Introduction générale

P. juliflora est un arbre arbustif de 3 à 12 m de hauteur et possède des branches étalées comme le montre la figure 1. C'est une plante originaire principalement du Mexique, du centre et du nord de l'Amérique du Sud. L'arbre est un envahisseur extrêmement agressif, en particulier dans les terres sèches sans gel ou dans les prairies semi-arides, que ce soit dans son aire de répartition d'origine ou là où il est introduit (Hussain et al., 2021; Kuwait & Nepal, 2019). Cet arbre peut pousser dans diverses conditions, allant des dunes de sable aux sols argileux, des sols alcalins aux sols salins, et avec des précipitations moyennes annuelles comprises entre 50 mm et 1500 mm. Il peut également supporter des températures élevées pouvant atteindre 50 °C dans l'air et 70 °C dans le sol (Noha et al., 2023). L'hybridation interspécifique de la plante favorise également son caractère envahissant dans plusieurs régions où elle a été introduite. Les propriétés fonctionnelles mentionnées de l'espèce renforcent sa capacité d'adaptation et favorisent son invasion dans de nombreux agro-écosystèmes et régions à travers le monde (Mohammed Mussa Abdulahi et al., 2017).



Fig 1: Plante *Prosopis juliflora*

Prosopis est un genre végétal considéré comme l'espèce végétale la plus envahissante au monde, et P. juliflora est considérée comme l'espèce la plus envahissante de ce genre (de Souza Nascimento et al., 2014). C'est ce qui a conduit à la déclaration de cette espèce comme mauvaise herbe agressive, tenace et nuisible dans plusieurs pays d'Afrique, notamment au

Kenya. La plante *P. juliflora* a été principalement introduite puis plantée au Kenya comme source de fourrage et de combustible, en particulier dans le cadre des programmes de bois de chauffage des années 1980. Ses graines ont été largement disséminées par les animaux de pâturage (Mwangi & Swallow, 2005; Pasiecznik, 2017). Cette plante est une espèce fixatrice d'azote et extrêmement résistante au sel et à la sécheresse, ce qui lui permet de supplanter rapidement de nombreuses autres espèces végétales. Ses épines et sa capacité à se développer en buisson lui permettent de bloquer rapidement différents chemins et de rendre une zone donnée impraticable (Eshetu, 2024; Pasiecznik, 2017).

La plante *Prosopis juliflora* possède des racines pivotantes profondes qui peuvent atteindre la nappe phréatique à plus de 50 mètres de profondeur, ce qui explique pourquoi les efforts visant à éliminer complètement cette plante ont échoué (Shiferaw et al., 2024). L'abattage des arbres s'est également révélé inutile, car ceux-ci repoussent toujours quelques semaines après avoir été coupés (Bokrezion, 2008). La propagation incontrôlée de cette espèce dans les terres arables empêche en réalité la plante d'atteindre son objectif initial, à savoir lutter contre la désertification, et elle est donc devenue une nuisance pour la communauté. En 2008, le ministre kenyan de l'Agriculture a déclaré que le *Prosopis juliflora* était devenu une mauvaise herbe mortelle et tenace et que les communautés environnantes devaient donc trouver des moyens de résoudre ce problème (Maundu et al., 2009).

La plante *Prosopis juliflora* a plusieurs utilisations. Elle sert notamment à l'alimentation, au fourrage, à l'apiculture, au combustible, aux fibres, aux colorants, aux alcools et à la médecine (Orwa et al., 2009). Différentes études phytochimiques sur *Prosopis juliflora* ont déjà été publiées et divers composés bioactifs ont également été identifiés. Dans de nombreuses études récentes, l'analyse phytochimique de diverses parties d'extraits de *P. juliflora* a révélé la présence de différentes classes de métabolites secondaires, notamment des composés phénoliques, des tanins, des flavonoïdes, des terpènes, des alcaloïdes et des stéroïdes. *P. juliflora* est largement connu pour la présence de flavonoïdes qui sont responsables de diverses propriétés biologiques et physicochimiques (J. Zhong et al., 2022). Parmi les flavonoïdes obtenus à partir de la plante, on trouve la quercétine, le kaempférol, la lutéoline, la catéchine, la 4'-O-méthylgallocatéchine et le mesquitol (S. C. Chepkwony, 2019; S. C. Chepkwony et al., 2020; Khandelwal et al., 2016; Sirmah et al., 2009). Sirmah et al. (2009) ont mis en évidence les quantités élevées de mesquitol (figure 2) présentes dans le bois de cœur de cette plante. Ainsi, si l'on considère les flavan-3-ols classiques, des métabolites secondaires connus pour leurs

propriétés antioxydantes chez les plantes, on pense que le mesquitol pourrait agir comme un agent antioxydant plus puissant grâce à ses deux fragments catéchol qui agissent comme des agents piégeurs de radicaux, limitant ainsi la poursuite de la réaction d'oxydation. Cela signifie que les antioxydants peuvent inhiber les espèces radicalaires impliquées dans les défauts polymères des cellules du bois, empêchant ainsi la décomposition du bois et favorisant sa protection (Das et al., 2024; Schultz & Nicholas, 2002).

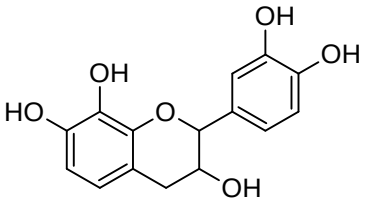


Fig 2: Structure du mesquitol [(2R,3S)-2-(3,4-dihydroxyphényl)-3,4-dihydro-2H-1-benzopyran-3,7,8-triol]

Les flavonoïdes, et plus particulièrement les flavan-3-ols, ont suscité beaucoup d'intérêt chez de nombreux chercheurs à travers le monde. Diverses études ont montré qu'ils présentent de nombreux bienfaits pour la santé, notamment des propriétés anticancéreuses, antibactériennes, anti-inflammatoires, antioxydantes et antivirales (Azam et al., 2011; Naik et al., 2023; Ullah et al., 2020). Les flavonoïdes peuvent également contribuer à réduire le taux de formation de radicaux libres dans le bois et, par conséquent, ils aident à inhiber l'activité de différents champignons phytopathogènes, tels que les champignons de pourriture blanche et de pourriture brune, qui affectent différentes essences de bois comme le hêtre (*Fagus sylvatica* L.) (Gao et al., 2024; Hammel et al., 2002; Pusztahelyi et al., 2015; Ribera & Zuñiga, 2012). De bons exemples de champignons de pourriture blanche et de pourriture brune sont respectivement *Trametes versicolor* (TV) et *Poria placenta* (PP) (Qi et al., 2023).

Les flavonoïdes étant des antioxydants naturels, ils peuvent donc jouer un rôle clé dans la réduction du stress oxydatif et des déséquilibres qui surviennent dans les réactions redox des cellules du bois associées aux espèces réactives de l'oxygène. La plupart des champignons qui affectent le bois utilisent des espèces réactives de l'oxygène (ERO), considérées comme un sous-ensemble de radicaux libres contenant de l'oxygène, pour affaiblir les parois cellulaires des plantes, ce qui entraîne différents défauts ou maladies des polymères des cellules du bois. (Castaño et al., 2018; Maraveas et al., 2021). Les ERO sont essentiellement des oxydants, ce

problème peut donc être facilement résolu à l'aide d'antioxydants. Différents composés dotés de bonnes propriétés antioxydantes, comme les flavonoïdes, présentent également de bonnes activités antifongiques (Joaquín-Ramos et al., 2020; Liu et al., 2025; Šovljanski et al., 2025).

Actuellement, l'utilisation de produits pétrochimiques issus de ressources fossiles ainsi que de biocides synthétiques, tels que le tébuconazole, a été étudiée pour ses effets négatifs en raison de leur niveau de toxicité. Il est donc nécessaire de remplacer ces composés par des alternatives, telles que des extraits contenant des flavonoïdes, afin de promouvoir la chimie verte, ce qui favorise la bioéconomie. L'utilisation de flavonoïdes permettrait de concevoir des produits qui minimisent ou éliminent l'utilisation et la production de substances dangereuses. Cela fait de la recherche visant à obtenir des alternatives naturelles renouvelables, non toxiques et biodégradables un domaine d'intérêt très important (Díaz Dellavalle et al., 2011; Gérardin et al., 2023; Kirker et al., 2024; Syofuna et al., 2012). Cependant, les flavonoïdes obtenus à partir des extraits ont une faible biodisponibilité et rencontrent des difficultés à franchir les barrières biologiques pour des applications dans les domaines cosmétique ou pharmaceutique. Il est donc nécessaire de modifier chimiquement les flavonoïdes afin de faciliter leur utilisation, leur formulation, leur passage à travers les membranes et d'ajouter d'autres propriétés biologiques (J. E. De Souza et al., 2015; Yuan et al., 2024). De plus, comme les flavonoïdes ont de bonnes propriétés antioxydantes, ils peuvent être mélangés à des fongicides synthétiques pour agir en synergie comme agent de préservation du bois pour les bois non durables en Europe, tels que les bois de hêtre (Gisi, 1996; Joseleau et al., 2022). Les fongicides synthétiques sont connus pour être toxiques pour les utilisateurs et leur utilisation continue a des effets néfastes sur la santé humaine et l'environnement (Díaz Dellavalle et al., 2011; Gérardin et al., 2023; Harris et al., 2001; Kirker et al., 2024). Pour résoudre ce problème, les fongicides synthétiques peuvent être mélangés à des extraits végétaux contenant des flavonoïdes naturels afin d'interagir de manière synergique et de former un nouveau fongicide plus sûr et moins toxique (Gisi, 1996; Joseleau et al., 2022).

De plus, tous les flavonoïdes possèdent différents groupes OH actifs sur leurs cycles, ce qui permet facilement de modifier chimiquement les composés à de nombreuses positions différentes, donnant ainsi lieu à un large spectre de dérivés flavonoïdes potentiels dotés de propriétés physiologiques utiles. (C. Li et al., 2023). Les quantités élevées de mesquitol, un composé flavan-3-ol, présentes dans le cœur du bois de la plante en tant que principal composant chimique, offrent la possibilité d'envisager des dérivés modifiés du mesquitol en

position OH aliphatique avec des propriétés améliorées, qui pourraient être utilisés pour différentes applications. Il est donc possible d'envisager la semi-synthèse de molécules antioxydantes lipophiles pour des applications dans la protection du bois. Dans ce contexte, la présente recherche est basée sur le mesquitol extrait de *P. juliflora* et sa modification afin d'obtenir des dérivés lipophiles ou amphiphiles pour faciliter la formulation, diminuer la lixiviabilité et améliorer l'activité antifongique du composé pour des applications dans la préservation du bois. De plus, l'amélioration de la lipophilie des dérivés du mesquitol peut aider à préserver les propriétés antioxydantes du composé semi-synthétisé pour des applications dans les industries pharmaceutiques et alimentaires.

2 Objectifs

a. Extraire et purifier le mesquitol à grande échelle à partir du *Prosopis juliflora* afin de démontrer la possibilité d'obtenir du flavan-3-ol de haute pureté à partir de cette plante.

L'extraction est une méthode principale qui aide à isoler le mesquitol à partir du matériel végétal. L'extraction consiste en fait à transférer les composés présents dans la plante d'un solvant à un autre, ce qui facilite la manipulation ou la concentration des composés. Dans cette étude, l'extraction a été réalisée à l'aide de cyclohexane et d'acétone, ce qui a permis d'éliminer de manière sélective les composés phytochimiques présents dans un mélange de composés de la plante. Selon la littérature, l'extrait d'acétone du bois de cœur du Prosopis juliflora contient un pourcentage élevé de mesquitol. (S. C. Chepkwony et al., 2020; Sirmah et al., 2009). Ainsi, l'extrait d'acétone a été purifié afin d'améliorer considérablement la qualité du mesquitol produit en éliminant toutes les impuretés. Une grande quantité de mesquitol était nécessaire pour mener à bien diverses réactions de semi-synthèse conduisant à des produits lipophiles ou amphiphiles pouvant être utilisés dans la préservation du bois, l'industrie pharmaceutique et l'industrie alimentaire.

b. Développer une méthode de synthèse de dérivés fonctionnalisés du mesquitol afin d'améliorer et/ou d'augmenter les propriétés du mesquitol.

L'utilisation de composés phytochimiques d'origine naturelle comme antioxydants dans les industries pharmaceutiques et alimentaires, ainsi que comme agents de préservation du bois, suscite un vif intérêt. Des études menées sur le mesquitol montrent que celui-ci possède de bonnes propriétés antioxydantes. Ces propriétés peuvent être préservées ou améliorées en

rendant le composé plus hydrophobe. L'hydrophobicité est considérée comme un avantage pour rendre les composés plus efficaces en tant qu'antioxydants (J. Zhong et al., 2022; Y. Zhong et al., 2012). Ainsi, la fonctionnalisation du mesquitol à l'aide de différents acides gras, acides aminés et anhydride succinique a permis d'obtenir des produits semi-synthétiques lipophiles ou amphiphiles qui présentaient de bonnes propriétés antioxydantes et contribuaient également à prévenir le lessivage du bois, réduisant ainsi le taux de décomposition du bois pendant sa preservation.

c. Étudier les propriétés physicochimiques et biologiques des différents composés obtenus à partir du mesquitol semi-synthétisé.

Les produits finaux obtenus présentent des propriétés hydrophobes supérieures à celles du mesquitol pur. Ils ont donc été testés pour leurs activités antifongiques et leur durabilité sur du bois de hêtre (*Fagus sylvatica* L.) afin de déterminer leurs capacités de lixiviation et de décomposition contre *Trametes versicolor* (champignon de pourriture blanche) et *Poria placenta* (champignon de pourriture brune). En outre, ils ont également été testés pour leurs propriétés antibactériennes, antioxydantes et gélifiantes en vue d'applications dans les industries pharmaceutique, cosmétique et alimentaire.

INTRODUCTION

1 General introduction

P. juliflora is a shrubby tree 3-12 m in height and has spreading branches as shown in figure 1. It is a plant native to mainly Mexico, Central as well as northern South America. The tree is an extremely aggressive invader, particularly in frost-free dry lands or in semi-arid grasslands whether in its native range or in where it is introduced (Hussain et al., 2021; Kuwait & Nepal, 2019). The tree can grow in various conditions that range from sand dunes to clay soils, in alkaline soils to saline soils and from 50 mm to 1500 mm mean rainfall per year. It can similarly withstand high temperatures from 50 °C in the air and 70 °C in the soil (Noha et al., 2023). The plant's interspecific hybridization similarly promotes its invasiveness in several regions it was introduced. The mentioned functional properties of the species enhance its adaptability and support its invasion across many agro-ecosystems and regions around the world (Mohammed Mussa Abdulahi et al., 2017).



Fig 1: *Prosopis juliflora* plant

Prosopis is a plant genus that is treated to have the worst invasive species of plants in the world, and *P. juliflora* is considered as the greatest invasive species of the genus (de Souza Nascimento et al., 2014). This is what led to the declaration of the species as an aggressive, stubborn and noxious weed in several countries in Africa, notably Kenya. *P. juliflora* plant was mainly introduced and then planted in Kenya as a source of fodder and fuel species, specifically during 1980s fuelwood programs. Its plant seeds were widely spread by the grazing animals

(Mwangi & Swallow, 2005; Pasiecznik, 2017). This plant is a nitrogen-fixing species as well as extremely salt and drought-tolerant species, thus it can quickly out-compete many other vegetation. Its thorniness and ability to spread into a bush permits it to rapidly block different paths and make a given area impassable (Eshetu, 2024; Pasiecznik, 2017).

Prosopis juliflora plant has deep penetrating taproots that are able to reach water tables to a depth of below 50 meters, and thus the efforts to completely eliminate the plant have failed (Shiferaw et al., 2024). Cutting down the trees has also proved to be futile since the tree always re-grow in after a few weeks from the time they have been cut down (Bokrezion, 2008). The uncontrolled spread of species into arable land actually makes the plant to fail its planned and proposed purpose of managing desertification thus it has become a nuisance to the community. In 2008, the Kenyan minister for agriculture stated that the *Prosopis juliflora* had become a deadly and stubborn weed and thus the surrounding communities needed ways to solve the problem (Maundu et al., 2009).

There are several uses of *Prosopis juliflora* plant. They include food, fodder, apiculture, fuel, fiber, dyestuff, alcohols and medicine (Orwa et al., 2009). Different phytochemical studies of *Prosopis juliflora* have been previously reported and various bioactive compounds have also been acknowledged. In many recent studies, phytochemical analysis of various parts of *P. juliflora* extracts showed diverse classes of secondary metabolites which include phenolics, tannins, flavonoids, terpenes, alkaloids and steroids. *P. juliflora* is widely known for the presence of flavonoids which are responsible for various biological and physicochemical properties (J. Zhong et al., 2022). Some of the flavonoids obtained from the plant include quercetin, kaempferol, luteolin, catechin, 4'-O-methylgallocatechin and mesquitol (S. C. Chepkwony, 2019; S. C. Chepkwony et al., 2020; Khandelwal et al., 2016; Sirmah et al., 2009). Sirmah et al. (2009) highlighted the high amounts of mesquitol (figure 2) in the heartwood part of this plant. Thus, considering the classical flavan-3-ols, secondary metabolites known to provide antioxidant roles in plants, it is believed that mesquitol could act as stronger antioxidant agent due to its two catechol moieties acting as radical scavenging agents limiting further oxidation reaction. This means that the antioxidants may inhibit radical species involved in wood cell polymer defects therefore preventing wood decays and promoting wood protection (Das et al., 2024; Schultz & Nicholas, 2002).

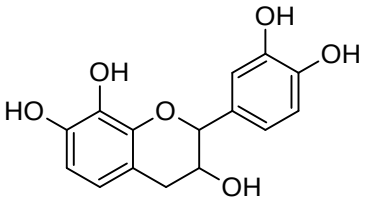


Fig 2: Structure of mesquitol [(2R,3S)-2-(3,4-Dihydroxyphenyl)-3,4-dihydro-2H-1-benzopyran-3,7,8-triol]

Flavonoids, more so flavan-3-ols, have generated lots of interests from many researchers around the world. Various studies have shown that they have many diverse health benefits, which include anticancer, antibacterial, anti-inflammatory, antioxidant and antiviral properties (Azam et al., 2011; Naik et al., 2023; Ullah et al., 2020). Flavonoids can also help in lowering the rate of formation of free radicals in wood and therefore, they assist in inhibiting the activity of different phytopathogenic fungi, such as white rot and brown rot fungi, which are affecting different species of wood like beech wood (*Fagus sylvatica* L.) (Gao et al., 2024; Hammel et al., 2002; Pusztahelyi et al., 2015; Ribera & Zuñiga, 2012). A good example of white rot and brown rot fungi are *Trametes versicolor* (TV) and *Poria placenta* (PP) respectively (Qi et al., 2023).

Since flavonoids are natural antioxidants, they can thus play a key role in reducing the oxidative stress and also the imbalances that happen in the wood cell redox reactions that are associated with reactive oxygen species. Most of fungi affecting woods utilize reactive oxygen species (ROS), which are considered as a subset of free radicals containing oxygen, to assist them in making the plant cell walls weak and this results to different wood cell polymer defects or diseases (Castaño et al., 2018; Maraveas et al., 2021). ROS are basically oxidants and therefore this problem can be solved easily using antioxidants. Different compounds having good antioxidant properties, like flavonoids, similarly exhibit good antifungal activities (Joaquín-Ramos et al., 2020; Liu et al., 2025; Šovljanski et al., 2025).

Currently, the use of petrochemicals from fossil resources as well synthetic biocides, such as tebuconazole, have been studied to possess negative effects because of their toxicity level. Thus, there is need to replace these compounds with alternatives, such as extractives containing flavonoids, to promote green chemistry, and this promotes bioeconomy. Use of flavonoids would lead to design of products that minimizes or eliminates the use and production of hazardous substances. This makes the research in obtaining renewable, non-toxic and

biodegradable natural alternatives a very critical area of interest (Díaz Dellavalle et al., 2011; Gérardin et al., 2023; Kirker et al., 2024; Syofuna et al., 2012). However, flavonoids obtained from the extractives have a low bioavailability and difficulties in crossing biological barriers for applications in cosmetic or pharmaceutical fields. Thus, there is need to chemically modify the flavonoids to facilitate their use, their formulation, their passage through membranes and add other biological properties (J. E. De Souza et al., 2015; Yuan et al., 2024). Additionally, since flavonoids have good antioxidant properties, they can be mixed with synthetic fungicides to act synergistically as a wood preservative for the non-durable woods in Europe such as beech woods (Gisi, 1996; Joseleau et al., 2022). Synthetic fungicides are known to be toxic to the users and their continuous use results to harmful impacts to the human health and environments (Díaz Dellavalle et al., 2011; Gérardin et al., 2023; Harris et al., 2001; Kirker et al., 2024). To solve this, the synthetic fungicides can be mixed with plant extractives containing natural flavonoids to interact synergistically forming a new fungicide which are safer and less toxic (Gisi, 1996; Joseleau et al., 2022).

Furthermore, all flavonoids possess different active OH groups on the rings, and this easily allows the compounds to be chemically modified at many different positions, thus resulting to a wide spectrum of probable flavonoid derivatives that have useful physiological properties (C. Li et al., 2023). The high quantities of mesquitol, a flavan-3-ol compound, present in the heartwood part of the plant as main chemical constitutes an opportunity to envisage modified mesquitol derivatives at aliphatic OH position with improved properties, which could be used for different applications. It is therefore possible to foresee hemi-synthesis of lipophilic antioxidant molecules for applications in wood protection. In this context, the present research is based on mesquitol extracted from *P. juliflora* and its modification to obtain lipophilic or amphiphilic derivatives to facilitate formulation, decrease leachability and improve antifungal activity of the compound for applications in wood preservation. Furthermore, improved lipophilicity of the mesquitol derivatives can help preserve the antioxidant properties of the semi-synthesized compound for applications in pharmaceutical and food industries.

2 Objectives

a. To extract and purify mesquitol in large scale from *Prosopis juliflora* to demonstrate the possibility of obtention of high purity flavan-3-ol from the plant.

Extraction is a principal method that helps in isolating mesquitol from the plant material. Extraction actually moves the compounds present in the plant from one solvent to another and this allows for easy manipulation or concentration of compounds. In this study, extraction was carried out using cyclohexane and acetone and this enabled selective removal of phytochemicals in a mixture of compounds in the plant. According to literature, acetone extract of the heartwood part of the *Prosopis juliflora* has got a high percentage of mesquitol (S. C. Chepkwony et al., 2020; Sirmah et al., 2009). Thus, acetone extract was purified to essentially increase the quality of mesquitol produced through elimination of any impurities. Large quantity of mesquitol was needed for carrying out various hemi-synthesis reaction leading to lipophilic or amphiphilic products that can be applied in wood preservations, pharmaceutical and food industries.

b. To develop a method for synthesis of functionalized mesquitol derivatives in order to improve and/or increase the properties of mesquitol.

There is a lot interest in the use of phytochemicals from natural sources as antioxidants for pharmaceutical and food industries, as well as wood preservatives. Studies done on mesquitol show that it has good antioxidant properties. These properties can be further preserved or improved by making the compound more hydrophobic. Hydrophobicity is considered as an advantage in making compounds more efficient antioxidant (J. Zhong et al., 2022; Y. Zhong et al., 2012). Thus, functionalization of mesquitol using different fatty acids, amino acids and succinic anhydride led to lipophilic or amphiphilic semi-synthesized products that had good antioxidant activities and also helped in preventing leaching of woods and thus reduced the rate of wood decay during wood preservations.

c. To study physicochemical and biological properties of the different compounds obtained from hemi-synthesized mesquitol.

The final obtained products have got more hydrophobic properties than pure mesquitol. Therefore, they were tested for antifungal activities and wood durability on beech wood (*Fagus sylvatica L.*) to determine their leaching and decay abilities against *Trametes versicolor* (white rot fungi) and *Poria placenta* (brown rot fungi). Furthermore, they were tested also for antibacterial, antioxidant and gelling properties for pharmaceutical, cosmetic and food industries applications.

CHAPTER 1: LITERATURE REVIEW

1.1 General review

Flavonoids have generated lots of interests from many researchers around the world. Various studies have shown that they have many diverse health benefits, which include anticancer, anti-inflammatory, antibacterial, antioxidant and antiviral properties (Azam et al., 2011; Naik et al., 2023; Ullah et al., 2020; J. Zhong et al., 2022). All flavonoids possess different active OH groups on the rings, and this easily allows the compounds to be chemically modified at many different positions, thus resulting to a wide spectrum of probable flavonoid derivatives that have useful physiological properties (C. Li et al., 2023). This research work is on a plant species called *Prosopis juliflora* that has been studied to contain various flavonoids. The study focusses on one of the phytochemicals from the plant known as mesquitol, which is a flavan-3-ol. Some studies have been carried out on this specific flavan-3-ol and thus chapter 2 discusses the literature reports of the plant and the compound.

1.2 Wood Parts

Wood is defined as an organic material that has fibrous, anisotropic and heterogeneous texture formed from a living organism called tree (Gonçalves & Margarido, 2015). It is the fibrous character of wood that powerfully impacts how it is utilized (Matuana & Stark, 2015). Thus, the characteristics of the fibrous cells of a tree and their arrangement in it influence properties as strength and shrinkage of wood as well as the grain pattern. Wood is mainly made up of the Bark, Cambium, Sapwood, Heartwood and Pith as shown in figure 3 (Ross, 2010; Woodard & Milner, 2016).

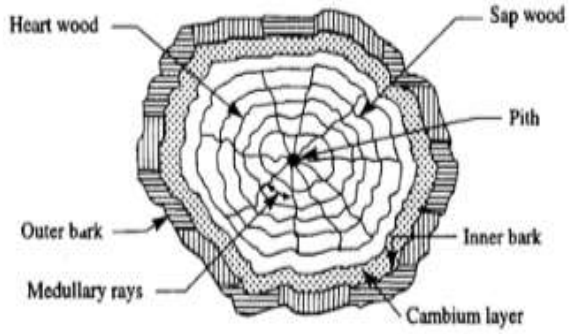


Fig 3: A cross-section of a tree trunk (Rajput, 2008)

The bark, is mainly divided into two; an outer corky dead part that has a thickness that vary significantly with tree age and species, and an inner thin living part that helps in carrying food from the leaves of the tree to its other growing parts of the tree. The pith is a small central tissue situated at the midpoint of tree branches, twigs and stems where the initial growth of wood occurs (Miller, 1999). Sapwood is made up of both dead and living tissue and it carries sap to the leaves from the roots. Heartwood is developed through a slow alteration in the sapwood and is often considered an inactive part of the tree (Asif, 2009). Cambium is a layer that is found on inside of the inner bark of a tree and forms bark cells. It can only be visible through the use of a microscope. As the tree increases in height, branching is introduced by lateral bud growth. The side branches of the tree are intergrown with the trunk wood as long as they are considered active. When the branch dies, its trunk stays and increases in diameter and covers that part of the branch, which projects from the tree trunk when it died. Later, the dead branches easily fall of the tree, and then the dead stubs grow to thickness and a clear wood is developed (Miller, 1999).

Maximum increase in thickness of wood is triggered by cambium cell division (Rashid et al., 2024). There is no growth and development in diameter that occurs in wood out of the zone of cambium; a new growth is mainly an addition and it involves growth of many new cells, but it does not mean further development of the old cells (Miller, 1999; Turley & Etchells, 2022). Mostly, new cells of wood are developed in the cambium and fresh bark cells are formed on the outside. Sapwood is made up of the dead cells and also the living cells and it is mostly used for in the food storage. Sapwood in the outer layers just close to the cambium helps in handling the transport of sap or water (Burley, 2004; Pallardy, 2010). The sapwood thickness often varies and its number of rings of growth increases with age (K. C. Yang & Hazenberg, 1991). It commonly varies from 4 cm to 6 cm in the thickness of the radius. It is a rule that the more energetically growing trees possess a wider sapwood. Numerous second-growth trees of marketable size comprise typically of sapwood (Miller, 1999).

The heartwood is made up of inactive cells that are not used in either conduction of water or storage of food. The change from sapwood of a tree to heartwood is followed with an increase in extractive contents of the wood. Normally, such extractives make the heartwood dark and thus gives the heartwoods their characteristic shade (S. Yang et al., 2025). In a number of

species, heartwood extractives help in making the wood resistant to any attack by insect or fungi. The extractives from the heartwood might similarly affect the wood through reduction of permeability, promoting slower drying of heartwood and making the wood harder to impregnate with various chemical preservatives (Burley, 2004; Zabel & Morrell, 2012). The heartwood extractives can also increase stability in altering the moisture conditions, and thus helps in slightly increasing the weight of wood. Nevertheless, when sapwood undergoes change to heartwood, mostly there is no cells being added or being taken away, nor are there any cells that change their shape. This means that the elementary wood strength is basically not influenced by the change from the cells of sapwood to those of the heartwood (Wiedenhoeft & Miller, 2005). Wood is like a three-D biopolymer composite made up of consistent network of lignin, cellulose and hemicelluloses as well as minor quantities of extractives and minerals (Miller, 1999; Niemz et al., 2023). The main chemical constituent of a living tree is just water, nevertheless on basis of being dry, all the cell walls of wood comprise largely of sugar-based polymers called carbohydrates (65%) and lignin (35%). Generally, the dry wood has got composition of elements of approximately 50% C, 6% H 44% O, and some small quantities of inorganics (Andersone et al., 2012).

Just as the name suggests, extractives found in wood, also called natural products are phytochemicals from wood, which can be extracted through the use of many solvents (Kirker et al., 2024; Santos et al., 2022). In various cases, these extractives can be classified by their extraction solvent, for instance, ether-soluble or water-soluble extractives (Abubakar & Haque, 2020; Andersone et al., 2012). Many extractives have undergone identification and, in various studies, their roles in a living tree have been well understood. But there are also a few other cases, when it is not clear why extractives are available in the wood. Many extractives have various applications in cosmetics, medicine and can be used as preservatives (Michalak, 2023; Valette et al., 2017). Other cases, the extractives are precursors to some chemical reactions, and others act as a defense mechanism for the plant (Qamer et al., 2021; Soleimani et al., 2024). Plant cell wall is a part of the heartwood, thus the extractives are consequently a collection of cell wall compounds that largely comprise of fatty alcohols, fatty acids, polyphenols (such as flavonoids), steroids, terpenes, waxes, resin acids and several other minor organic chemicals (Morel-Rouhier, 2021). These compounds occur as monomers, dimers as well as polymers (Andersone et al., 2012).

1.3 Flavonoids

Flavonoids generally have a 15-carbon flavone skeleton structure, C6-C3-C6, whereby there is two benzene rings (ring A and ring B) attached together by a 3-carbon pyran ring (ring C) as shown in figure 4. The catechol B-ring position on the pyran ring C and the number as well as the position of OH groups on the catechol group of ring B affects the antioxidant activity ability as well as other biological activities for the flavonoid. Notably, most of flavonoids are commonly located in the leaves, fruits, flowers, bark, vegetables and seed coats of many plants (Chen et al., 2023; Dias et al., 2021; Nagar et al., 2023).

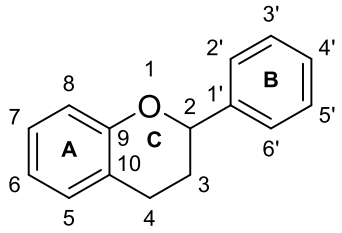


Fig 4: Basic structure of flavonoids

1.3.1 Flavonoids classification

Flavonoids can be classified into 7 major classes based on their structures, namely: chalcones, flavanones, flavones, isoflavones, flavonols, flavan-3-ols, and anthocyanins (Chen et al., 2023).

1.3.1.1 Chalcones

Chalcones are mainly 1,3-diaryl-2-propen-1-ones. They are naturally known open-chain flavonoids that carry up to 3 modified or even unmodified C_5-, C_{10}-, and C_{15}-prenyl moieties on both of their ring A and ring B. They are widely distributed in *Moraceae, Fabaceae, Cannabaceae* and *Zingiberaceae* families (K. Zhou et al., 2021). Chalcones show various pharmacological effects, which include antibacterial, antioxidant, anthelmintic, antiprotozoal, anticancer, antiulcer and antiviral effects (Goyal et al., 2021; P. Sharma et al., 2022). They are considered to be the main precursors of flavonoids. The chalcones structural features are simply created from simple aromatic compounds. Examples include butein and naringenin chalcone as shown in figure 5 (Dziągwa-Becker et al., 2024; Jasim et al., 2021; Villa et al., 2024).

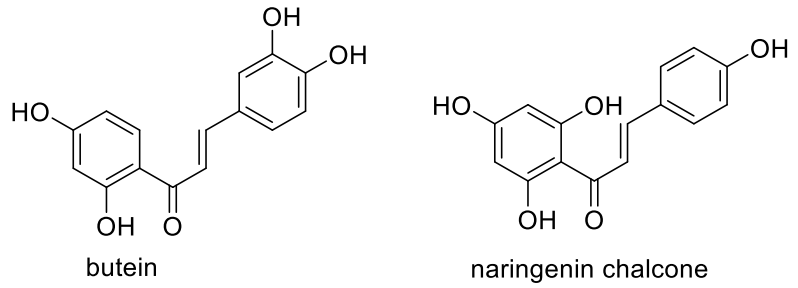


Fig 5: Structure of butein and naringenin chalcone

1.3.1.2 Flavones

Flavones are considered as one of the largest classes of flavonoids. They are made up of 4H-chromen-4-one that carries a phenyl substituent at position 2 of the flavonoid structure. They frequently appear as 7-*O*-glycosides, and they are plenty in celery, red pepper, mint, ginkgo and parsley. Two common examples are luteolin and apigenin as shown in figure 6 (Hostetler et al., 2017; Panche et al., 2016).

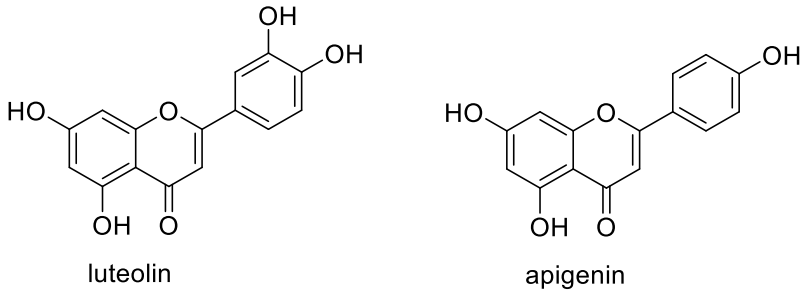


Fig 6: Structures of luteolin and apigenin

1.3.1.3 Flavonols

Flavonols are easily identified by their unique substitutions in ring A and ring B that are linked by a 3-carbon chain. They are sometimes referred to as 3-hydroxy flavone. They bear their OH groups at position 5 and position 7 of ring A. Most flavonols are available in the epidermal cells to help in protection of DNA against lots of induced damages caused by UV light. A few examples include quercetin, myricetin, fisetin, morin and kaempferol as shown in figure 7 (Panche et al., 2016; Tahir et al., 2021; Zhao et al., 2025).

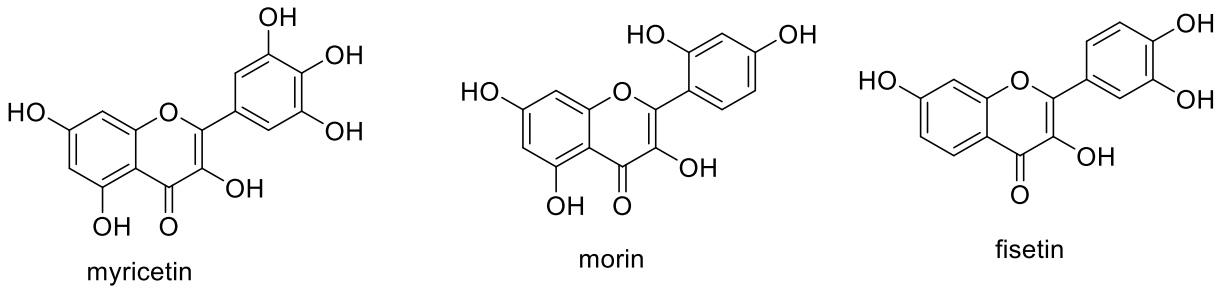


Fig 7: Structure of myricetin, morin and fisetin

1.3.1.4 Flavanones

Flavanones are also referred to as dihydroflavones. They have a saturated ring C. The saturation of double bond between positions 2 and position 3 in the ring C is what shows the main structural difference between flavanones and the rest of the classes of flavonoids. Flavanones possess the OH groups at position 5 and position 7 in ring A and either hydroxyl or methoxy substituents at the positions C3 or C4 of the ring B. They are mostly distributed in various citrus fruits, such as oranges, lemons, clementines, limes and grapes. Flavanones examples include naringenin, hesperetin and eriodictyol as shown in figure 8 (Chen et al., 2023; Panche et al., 2016).

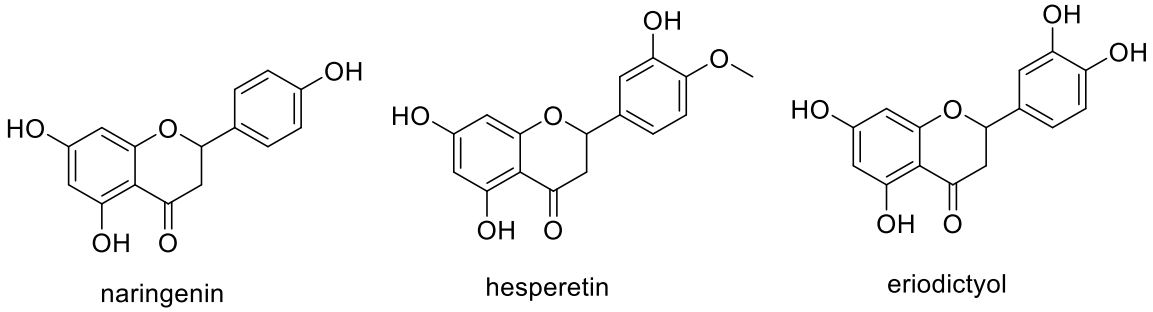


Fig 8: Structures of naringenin, hesperetin and eriodictyol

1.3.1.5 Isoflavones

Isoflavones mostly possess the ring B at the position C3 of the heterocyclic ring C of the C6–C3–C6 (diphenyl propane) backbone. This mainly shows their main chemical as well as structural difference from the other classes of flavonoids. They are characteristic metabolites for various leguminous plants and thus they are important in microbial signaling and nodule

induction in legumes (Chen et al., 2023; Lim et al., 2020). Their molecular structure can be related to that of the animal's estrogens. They are thus phytoestrogens that show potent estrogenic activity. Additionally, isoflavones have a strong antioxidant activity and this can help in decreasing the risk of getting cancers by hindering free radical-induced DNA damage (Mori-Yasumoto et al., 2021). A few examples include glycitein, daidzein and genistein as shown in figure 9 (Panche et al., 2016).

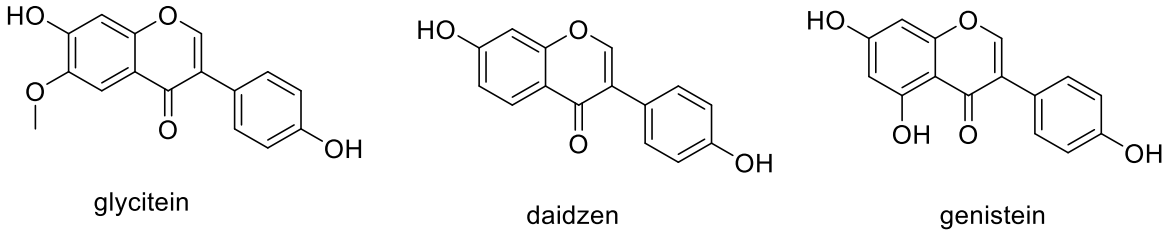


Fig 9: Structures of glycitein, daidzein and genistein

1.3.1.6 flavanols

Flavanols, mostly referred to as flavan-3-ols, possess the OH group at position 3 in the ring C. They do not have a double bond between position 2 and position 3 in the ring C (Panche et al., 2016). Several flavanols, such as catechin, epicatechin, epicatechin gallate, epigallocatechin gallate and mesquitol as shown in figure 10, largely occur in green tea, red wine and various fruits namely apples, blueberries and bananas (Godos et al., 2024; Rucker et al., 2014). Mesquitol is also found in roots, stem and leaves of plants such as *prosopis juliflora* (S. C. Chepkwony, 2019; S. C. Chepkwony et al., 2020; Sirmah et al., 2009). Flavanols are known to have good antioxidant activities (Aron & Kennedy, 2008; Duan et al., 2022). A flavanol-rich diet can help in promoting permanent development of endothelial function as well as prevent the advancement of cardiovascular diseases (Rees et al., 2018).

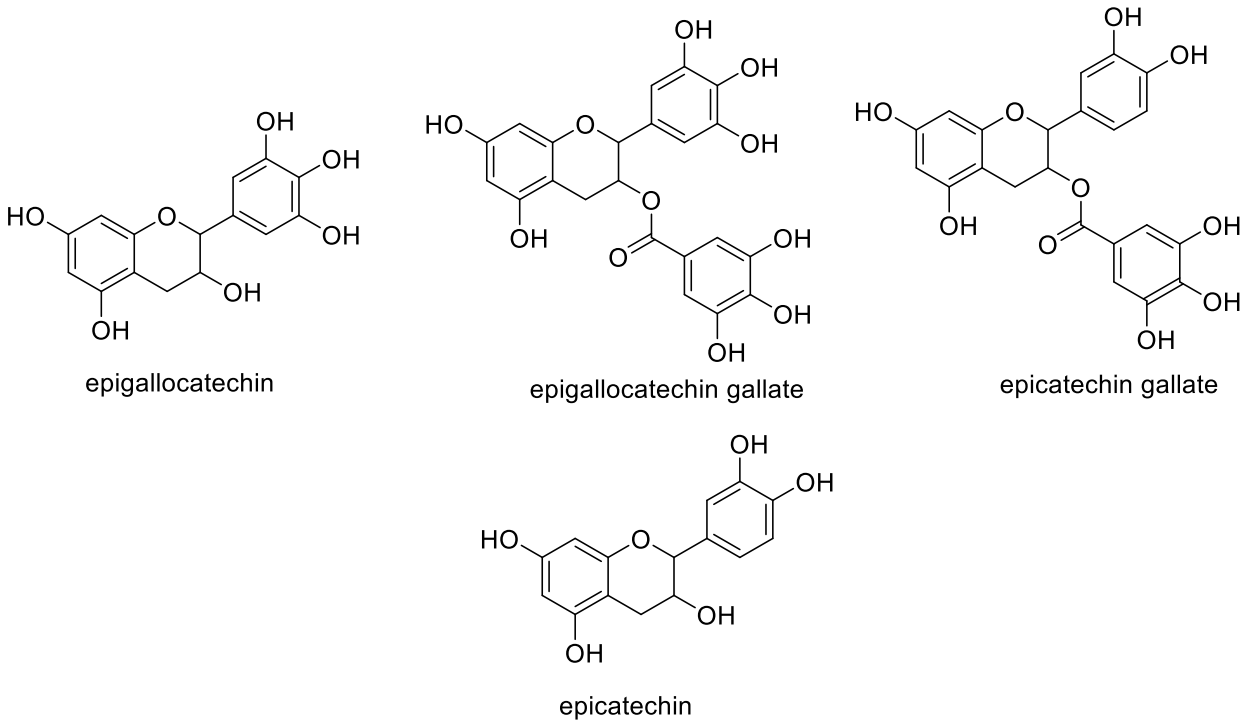


Fig 10: Structures of epigallocatechin, epigallocatechin gallate, epicatechin gallate and epicatechin

1.3.1.7 Anthocyanins

Anthocyanins are a group of water-soluble flavonoids mostly found in fruits and vegetables. They are glycosylated polyphenolic flavonoids thus they can be referred to as glycosides of anthocyanidins (Rauter et al., 2018). They have a range of colors, such as orange, purple, blue and red, and this depends on the micro-environmental pH of the flowers, fruits, as well as vegetative tissues (Chen et al., 2023; Khoo et al., 2017; S. Sharma et al., 2024). The number and positions of methoxy and hydroxy groups available as substituents in their structures lead to various classes of anthocyanins. Therefore, more than 650 anthocyanins have been identified and characterized in various plants. Anthocyanins examples include cyanidin, delphinidin, petunidin, malvidin, pelargonidin, and peonidin as shown in figure 11 (Aizza et al., 2019; Dias et al., 2021; Nutricati et al., 2025). Their antioxidant ability is linked to their ring orientation as well as the number and position of free OHs around the pyrone ring (Ali et al., 2016; Sadowska-Bartosz & Bartosz, 2024). This class of flavonoids play key roles in visual acuity, decomposition of cholesterol, and lowers the risk of cardiovascular diseases in human beings. They are also normally used as food colorants (Ma et al., 2025; Mozos et al., 2021).

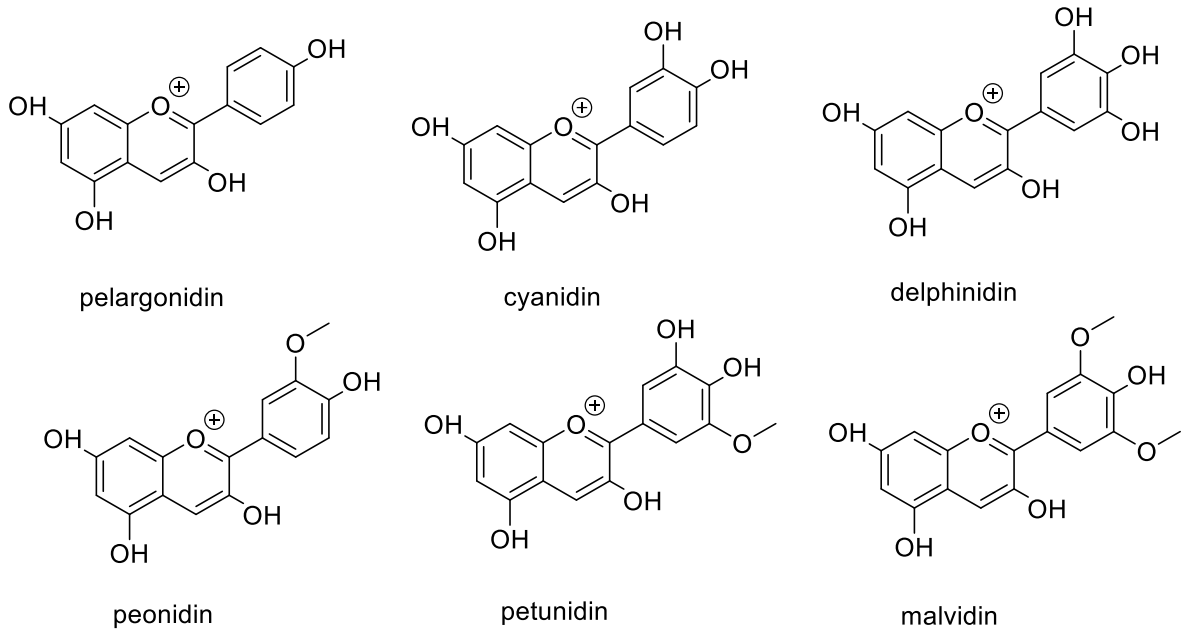


Fig 11: Structures of pelargonidin, cyanidin, delphinidin, peonidin, petunidin and malvidin

1.3.2 Flavonoids nomenclature

Flavonoids are classes of compounds with C_6-C_3-C_6 carbon framework as shown in figure 12 below. The framework shows the chromane structure or that of a 1-benzopyran, known as chromene that is a fusion of benzene ring (ring A) and the 3,4-dihydro-2H-pyran, known as pyran (ring C), together with a substituted phenyl group (ring B) on the ring C (Rauter et al., 2018).

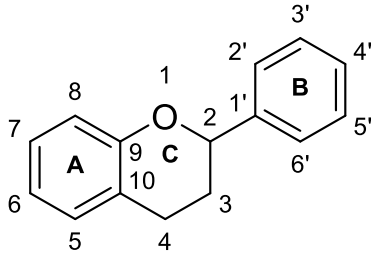


Fig 12: Basic C_6-C_3-C_6 carbon framework

1.3.2.1 Flavans

The C_6-C_3-C_6 carbon framework shown above gives flavonoids' basic structure called flavans. They are compounds having a 2-phenyl-3,4-dihydro-2*H*-1-benzopyran structure, that can further

be substituted into more classes of flavonoids, such as flavan-3-ols, flavan-4-ones as well as flavan-3,4-diols. Flavan-3-ols are compounds that originate from 2-phenyl-3,4-dihydro-2*H*-1-benzopyran-3-ol structure and flavan-4-ones are compounds that originate from 2-phenyl-2,3-dihydro-4*H*-1-benzopyran-4-one structure. These compounds are normally referred to as flavanols and flavanones, correspondingly. Flavan-3,4-diols are formed from 2-phenyl-3,4-dihydro-2*H*-1-benzopyran-3,4-diol structures. Figure 13 below shows the skeleton structures of the three classes of compounds that originate from flavans, namely flavan-3-ols, flavan-4-ones and flavan-3,4-diols.

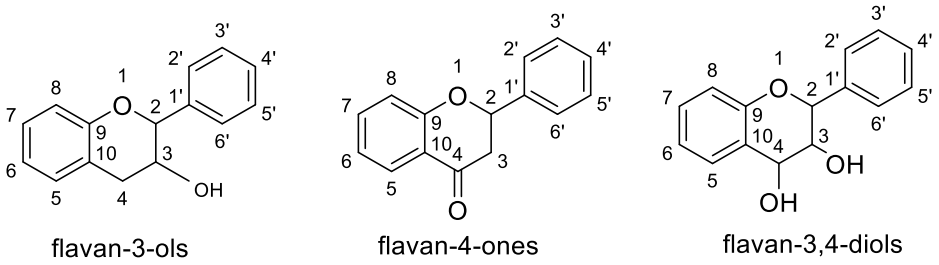


Fig 13: Skeleton structures of flavan-3-ols, flavan-4-ones and flavan-3,4-diols

1.3.2.2 Flavones and flavonols

Flavones and flavonols are compounds that originate from 2-phenyl-4*H*-1-benzopyran-4-one structure (also referred to as 2-phenyl-4*H*-chromen-4-one structure). Flavonols are basically 3-hydroxyflavones thus they are compounds having 3-hydroxy-2-phenyl-4*H*-1-benzopyran-4-one structure (or 3-hydroxy-2-phenyl-4H-chromen-4-one structure) as shown in figure 14.

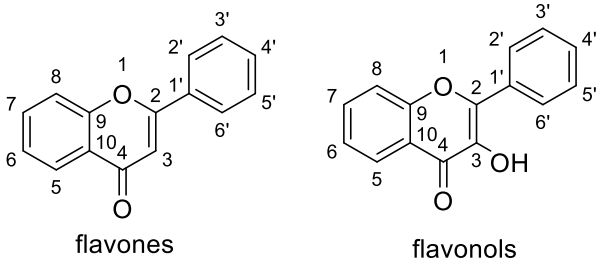


Fig 14: Skeleton structures of flavones and flavonols

1.3.2.3 Anthocyanidins and anthocyanins

Anthocyanidins are considered to be compounds that originate from a flavylium ion (2-phenyl-1-benzopyrilium ion or 2-phenylchromenylium ion). Anthocyanins are actually anthocyanidin-3-glycosides. Anthocyanidins together with anthocyanins represent a large assembly of pigments of plants. Figure 15 below shows the skeleton structure of anthocyanin.

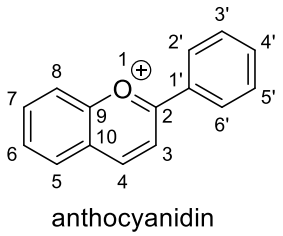


Fig 15: Skeleton structure of anthocyanidin

1.3.2.4 Isoflavans and isoflavones

Isoflavans are compounds that originate from 3-phenyl-3,4-dihydro-2*H*-1-benzopyran as the parent structure. Sometimes they are substituted. Isoflavones have the basic structure of 3-phenyl-4*H*-1-benzopyran-4-one (also known as 3-phenyl-4*H*-chromen-4-one). They can have substituent groups too from their basic structures shown in figure 16.

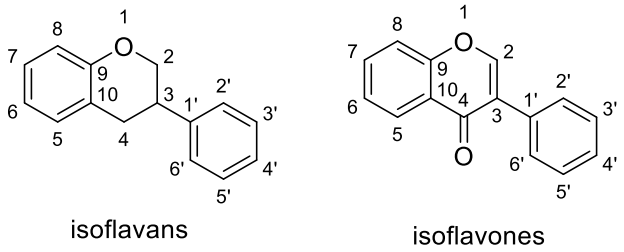
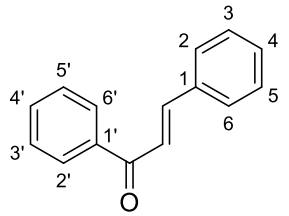


Fig 16: Skeleton structure of isoflavans and isoflavones

1.3.2.5 Chalcones

Chalcones basic structure is (2*E*)-1,3-diphenylprop-2-en-1-one as drawn in figure 17. A number of them are considered to be intermediates in the flavonoid's biosynthesis, whereby the pyran

group (ring C) has not yet been formed. Therefore, chalcones are structurally and biogenetically related to flavonoids.

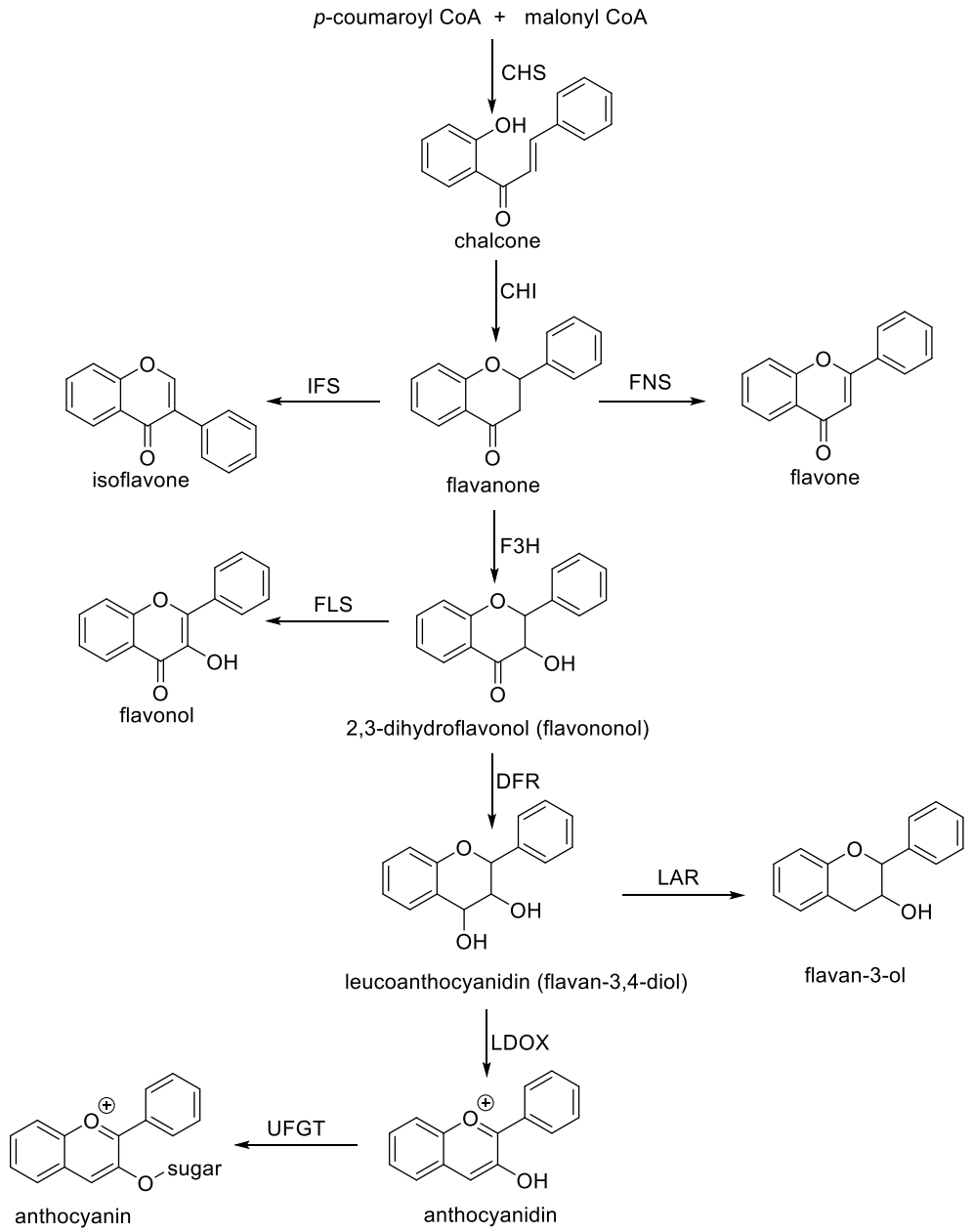


chalcones

Fig 17: Skeleton structure of chalcones

1.3.3 Flavonoids biosynthesis

Flavonoid biosynthesis occurs through the phenylpropanoid pathway, which involves the condensation reaction of one 4-coumaroyl-CoA and three malonyl-CoA, leading to formation a yellow-colored molecule called chalcone (mostly naringenin chalcone) through an enzyme called chalcone synthase (CHS). CHS is the main rate-limiting enzyme for the biosynthesis of flavonoids. In many plants, chalcones are not the final products since the pathway continues with other enzymatic steps to produce other classes of flavonoids, namely flavanones, flavones, isoflavones, dihydroflavonols, flavonols, flavanols and, finally, anthocyanins, which is the main water-soluble pigments in fruits, flowers, leaves and root crops such as beets. The formed chalcone is isomerized using chalcone isomerase enzyme (CHI) to generate flavanones. These are central intermediate products; thus, the pathway separates into many side branches, each leading to the different flavonoids through different enzymatic actions as shown in scheme 1. From the chemical point of view, the end product anthocyanins are mainly considered as the anthocyanidins O-glycosides (Falcone Ferreyra et al., 2012; Ku et al., 2020).



Scheme 1: Biosynthesis of flavonoids [CHS: chalcone synthase; CHI: chalcone isomerase; FNS: flavone synthase; IFS: isoflavone synthase; F3H: flavanone 3-hydroxylase; FLS: flavonol synthase; DFR: dihydroflavonol 4-reductase; LDOX: leucoanthocyanidin dioxygenase; LAR: leucoanthocyanidin reductase; UFGT: UDP-glucose: flavonoid-3-O-glycosyltransferase] (Ku et al., 2020)

1.3.4 Sources of flavonoids

Flavonoid-rich foods are referred to as superfoods. This is because of their amazing health benefits. They include many plant origin foods, such as tea, vegetables, fruit, grains, wine, legumes and nuts. Wine and tea are considered as the main nutritional sources of flavonoids in different western as well as eastern societies, respectively. In addition, leafy vegetables, apples, onions, cherries, berries, citrus fruits and soybeans are considered also a very vital source of flavonoids for the human diet (Panche et al., 2016; Waheed Janabi et al., 2019).

1.3.5 Extraction, isolation, and characterization of flavonoids

The extraction, isolation together with characterization of flavonoids have been successfully done from natural products by phytochemists through the use of applicable techniques as well as new approaches. Most of the flavonoids have various pharmacological properties such as antioxidant and antifungal properties that can be applied in food, cosmetic, and pharmaceutical industries. To obtain these flavonoids, there is need to have comprehensive know-how of the extraction processes that is appropriate to obtain a high quality and pure extracts. Thus, it is important to come up with new extraction techniques or improve the old methods (Dias et al., 2021).

1.3.5.1 Extraction

Flavonoids obtained from plants can be extracted from different parts of the plant like roots, leaves, barks, flowers, woods and fruits. The plant samples are usually dried and finely ground prior to the extraction process to help in facilitating the extraction yields through increased solubility as well as preserving the chemical constituents of the plants. The dried plant samples contain lots of flavonoids compared to fresh plant samples. Noticeably, the finely ground samples continuously give higher extraction yields because the solvent has much contact with surface plant constituents when the samples have smaller particles. The yields of extraction of flavonoids from various natural products are similarly affected by other factors, like time, temperature, and ratio of mixing solvents (M. D. Awouafack et al., 2017; Chaves et al., 2020; Shi et al., 2022).

There are many methods for extraction of flavonoids from plant materials. They comprise of maceration, infusion, decoction, percolation, Soxhlet (or hot continuous extraction), microwave-

assisted extraction and ultrasound-assisted extraction (M. D. Awouafack et al., 2017; Bitwell et al., 2023). Most commonly used solvents during extraction are water, methanol, ethanol, n-butanol, acetone, chloroform, ethyl acetate, and many others. Polar solvents are mainly used in obtaining flavonoid glycosides, while the other non-polar solvents help in extracting mainly their aglycones (M. D. Awouafack et al., 2017). Most research done in the flavonoids extraction in plant materials have been done through infusion and maceration (Munhoz et al., 2014; Vongsak et al., 2013). Maceration is actually very simple but a very old extraction technique. It is mostly done at room temperature. The method involves soaking of the powdered plant materials in solvent (such as water, alcohol, oil or any other preferred organic solvent) for longer duration of time. Such prolonged soaking period often breaks the cell wall and helps in driving the plant constituents into the solvents (Anandharamakrishnan & Parthasarathi, 2023). Acetone, methanol and ethanol are considered to be best solvents for extraction of flavonoids. Acetone was described to be the best solvent for extraction of flavonoids from *Tagetes patula* and *Momordica charantia* (Munhoz et al., 2014; Tan et al., 2014). Many extracted products that are intended for human consumption are often made through maceration technique, while products that are made purposely for experimental testing are made through the use of other techniques in addition to the maceration technique (Abubakar & Haque, 2020).

Decoction and infusion are closely related. Infusion is an extraction method for chemical compounds through making the plant material to remain suspended in a solvent like water, alcohol or oil over a period of time. Maceration is sometimes referred to as simple infusion (Turatbekova et al., 2023). Decoction mainly involve first drying of the plant materials followed by mashing, slicing, as well as cutting the plant materials to permit maximum dissolution of the compounds. This is finally followed boiling in water to help in extract of volatile organic compounds plus any other chemical substances of interest. Decoction is more simple, convenient and cheap extraction method and thus it can easily be used in poorly-equipped research laboratory. Report on decoction methods show that it has the highest concentration of phenolic compounds such as flavonoids as compared to infusion (Martins et al., 2015).

Percolation is a technique of extraction whereby the plant is put in a narrow cone-shaped vessel that is hollow on both ends, referred to as a percolator extractor, and then a specific solvent of interest is passed through percolator with the plant material for maybe 24 hours - 72 hours (Abubakar & Haque, 2020). This extraction process is done mostly at room temperature as the solvent is passed between plant materials dropwise, whereas the extracted compounds pass

from the plant material to the extractant due to their diffusion and dissolution process. The flavonoid extracts prepared by percolation or infusion technique need obligatory purification (Turatbekova et al., 2023).

Soxhlet extraction technique utilizes the principles of tapping and recirculation to endlessly extract plant constituents from the plant materials using fresh solvents (Figure 18). Compared to methods of extraction like percolation and maceration, the Soxhlet extractor has got many advantages. It is considered to be a very effective automated method that needs less time and solvent. An example of this technique is the flavonoids extraction from *Vernonia cinerea* leaves through the use of ethanol that takes less time, approximately 2 hours (Alara et al., 2018). Nevertheless, it is vital to consider that long extraction periods and high temperatures are able to increase the probability of thermal degradation (Turatbekova et al., 2023).

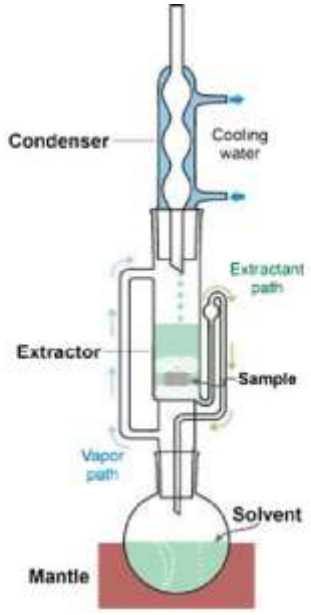


Fig 18: Soxhlet extractor (Dabbs et al., 2006)

Ultrasound-assisted extraction (UAE) is also another method that is extensively studied and documented for the effective extraction of flavonoids. Ultrasonic transducers make use of electrical energy to help in producing ultrasound. Then, the intermolecular interaction between the plant matrix and flavonoids are destroyed because of collapse of cavitation bubbles. The solvent extraction process is referred to as solid-liquid mass transfer process. Ultrasound destroys the plant cell walls thus permitting solvents to effortlessly enter the plant samples to

promote mass transfer. Therefore, UAE can typically attain exceptional extraction efficiencies (Zhu et al., 2024). Cui et al (2022) report on successful extraction of flavonoids from the *Astragalus membranaceus* stems and leaves using Ultrasound-assisted extraction (Cui et al., 2022). Another study showed that ultrasound-assisted ethanol extraction can be used in extraction of flavonoids from the fruit bodies of *Sanghuangporus vaninii* (Lin et al., 2023).

Considering other methods, the microwave-assisted extraction (MAE) goes through many steps compared to the conventional extraction methods, such as percolation, maceration and Soxhlet extraction. These steps are solvent penetration into the solid matrix, the breakdown of compounds through solubilization, the solute transportation to the outside of the solid matrix, the solute migration from the external solid surface of the into the solution, the extract movement with respect to the solid, as well as the separation and finally the discharge of the solid and extract. A study on flavonoids extracted from *Perilla frutescens* leaves showed that MAE can be a good alternative method of extraction compared to Soxhlet. MAE needs less quantity of solvents and consumes less time (Darvishzadeh & Orsat, 2022; Shao et al., 2012). MAE is actually an advanced extraction method and its efficiency is affected by the behavior, polarity and nature of solvents as well as compounds that are analyzed under the conditions of the microwave (Orsat & Routray, 2017).

1.3.5.2 Isolation

Isolation is the process of separation of individual compounds from a particularly very complex matrix. Separation of different flavonoids from their natural sources can be done by repetitive and sequential chromatography methods like column chromatography (CC), preparative thin-layer chromatography (prep. TLC), high-performance liquid chromatography (HPLC), medium-pressure liquid chromatography (MPLC), amongst others. Column chromatography uses stationary phases that can either be normal or reverse phase Sephadex or silica gels (M. D. Awouafack et al., 2017). The open CC is considered as the current easily used isolation methods for flavonoids. The choice of very good solvents system to be used in the mobile phase is vital and ought to be prepared from the TLC analysis of the flavonoid-rich crude extract. In other cases, some key separation fractions can directly be isolated using prep. TLC or Sephadex LH-20 gel if the extracts do not comprise of complex flavonoids mixtures. In the process of doing separation, constituents from the extract are able to have closer retention factors (Rf) values considering their difference in polarities. To have a good separation process,

a change in adsorbent phase to either prep. TLC or open CC in some cases is very important (M. D. Awouafack et al., 2017; Susanti et al., 2024). A number of studies reported the isolation of different flavonoids by means of CC. Flavonoids from the *Mitracarpus hirtus* plant leaves were isolated using CC (Musa, 2015). A few dihydrochalcones from *Eriosema glaumerata*, polyhydroxylated flavones from *Eriosema robustum* having good antioxidant activities, an isoflavanol from *Kotschya strigosa* and isoflavones from the leaves, stem and roots of *Erythrina schliebenii* were also isolated using CC (M. Awouafack et al., 2008; M. D. Awouafack et al., 2013, 2016; Nyandoro et al., 2017).

Considering HPLC, there are reverse and preparative HPLC (prep. HPLC), and both are used to obtain a high purity flavonoid. Reverse phase HPLC makes use of a polar mobile phase and a non-polar stationary phase to help in separation of mixtures of compounds based on their hydrophobicity. Prep. HPLC is used for purification of a large number of mixtures of compounds for further research. However, prep. HPLC method has widely been utilized for isolation of various polyphenols, such as flavonoids. The suitability of the technique is its high absorption ability in UV detector during the process of isolation (M. D. Awouafack et al., 2017). The method is similarly linked with high analytical version that helps in qualitative characterization of different polyphenols in the analyzing extracts or samples. Prep. HPLC has been used to isolate gallocatechin, a flavanol, from *Alphitonia neocaledonic* and orientin and isoorientin, flavones, from *Mauritia flexuosa* (D. M. De Oliveira et al., 2013; Muhammad et al., 2015). HPLC is different from MPLC. HPLC uses smaller particles as well as high pressure thus it offers superior separation, whereas MPLC makes use of larger particles and lower pressures. MPLC, therefore, permits faster flow rates and can use larger volumes of samples as compared to HPLC (Susanti et al., 2024).

The application of other methods, like centrifugal thin layer chromatography (CTLC), which uses centrifugal force for separation of larger quantities of samples in a thin layer adsorbent material; and high speed counter current chromatography (HSCCC) that is a liquid-liquid separation method based on the partition coefficients of two different immiscible liquid phases; has similarly resulted to the isolation of many different flavonoids (M. D. Awouafack et al., 2017). In a particular study, isolation of flavonoid fractions from *Ricinus communis* leaves was done by CTLC (N. Choudhary & Arora, 2013). In other cases, different studies have used a combination of these methods to help isolate the flavonoids. Rutin, a flavonol, has been isolated through the use of a combination of HPLC and MPLC from *Scrophularia atrata* (Dawa et al., 2021). A

combination of prep. HPLC and HSCCC was used in isolating quercetin-3-O-neohesperidoside, vitexin and orientin, three flavonoid glycosides, from *Trollius ledebourii* plant (X. Zhou et al., 2005). Some flavonoids can similarly be isolated as enantiomers using prep. HPLC from natural products, such as Lachnoisoflavones A and Lachnoisoflavones B from *Crotalaria lachnophora* (M. D. Awouafack et al., 2011). Figure 19 below shows a few flavonoids isolated in literature from the plant.

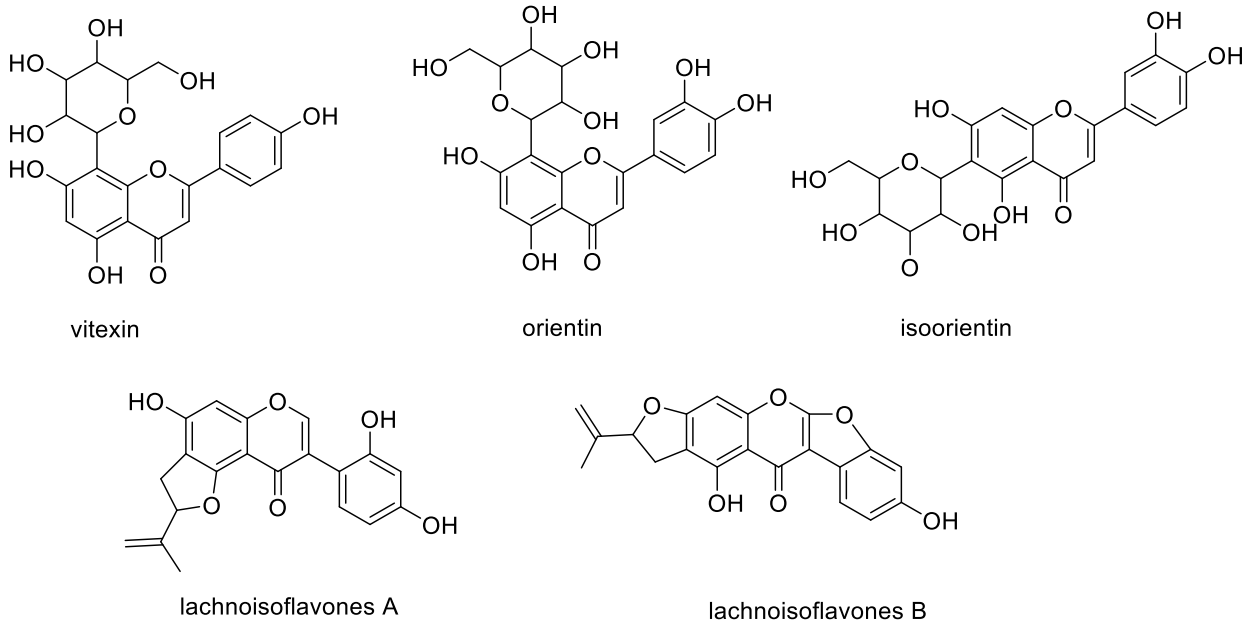


Fig 19: Some flavonoids isolated from different plants

1.3.5.3 Structure characterization

The characterization of different structures of flavonoids is mainly the interpretation of their spectroscopic spectra attained through using various methods like nuclear magnetic resonance (NMR), ultra-violet (UV), infrared (IR) and mass spectrometry (MS). Additionally, physical properties, such as circular dichroism (CD) and melting point (MP) are very important for full structure characterization of any isolated flavonoid. The NMR spectroscopy is primarily made up of 1D analysis and 2D analysis (M. D. Awouafack et al., 2017). In 1D NMR analysis, there is proton (^{1}H) analysis, carbon-13 (^{13}C) analysis as well as distortionless enhancement by polarization transfer experiment (DEPT) analysis, which gives information regarding signals of protons and carbons and also type of carbons such as C, CH, CH_2 or CH_3 in the flavonoid structure during structure elucidation. ^{1}H NMR spectrum shows the number, known as the

integration value, as well as the type of proton in the flavonoid involved. The proton (^{1}H) chemical shift (δ_H) values are typically showed from 0 (TMS reference standard value) to 14 ppm whereas the carbon-13 (^{13}C) chemical shift (δ_C) values occur from 0 to 220 ppm. For example, the ^{1}H chemical shift values for H-3 (Flavanone) appear at 2-3 ppm, for A- and B-ring protons appear at 6-8 ppm and for H-2 (isoflavone) appear at 8-8.5 ppm. The ^{13}C chemical shift values for C=O and C-4 (flavanol) appear at 170-210 ppm and 28-35 ppm respectively. On the other hand, the 2D NMR includes the proton-proton correlated spectroscopy (1H 1H COSY), heteronuclear single quantum coherence (HSQC) or the heteronuclear multiple quantum coherence (HMQC), the heteronuclear multiple bond connectivity (HMBC), the rotative-frame overhauser effect spectroscopy (ROESY), the nuclear overhauser effect spectroscopy (NOESY) and also the total correlated spectroscopy (TOCSY) experiments. In many cases, the ^{13}C flavonoids data are easily assigned from HMBC as well as HMQC spectra (M. D. Awouafack et al., 2017; Lenny et al., 2013; T. Mabry et al., 2012).

The IR spectroscopy shows less but very important information in the characterization of flavonoids structure. The hydroxylated chalcones or dihydrochalcones, flavones and isoflavones have a large absorption band approximately 3300–3600 cm^{-1} because of the presence of OH groups. Furthermore, there is an intense absorption band that is characteristic for all flavonoid C=O, which appears around 1680 cm^{-1} and can move to around 1620 cm^{-1} when the OH is chelated with a carbonyl group. There is also a sharp and very intense absorption band that appears between 1500 cm^{-1} and 1600 cm^{-1} because of the aromatic double bonds from the aromatic rings (M. D. Awouafack et al., 2017; Krysa et al., 2022). For the UV spectroscopy, there is two maxima absorption bands observed at approximately 300-350 nm, which corresponds to band I from A-ring and 240-280 nm, which corresponds to band II from B-ring for the flavonoids (Nistor & Butnariu, 2023).

MS is the other technique used in structure characterization. It helps in determining the molecular weight by understanding the distribution of substituent groups between the A-ring and B-ring of flavonoids. It can also help in determining of the site as well as the nature of attachment of different sugars in flavonoid O-glucosides and C-glucosides. The basic flavonoid nucleus has a molecular weight of 224 a.m.u. for chalcones and flavanones; 222 a.m.u. for aurone, flavones and isoflavone; 238 a.m.u. for flavonols; and 240 a.m.u. for dihydroflavonols. Determination of the molecular weight of an unidentified flavonoid involves addition of a.m.u of all its substituent groups [for example, 16 a.m.u for -OH group and 30 a.m.u. for -OCH3 group]

to the corresponding molecular weights of the basic flavonoids above. The loss of a number of ion-fragments from the molecular ion is actually characteristic of the flavonoids mass spectra. Peaks attained in this process of fragmentation show correctly the matching ion-fragments, which are that are stated as mass-to-charge ratio (m/z). The exact molecular weight for every ion-fragment can be measured to approximately 0.0001 mass unit when the MS machine is working in high resolution. The mentioned information allows for easy calculation of the exact molecular formula obtained from the molecular ion-fragments and ion peaks. A precondition for effective MS analysis is that the flavonoid ought to be adequately volatile when it is under high vacuum in the spectrometer. Many aglycones are appropriately volatile at the probe temperature (100-230 °C). Higher temperatures are required for the very polar flavonols and polyhydroxyflavones. Anthocyanidins and glycosides, nevertheless, are not adequately volatile and ought to thus be derivatized to enhance their volatility ability through either perdeuteromethylation, permethylation or trimethylsilylation process. The determination of structures of different flavonoids remains a key source of many drug discoveries (M. D. Awouafack et al., 2017; Cuyckens & Claeys, 2004; Hagazy & Mulata, 2016; T. J. Mabry & Markham, 1975).

1.4 The species Prosopis juliflora

The species *Prosopis juliflora* is an evergreen shrub that is considered amongst the most invasive species in many arid and semi-arid regions around the world. It originated from Mexico, the Caribbean and South America. It has been recognized as an invasive weed species in Australia, Africa, Asia and elsewhere (Hussain et al., 2021; Pasiecznik, 2017; Patnaik et al., 2024). The plant is widespread and hyperaccumulates in an area very fast (Bezaredie et al., 2023). It is a phreatophytic as well as a perennial tree species (Choge et al., 2022). It is a member of *Fabaceae* family as shown in the taxonomical classification in table 1 below.

Table 1: Taxonomical classification of *Prosopis juliflora* (Yadav & Rana, 2020)

Kingdom	**Plantae**
Phylum	Angiosperms
Class	Dicots
Order	Fabales
Family	Leguminosae or Fabaceae
Subfamily	Mimosoideae
Genus	*Prosopis*
Species	*Juliflora*

1.4.1 Botanical information

The species is mostly large, thorny, deciduous, and deep-rooted. It grows up to approximately 12 m in height and has a trunk diameter of around 1.2 m containing the sapwood, heartwood, pith and barkwood, but all these depend on the tree variety and climatic conditions (Balcha, 2022). It also has a rough, thick, gray-green bark that forms scale as it ages. The plant is usually multi-stemmed with various sharp thorns measuring up to 5 cm. The plant's leaves are light-green, deciduous, bi-pinnate, combined with around 10 to 20 leaflets (Sundhararajan et al., 2023). It forms flowers soon after the development of its leaves. The flowers are 5 to 10 cm in length. The ends of the branches have elongated green-yellow cylindrical spikes that appear in groups of 2 to 5. The plant pods are 20-30 cm long and contain around 10 to 30 seeds for every pod (Venkatachalam et al., 2021). A mature mesquite tree is capable of producing many thousands of seeds that remain viable for a maximum of 10 years. The tree reproduction is through seeds, but not vegetatively. Its seeds are spread by mostly cattle and other animals that eat the pods and spread the tree seeds from the pods in their droppings (Byalt & Korshunov, 2021; Hussain et al., 2021; Simon, 2021). The roots of the plant are capable of growing to a great depth of 53 m (175 feet) as it searches for water (Shiferaw et al., 2024).

1.4.2 Distribution in Kenya

The first documented *Prosopis juliflora* case in Kenya happened in 1973 in Bamburi, a place in Mombasa County (Mungoche et al., 2025). The following years, many *Prosopis juliflora* plants were introduced on a large cultivation and thus extensive scale started in 1983 in the Bura region in Taita Taveta County and Baringo county. The species later became a major invader of

other dry parts of the country, which included Marsabit, Garissa, Mandera, Mwingi, Tana River, and Wajir counties (Kihungu, 2022). The large-scale cultivation of plant was encouraged by the NGOs and government agencies, who endorsed the plant because of its suitability to survive during the dry seasons. In 2008, the Kenyan minister for agriculture stated that the *Prosopis juliflora* had become a deadly and stubborn weed and thus the government needed ways to solve the problem (Maundu et al., 2009).

1.4.3 Distribution around the world

Prosopis juliflora is a shrub which is native to Mexico, South America and the Caribbean. It is an evergreen shrub which has extensive root system which can reach up to 40 cm in just eight weeks, and grows quickly after germination (Mohammed Mussa Abdulahi et al., 2017). This characteristic of the plant helps it to invade new regions. The plant is native to countries such as Costa Rica, El Salvador, Quatemala, Honduras, Mexico, Nicaragua, Panama, Colombia and Venezuela. It was later introduced by the early man many years in countries like Brazil, French Polynesia, Austrailia, Hawaii (USA), Vietnam, Yemen, United Arabs Emirates, Iran, Iraq, Israel, Jordan, Kuwait, Nepal, Oman, Pakistan, Philippines, Qatar, Saudi Arabia, Sri Lanka, Taiwan, Thailand, India, Indonesia, Cambodia, Uganda, Kenya, Tanzania, Tunisia, Sudan, South Africa, Senegal, Nigeria, Niger, Morrocco, Mali, Mauritania, Ghana, Ethiopia, Egypt, Namibia, Chad, Djibouti, Eritrea and Algeria amongst others. To the countries the plant was introduced, it became invasive in Kenya, Ethiopia, Egypt, Chad, Eritrea, Mauritania, Namibia, Nigeria, Senegal, South Africa, Tanzania, Sudan, Uganda, India, Israel, Iraq, Kuwait, Jordan, Oman, Pakistan, Sri Lanka, Saudi Arabia, United Arab Emirates, Yemen, Australia, Brazil, Hawaii (USA), Colombia and Venezuela. It has become established as a weed in Asia, Australia, and elsewhere because it is fast-growing, nitrogen-fixing, and tolerant to arid conditions and saline soils (Mohammed Mussa Abdulahi et al., 2017; Mungoche et al., 2025; Pasiecznik, 2017).

1.4.4 Uses of *Prosopis juliflora* plant

There are several uses of *Prosopis juliflora* plant around the world. They include food, fodder, apiculture, fuel, fiber, dyestuff, alcohols and medicine (Aguirre–Loredo, 2025; Orwa et al., 2009).

- Foodstuff: A rich, tasty flour can be prepared from crushed pods with no seeds. The embryos and cotyledons when pulverized produce a flour full of proteins and sugar

suitable for people with diabetes. Other literatures show that *P. juliflor*a pods are utilized in the preparation of bread, coffee, sweets and syrup. The pods are then processed to help in improving the flavor.

- Fodder: For dairy farming, the flour may produce approximately 60% of concentrate rations. The plant is fed unmixed to sheep in South Africa. It should be noted that ripe pods compose of approximately 14% crude protein. Additionally, the short-fibred parts are appropriate for poultry and pigs. The leaves are used as fodder by goats in Baringo, Kenya.
- Apiculture: The plant is a key source of honey in many countries such as Jamaica, Bolivia, Pakistan, Australia and Sri Lanka.
- Fuel: The plant's crooked stems as well as branches are used generally in making good firewood and for provision of excellent charcoal. For instance, the species wood is widely used in the USA as a source of barbecue fuel and approximately 30% of the charcoal that are commercialized for this purpose comes from *P. juliflora* from the northern Mexico's Sonora Desert.
- Fiber: A large percentage of the plant's species is used for production of fiber. The produced fiber can be used in manufacturing paper, hardboard and paperboard.
- Timber: The seasoned wood can be utilized in producing fencing posts, wood for furniture, corrals and crafts. The wood is hardly ever used in the construction industry since most of its trunks are not growing straight enough or long enough.
- Gum or resin: The heartwood part of the plant contains good quantities of extractable polyphenolic compounds that can be isolated as a unique flavonol compound to be utilized in the formation of another phenol-formaldehyde polymeric resins. A gum, which is the same in properties to the gum arabic from *Acacia senegal*, regularly projects from the stem as well as older branches.
- Tannin or dyestuff: This product can be extracted from *P. juliflora*; however, the yield is just approximately 10%. Tannin can similarly be extracted from the plant as a byproduct when the plant's wood is treated for other uses, like animal rations.
- Alcohol: In countries such as Chile, Peru and Argentina, the pods are importantly used producing alcoholic beverages like cocktails.
- Medicine: *P. juliflora* syrup that is prepared from ground plant pods has many values medicinally. The syrup is offered to children who show weight deficiency or motor development retardation; it is thought to be increasing lactation in children. Tea can also

be made from *P. juliflora* and it is believed to be good for skin lesions and any digestive disturbances.

1.4.5 Biological activities of *Prosopis juliflora*

The genus *Prosopis* is a commercially vital plant group. They have been utilized from long time ago, mainly for medicinal aims (Ravhuhali et al., 2021; Sharifi-Rad et al., 2019). Conventionally, the gum, smoke and pasters from pods and leaves of this genus, which includes species such as *Prosopis juliflora*, have been used for anticancer, antimicrobial, anti-inflammatory and antidiabetic purposes (Henciya et al., 2017). Compounds from *Prosopis juliflora*, namely flavonoids, quinones, tannins, alkaloids or many other phenolic compounds have showed potentials in many biofunctions, like analgesic, antibiotic, anthelmintic, antiemetic, antioxidant, antiprotozoal, antiulcer and antimalarial activities (Aguirre–Loredo, 2025; Henciya et al., 2017; Sirmah et al., 2011). Crude extracts of *P. juliflora* have shown different classes of secondary metabolites mentioned above that have exceptional and combined antifungal and therapeutic traits (Naik et al., 2023). Some pure compounds, such as an alkaloid juliflorine, from *Prosopis juliflora* also helps in curing Alzheimer disease through inhibition of acetylcholineesterase in the cholinergic brain synapses (M. I. Choudhary et al., 2005). Ethanolic extracts from the fruits and flowers of *P. juliflora* showed IC50 value of 24 µg/ml and they have got good antiplasmodial properties (Simonsen et al., 2001). Ethanol extracts from the leaves have got good anthelmintic activity as compared to the roots. The leaves contain compounds such as condensed tannins, alkaloids and saponins that are responsible for the anthelmintic activity (Rechab, 2017). In another study, acetone extract of the plant had good larvicidal activity during the control of Aedes albopictus mosquito (Yadav & Rana, 2020). Some indirect medicinal use of *Prosopis juliflora* include the presence of high antioxidant activity in “mesquite” honey and pollens of the plant (Almaraz-Abarca et al., 2007; Henciya et al., 2017).

Fungal pathogens namely *Rhizoctonia bataticola, Colletotrichum gloeosporioides* and *Alternaria alternata,* and bacterial food borne pathogens namely *Escherichia coli, Staphylococcus aureus* and *Salmonella enterica* are excellently suppressed by *Prosopis juliflora* acetone and water extracts (Naik et al., 2023). The ethanolic and aqueous extracts of the *P. juliflora* leaves are considered to have good antibacterial and antifungal activities (Saleh & Abu-Dieyeh, 2021). This good antimicrobial activities can be utilized in identification of bioactive natural products, which can serve as leads in the development of new pharmaceuticals. Thus, a deeper study of

Prosopis juliflora plant pure compounds can also result into the advancement of natural substitute antimicrobial compounds against food borne and plant pathogens.

1.4.6 Phytochemical information

Different phytochemical studies of *Prosopis juliflora* have been previously reported and various bioactive compounds have also been acknowledged. In many recent studies, phytochemical analysis of various parts of *P. juliflora* extracts showed diverse classes of secondary metabolites, which include phenolics, tannins, flavonoids, terpenes, alkaloids and steroids as shown in table 2 below (Singh, 2012; Yadav & Rana, 2020).

Table 2: Phytochemical information of *Prosopis juliflora* (Singh, 2012)

Phytochemicals	*Leaf*	*Pod*	*Flower*	*Stem*	*Root*
Tannin	+	+	-	-	++
Phenolics	+++	+++	+++	+	++
Flavonoids	+++	++	+++	+	++
Cardiac glycosides	-	-	-	-	-
Alkaloids	++	+++	++	-	+
Terpenes	++	++	+	+	++
Steroids	+++	++	+	+	+
Saponin	-	-	-	-	+

Key: +++ = high concentration, ++ = moderate concentration, + = low concentration, - = absent

P. juliflora is widely known for the presence of flavonoids which are responsible for various activities, such as antioxidant activity and antifungal activities. Figure 20 shows some of the flavonoids obtained from the plant, which include quercetin, kaempferol, luteolin, catechin, 4'-O-methylgallocatechin and mesquitol (S. Chepkwony et al., 2022; Khandelwal et al., 2016; Sirmah et al., 2009).

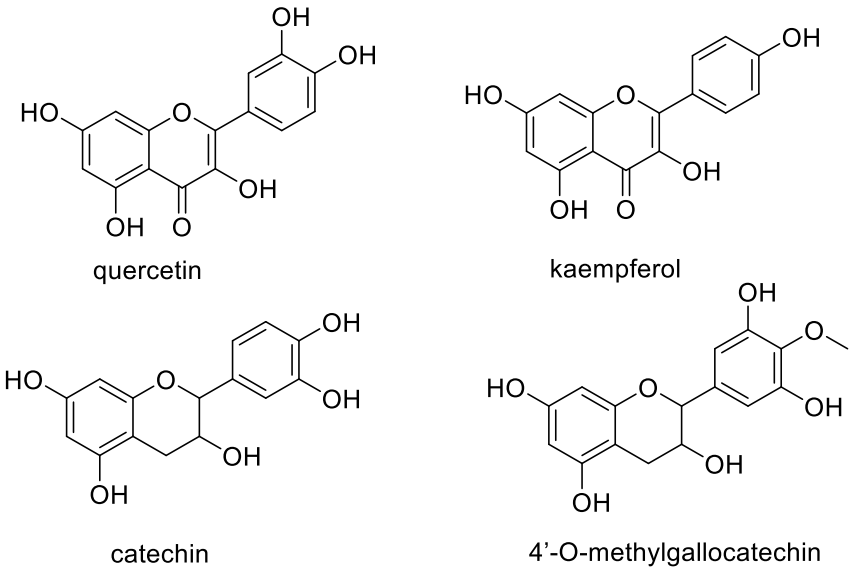


Fig 20: Structure of some flavonoids from *Prosopis juliflora*

CHAPTER 2: MATERIALS AND METHODS

2.1 Reagents and apparatus

2.1.1 Reagents

Acetone, ethyl acetate, ethanol, cyclohexane, hexane, toluene, dichloromethane (DCM), acetonitrile (ACN), methanol, triethylamine (TEA), amongst other solvents used in this study were analytical grade and they were all obtained from Sigma-Aldrich (Darmstadt, Germany). All the reagents used in the research namely catechin, caproic acid, caprylic acid, lauric acid, myristic acid, palmitic acid, DMAP, DCCI, BOP, TBAI, Pd/C, benzyl bromide, tebuconazole, malt extract, nutrient agar and HCl were commercially obtained and were not purified whatsoever. $NaHCO_3$, NaCl, NaOH, K_2CO_3 and $MgSO_4$ salts were bought from Sigma-Aldrich (Darmstadt, Germany). The distilled water used was from the laboratory water distiller.

2.1.2 Apparatus

The apparatus used in this study include: measuring cylinders, round bottomed flasks, conical flasks, volumetric flasks, thermometer, desiccator, fritted Pyrex 3 glass funnel, filter papers, filter funnels, beakers, separating funnels, pipettes, weighing balances, test tubes, brushes for test tubes, wash bottles, petri dishes, etc.

2.2 Analysis

Different analysis tools were used for this particular project. They include TLC plates, NMR machine, FT-IR machine and Kofler heating bench.

2.2.1 TLC analysis

Analytical TLC was carried out using Merck silica gel 60 F 254 plate and phosphomolybdic acid and UV lamp (detection performed at 254 nm) were used to visualize the TLC plates.

2.2.2 NMR analysis

The ^{1}H NMR and ^{13}C NMR spectra were carried out using a Bruker 400 MHz NMR machine (Billerica, Massachusetts, USA) and they were recorded using acetone, chloroform and methanol deuterated solvents. The chemical shifts (δ) were given in parts per million (ppm) and the coupling constants (J) was given in hertz (Hz). The chemical shifts were stated comparative

to the solvent peak. Multiplicities of the peaks in the NMR were documented as singlets (s), doublets (d), doublets of doublets (dd), triplet of doublets (td), quartets (q) and as multiplets (m).

2.2.3 FT-IR analysis

FT-IR spectra were recorded using a Perkin Elmer FT-IR spectrophotometer (Waltham, MA in USA) for analysis of the functional groups of compounds by taking measurements of the absorption of IR light. The named Perkin Elmer FT-IR spectrophotometer analyses samples using Attenuated Total Reflectance (ATR) method whereby the solid samples are put directly onto the crystal and then pressed to allow for a good contact for analysis.

2.2.4 Melting point analysis

Melting point of different compounds were determined using Kofler heating bench by putting the compounds on a metal bar having a calibrated temperature gradient, and spreading them in a thin line along the heated bar. The compounds melt only at the point in which the heating bench temperature is equal to its melting point. This analysis allows for direct recording of the melting point along the heating bar.

2.3 Sample preparation

Prosopis juliflora plant samples were collected mainly from Baringo County (latitude 0°35′0” N, longitude 36°00′32.5” E) in Kenya. The stem parts of *Prosopis juliflora* plant were cut down and heartwood part of the wood obtained from the sample. The heartwood parts were air dried under the shade and then ground into fine powders using a Fritsch pulverisette 9 laboratory vibrating cup mill (Idar-Oberstein, Germany) at 1100 RPM for 3 minutes and passed through a 115-mesh for sieving. The obtained fine plant sample as shown in figure 21 was dried overnight in the desiccator before extraction.



Fig 21: Powdered *Prosopis juliflora* plant sample

2.4 Extraction methods

In this particular research, two different extraction methods were studied using the heartwood part of the plant from Baringo County, Kenya. The two extraction methods were Soxhlet extraction and maceration extraction. Each extraction method produced different yields that were calculated in percentage using the formula below:

$$\% \text{ yield} = \frac{\text{mb}}{\text{ma}} \text{ x } 100$$

Where ma is the mass of the sample powder before extraction and mb is the mass of the extractives after extraction.

2.4.1 Soxhlet extraction method

For this extraction, triplicate extractions were carried out on a one-liter Soxhlet apparatus (figure 22). 60 g of plant sample powder was used together with two solvents namely cyclohexane and acetone. The volume of each solvents used was 600 ml. Cyclohexane extraction was done for 2 hours and acetone extraction was done for 6 hours. One cycle of extraction was completed after every 5 minutes. Timing of extraction period began just after the first extraction cycle was completed. The two solvent extracts were thus concentrated at low pressure through the use of

Büchi rotavapor R-200 (Flawil, Switzerland) to produce a yellow extract for cyclohexane extraction and a dark-brown extract for acetone extraction.

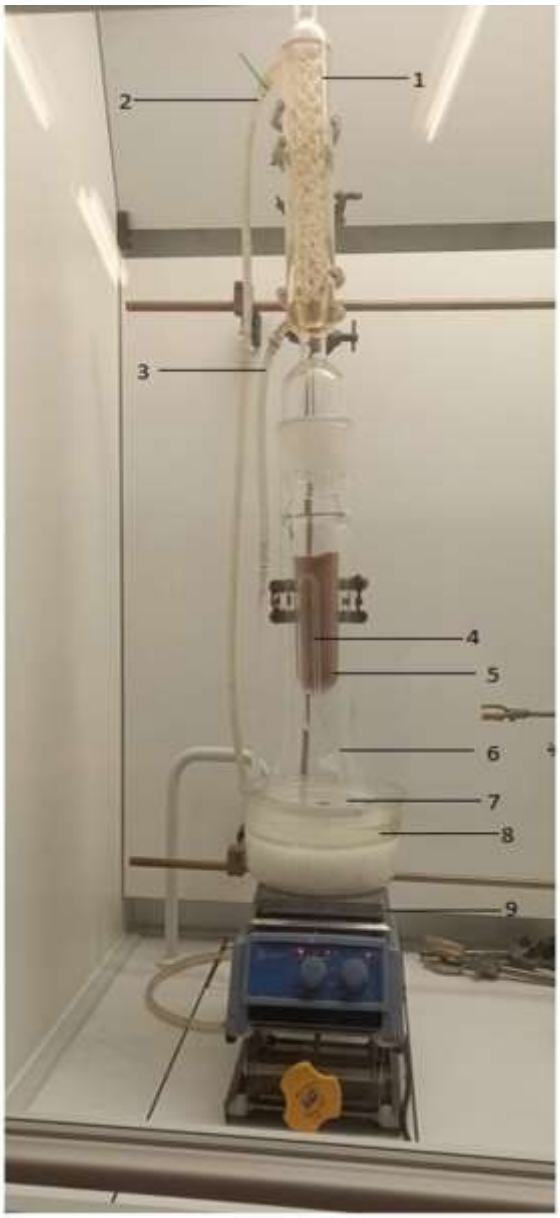


Fig 22: Soxhlet extraction set-up (1. condenser, 2. water outlet, 3. water inlet, 4. siphon arm, 5. thimble, 6. solvent holding flask, 7. extraction solvent, 8. oil bath and 9. heat source)

2.4.2 Maceration extraction method

Triplicate maceration extractions were carried out using a rotavapor machine set up as shown in figure 23. The rotavapor helped in provision of the mechanical stirring of the process at 80 RPM and 40°C. Two solvents were used for this study namely cyclohexane and acetone. 60 g of the solid plant sample was dissolved in 600 ml of solvent. Cyclohexane solvent extraction was done for 2 hours and acetone solvent extraction was done for 6 hours.



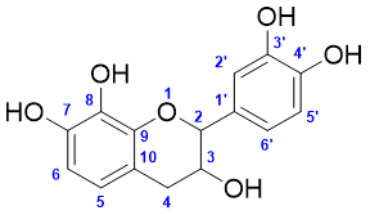
Fig 23: Maceration extraction set-up

2.5 Purification

Purification of mesquitol, which is labelled as compound [**1**], was done through slow wash of the acetone extract with 3:7 cyclohexane and ethyl acetate (*v*/*v*) solvent mixture using a silica-bed fritted glass Pyrex Buchner funnel with fine porosity under low pressure. This is a slow wash purification and took 1–2 h. After purification, the obtained filtrates that contained mesquitol in form of collected fractions were concentrated on a Büchi rotavapor R-200. The obtained yields of mesquitol were also calculated in percentage using the formula below:

$$\% \text{ yield} = \frac{\text{md}}{\text{mc}} \text{ x } 100$$

Where mc is the mass of the sample powder before purification and md is the mass of pure mesquitol after purification. The final product was confirmed to be mesquitol through FTIR, ^{1}H NMR, ^{13}C NMR and melting point analysis.



Compound [**1**]

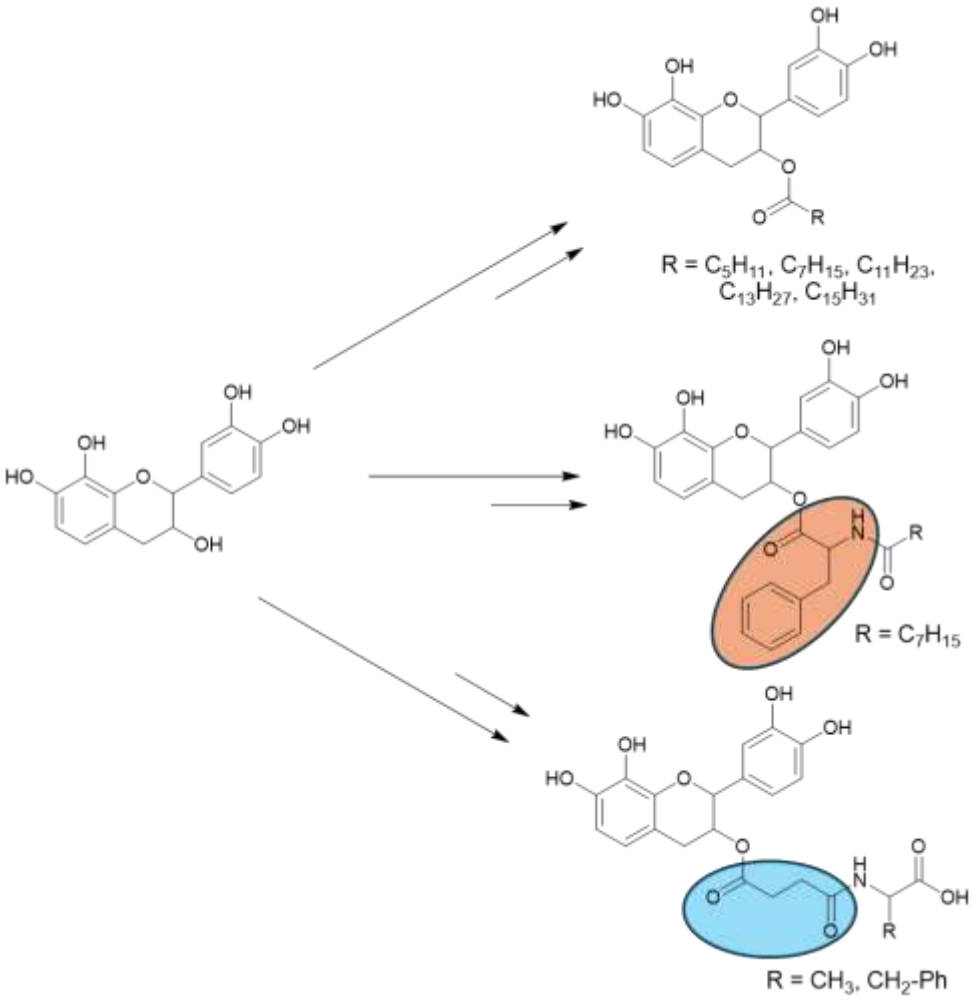
MW: 290.26 g/mol; Color: yellow; MP: 80 °C; Percentage yield: 62-66 %

^{1}H NMR (400 MHz, Acetone-d6) δ (ppm): 7.51 (1H, s, H^{OH}), 7.48 (1H, s, H^{OH}), 7.44 (1H, s, H^{OH}), 7.31 (1H, s, H^{OH}), 6.73–6.62 (3H, m, $H^{2', 5', 6'}$), 6.27-6.25 (2H, m, $H^{5, 6}$), 4.50 (1H, d, *J* 7.6 Hz, H^{2}), 3.90 (1H, td, *J* 5.3 and 6.8 Hz, H^{3}), 3.90 (1H, s, H^{OH}), 2.80 (1H, dd, *J* 5.2 and 15.7 Hz, H^{4}), 2.60 (1H, dd, *J* 8.5 and 15.7 Hz, H^{4}).

^{13}C NMR (400 MHz, Acetone-d6) δ (ppm): 82.18 (C-2), 67.52 (C-3), 32.77 (C-4), 108.18 (C-5), 119.3 (C-6), 144.77 (C-7), 144.91 (C-8), 132.57 (C-9), 112.43 (C-10), 131.00 (C-1′), 119.05 (C-2′), 143.96 (C-3′), 142.66 (C-4′), 114.86 (C-5′), 114.35 (C-6′).

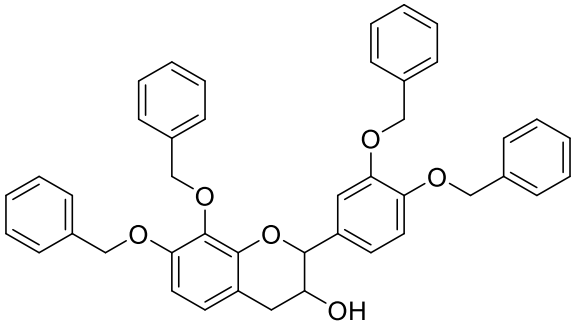
FTIR (υ_{max} cm^{-1}): 3500-3100 (O-H stretch, alcohol), 2950-2850 (C-H stretch, aliphatic), 1610, 1690 (C=C stretch, aromatic).

2.6 Hemi-synthesis reactions



Scheme 2: Target molecules for the study

Many chemical reactions in this section involved the hemi-synthesis reactions of mesquitol as shown in scheme 2. These reactions were carried to chemically modify pure mesquitol to be suited for various physicochemical and biological applications. The reactions were carried out at the aliphatic OH position of the mesquitol using different carboxylic acids and amides to form different esters. The formed mesquitol derivatives and their intermediates were thus characterized using ^{1}H and ^{13}C NMR analysis, FT-IR spectroscopy and melting points determination for solids. The summary of the experimental procedures for each hemi-synthesis reaction together with the characterization of each obtained compound from compound **[2]** to compound **[22]** are given below:



Compound [**2**]

1 g of mesquitol was dissolved in 40 ml of dry acetone. TBAI (0.3 eq), K_2CO_3 (8 eq) and benzyl bromide (6 eq) were added in that order in a round bottomed flask. The mixture was dissolved in the solvent under nitrogen environment through continuous agitation using a magnetic stirrer. The reaction was carried out for 24 hours at room temperature. At the end of the reaction, the reaction mixture was filtered using filter paper and filter funnel and the filtrate evaporated under vacuum using Büchi rotavapor R-200. The obtained solid residue was dissolved in 100 ml of ethyl acetate and then washed two consecutive times with 30 ml of saturated $NaHCO_3$ and once with 30 ml saturated NaCl. Organic phase was separated from the aqueous phase using a separating funnel. The organic phase was dried by using anhydrous $MgSO_4$, filtered and finally concentrated to dryness using Büchi rotavapor R-200. A solid crude product was formed that was purified through a classical column on silica gel using 9:1 cyclohexane and ethyl acetate solvent mixture. Visualization was done by the UV lamp and phosphomolybdic acid which revealed compound [**2**] at an Rf of 0.48 with an eluent system of 7:3 cyclohexane and ethyl acetate solvent mixture. Purification was also done through slow recrystallization of the crude product overnight using 2:1 hexane and ethyl acetate solvent mixture. The characterization of compound is given below.

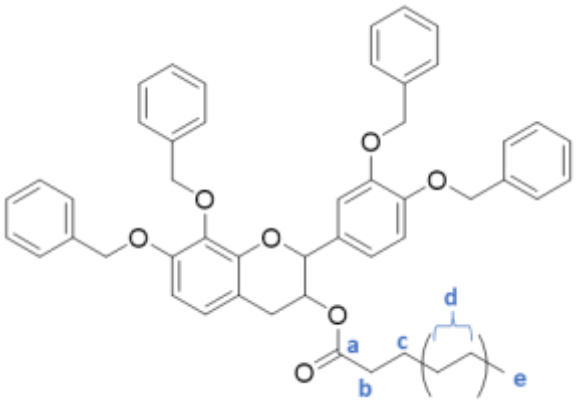
MW: 650.76 g/mol; Color: white; MP: 150 °C; Percentage yield: 62 %

^{1}H NMR (400 MHz, Acetone-d6) δ (ppm): 7.40-7.08 (20H, m, H^{Ph}), 7.07-6.91 (3H, m, $H^{2', 5', 6'}$), 6.61-6.54 (2H, m, $H^{5, 6}$), 5.04-4.89 (8H, m, $H^{C\underline{H}Bn}$), 4.66 (1H, d, *J* 7.35 Hz, H^{2}), 3.97 (1H, td, H^{3}), 2.84 (1H, dd, *J* 4.9 and 20.5 Hz, H^{4}), 2.65 (1H, dd, *J* 8.1 and 21.7 Hz, H^{4})

^{13}C NMR (400 MHz, Acetone-d6) δ (ppm): 81.94 (C-2), 67.18 (C-3), 33.04 (C-4), 107.21 (C-5), 123.78 (C-6), 148.94 (C-7), 151.23 (C-8), 138.53 (C-9), 115.39 (C-10), 132.81 (C-1′), 120.56 (C-

2′), 148.88 (C-3′), 148.38 (C-4′), 114.50 (C-5′), 114.04 (C-6′), 74.49-70.75 (C-CHBn), 128.33-127.46 (CH-Ph), 137.84-136.89 (C-Ph).

FTIR (υ_{max} cm^{-1}): 3550-3300 (O-H stretch, alcohol), 3100-3000 (C-H stretch, aromatic), 2950-2850 (C-H stretch, aliphatic), 1610 (C=C stretch, aromatic).



Compound [**3**]

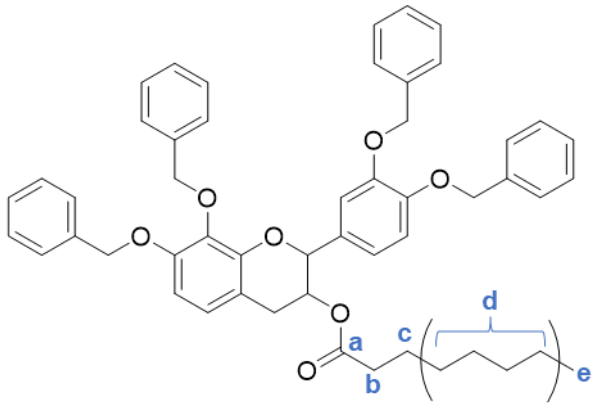
1 g benzylated mesquitol was dissolved in 50 ml anhydrous acetonitrile in a round-bottomed flask. DMAP (1.5 eq) was added very fast followed by DCCI (1.5 eq) and finally caproic acid (1.5 eq) under nitrogen environment. The reaction was then carried out at 80 °C reflux system for 12 hours. The reaction mixture was cooled down to room temperature and the formed product was filtered. The reaction flask was rinsed thoroughly with acetone to recover everything. The obtained filtrate was concentrated to dryness on the Büchi rotavapor R-200 to obtain a solid product. The product was dissolved in ethyl acetate then washed thrice with saturated NaCl. An organic phase was obtained that was dried with anhydrous $MgSO_4$. The obtained organic phase was concentrated in the rotavapor and a solid crude product formed. The crude product was then purified through a classical column on silica gel using 9:1 cyclohexane and ethyl acetate solvent mixture to obtain compound [**3**]. Visualization was done by the UV lamp and phosphomolybdic acid which revealed the ester at an Rf of 0.43 with an eluent system of 9:1 cyclohexane and ethyl acetate. The characterization of compound is given below.

MW: 748.90 g/mol; Color: brownish-white; MP: 54 °C; Percentage yield: 75 %

^{1}H NMR (400 MHz, Acetone-d6) δ (ppm): 7.25-7.08 (20H, m, H^{Ph}), 7.07-6.84 (3H, m, $H^{2', 5', 6'}$), 6.64-6.56 (2H, m, $H^{5, 6}$), 5.02-4.92 (9H, m, H^{2}, $H^{\underline{CH}Bn}$), 5.16 (1H, td, H^{3}), 2.82 (1H, dd, *J* 4.9 and 21.4 Hz, H^{4}), 2.68 (1H, dd, *J* 7.0 and 23.2 Hz, H^{4}), 2.05 (2H, m, H^{b}), 1.33 (2H, m, H^{c}), 1.10-1.01 (4H, m, H^{d}), 0.68 (3H, t, H^{e})

^{13}C NMR (400 MHz, Acetone-d6) δ (ppm): 81.94 (C-2), 67.18 (C-3), 30.07 (C-4), 107.21 (C-5), 123.78 (C-6), 148.94 (C-7), 151.23 (C-8), 138.53 (C-9), 115.39 (C-10), 132.81 (C-1′), 120.56 (C-2′), 148.88 (C-3′), 148.38 (C-4′), 114.50 (C-5′), 114.04 (C-6′), 74.49-70.75 (C-<u>C</u>H2Bn), 128.33-127.46 (<u>C</u>H-Ph), 137.84-136.89 (<u>C</u>-Ph), 171.99 (C-a), 33.80 (C-b), 28.64 (C-c), 24.43-22.07 (C-d), 13.28 (C-e)

FTIR (υ_{max} cm^{-1}): 3100-3000 (C-H stretch, aromatic), 2950-2850 (C-H stretch, aliphatic), 1728 (C=O stretch, ester), 1607 (C=C stretch, aromatic).



Compound [**4**]

The same reaction for compound [**3**] was carried out again, however with caprylic acid (1.5 eq) in place of caproic acid. After purification via column chromatography, visualization was done by the UV lamp and phosphomolybdic acid which revealed compound [**4**] at an Rf of 3.8 with an eluent system of 9:1 cyclohexane and ethyl acetate solvent mixture. The characterization of compound is given below.

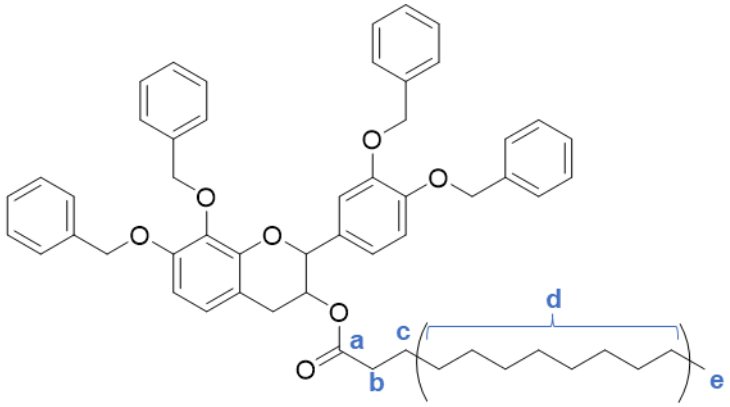
MW: 776.95 g/mol; Color: white; MP: 74-78 °C; Percentage yield: 81 %

^{1}H NMR (400 MHz, Acetone-d6) δ (ppm): 7.36-7.23 (20H, m, H^{Ph}), 7.23-7.00 (3H, m, $H^{2', 5', 6'}$), 6.80-6.72 (2H, m, $H^{5, 6}$), 5.18-5.08 (9H, m, H^{2}, $H^{\underline{CH}Bn}$), 5.32 (1H, td, H^{3}), 2.96 (1H, dd, *J* 4.9 and

21.1 Hz, H^4), 2.84 (1H, dd, *J* 6.9 and 23.1 Hz, H^4), 2.21 (2H, m, H^b), 1.48 (2H, m, H^c), 1.31-1.15 (8H, m, H^d), 0.86 (3H, t, H^e)

^{13}C NMR (400 MHz, Acetone-d6) δ (ppm): 78.30 (C-2), 68.84 (C-3), 31.48 (C-4), 107.36 (C-5), 123.78 (C-6), 148.98 (C-7), 151.53 (C-8), 137.56 (C-9), 113.74 (C-10), 131.56 (C-1′), 119.84 (C-2′), 148.80 (C-3′), 148.40 (C-4′), 114.56 (C-5′), 113.45 (C-6′), 74.52-70.70 (C-CH2Bn), 128.34-127.42 (CH-Ph), 137.70-136.80 (C-Ph), 171.90 (C-a), 33.84 (C-b), 28.79 (C-c), 28.68-22.36 (C-d), 13.42 (C-e)

FTIR (υ_{max} cm^{-1}): 3100-3000 (C-H stretch, aromatic), 2950-2850 (C-H stretch, aliphatic), 1732 (C=O stretch, ester), 1606 (C=C stretch, aromatic).



Compound [**5**]

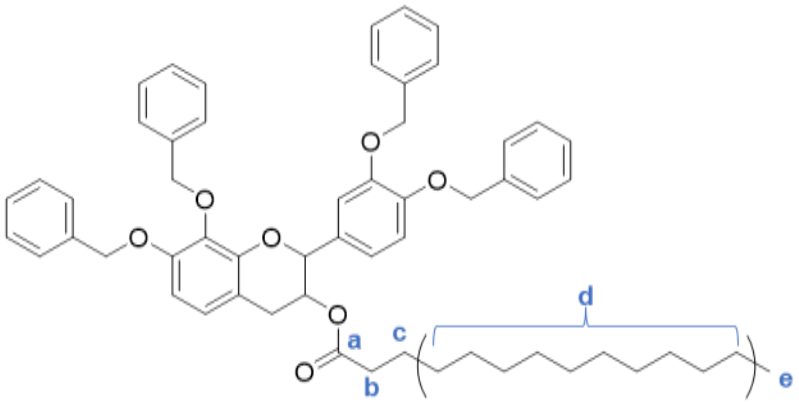
The same reaction for compound [**3**] was carried out again, however with lauric acid (1.5 eq) in place of caproic acid. After purification via column chromatography, visualization was done by the UV lamp and phosphomolybdic acid which revealed compound [**5**] at an Rf of 0.61 with an eluent system of 4:1 cyclohexane and ethyl acetate solvent mixture. The characterization of compound is given below.

MW: 833.06 g/mol; Color: white; MP: 86 °C; Percentage yield: 82 %

^{1}H NMR (400 MHz, Acetone-d6) δ (ppm): 7.19-7.08 (20H, m, H^{Ph}), 7.07-6.87 (3H, m, $H^{2', 5', 6'}$), 6.64-6.57 (2H, m, $H^{5, 6}$), 5.03-4.93 (9H, m, H^2, $H^{CH}Bn$), 5.16 (1H, td, H^3), 2.81 (1H, dd, *J* 4.9 and 21.1 Hz, H^4), 2.64 (1H, dd, *J* 7.0 and 23.1 Hz, H^4), 2.05 (2H, m, H^b), 1.32 (2H, m, H^c), 1.13-1.06 (16H, m, H^d), 0.73 (3H, t, H^e)

^{13}C NMR (400 MHz, Acetone-d6) δ (ppm): 78.30 (C-2), 68.84 (C-3), 31.73 (C-4), 107.36 (C-5), 123.78 (C-6), 149.09 (C-7), 151.53 (C-8), 137.73 (C-9), 113.74 (C-10), 131.56 (C-1′), 119.85 (C-2′), 148.98 (C-3′), 147.90 (C-4′), 114.56 (C-5′), 113.46 (C-6′), 74.54-70.70 (C-CH2Bn), 128.34-127.41 (CH-Ph), 137.69-136.81 (C-Ph), 171.98 (C-a), 33.87 (C-b), 29.44 (C-c), 29.12-22.43 (C-d), 13.42 (C-e)

FTIR (υ_{max} cm^{-1}): 3100-3000 (C-H stretch, aromatic), 2950-2800 (C-H stretch, aliphatic), 1729 (C=O stretch, ester), 1612 (C=C stretch, aromatic).



Compound [**6**]

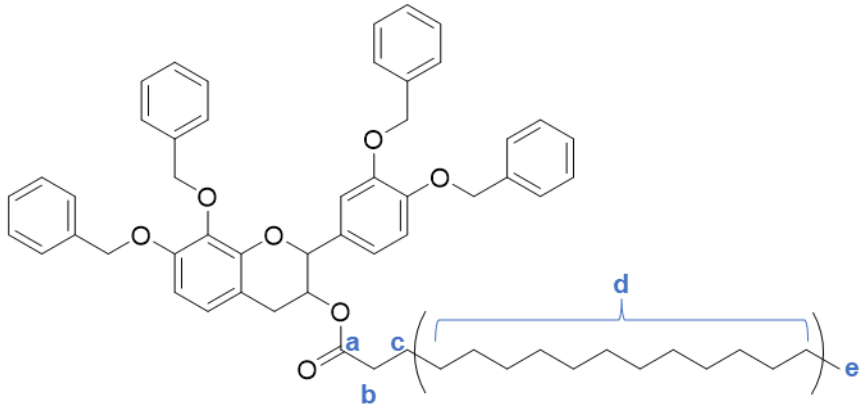
The same reaction for compound [**3**] was carried out again, however with myristic acid (1.5 eq) in place of caproic acid. After purification via column chromatography, visualization was done by the UV lamp and phosphomolybdic acid which revealed compound [**6**] at an Rf of 0.56 with an eluent system of 4:1 cyclohexane and ethyl acetate solvent mixture. The characterization of compound is given below.

MW: 861.11 g/mol; Color: white; MP: 88 °C; Percentage yield: 78 %

^{1}H NMR (400 MHz, Acetone-d6) δ (ppm): 7.19-7.09 (20H, m, H^{Ph}), 7.07-6.76 (3H, m, $H^{2', 5', 6'}$), 6.55-6.43 (2H, m, $H^{5, 6}$), 5.03-4.96 (9H, m, H^{2}, H^{CHBn}), 5.15 (1H, td, H^{3}), 2.77 (1H, dd, *J* 4.5 and 20.6 Hz, H^{4}), 2.62 (1H, dd, *J* 6.1 and 22.3 Hz, H^{4}), 2.07 (2H, m, H^{b}), 1.37 (2H, m, H^{c}), 1.23-1.04 (20H, m, H^{d}), 0.78 (3H, t, H^{e})

^{13}C NMR (400 MHz, Acetone-d6) δ (ppm): 78.28 (C-2), 69.14 (C-3), 32.02 (C-4), 107.56 (C-5), 123.85 (C-6), 148.94 (C-7), 151.56 (C-8), 137.98 (C-9), 113.58 (C-10), 131.56 (C-1′), 119.73 (C-2′), 147.83 (C-3′), 147.80 (C-4′), 114.91 (C-5′), 113.32 (C-6′), 75.16-71.33 (C-CH2Bn), 128.65-127.34 (CH-Ph), 137.42-136.83 (C-Ph), 173.10 (C-a), 34.42 (C-b), 29.80 (C-c), 29.74-22.80 (C-d), 14.24 (C-e)

FTIR (υ_{max} cm^{-1}): 3100-3000 (C-H stretch, aromatic), 2950-2850 (C-H stretch, aliphatic), 1731 (C=O stretch, ester), 1608 (C=C stretch, aromatic).



Compound [**7**]

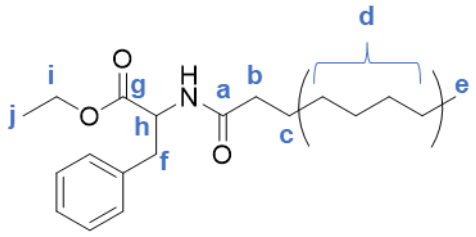
The same reaction for compound [**3**] was carried out again, however with palmitic acid (1.5 eq) in place of caproic acid. After purification via column chromatography, visualization was done by the UV lamp and phosphomolybdic acid which revealed compound [**7**] at an Rf of 0.53 with an eluent system of 4:1 cyclohexane and ethyl acetate solvent mixture. The characterization of compound is given below.

MW: 889.16 g/mol; Color: white; MP: 88-90 °C; Percentage yield: 85 %

^{1}H NMR (400 MHz, Acetone-d6) δ (ppm): 7.38-7.18 (20H, m, H^{Ph}), 7.10-6.84 (3H, m, $H^{2', 5', 6'}$), 6.64-6.56 (2H, m, $H^{5, 6}$), 5.03-4.93 (9H, m, H^{2}, H^{CHBn}), 5.16 (1H, td, H^{3}), 2.80 (1H, dd, *J* 4.9 and 21.2 Hz, H^{4}), 2.67 (1H, dd, *J* 7.0 and 23.1 Hz, H^{4}), 2.06 (2H, m, H^{b}), 1.31 (2H, m, H^{c}), 1.19-0.99 (24H, m, H^{d}), 0.74 (3H, t, H^{e})

^{13}C NMR (400 MHz, Acetone-d6) δ (ppm): 78.32 (C-2), 68.85 (C-3), 31.74 (C-4), 107.36 (C-5), 123.78 (C-6), 149.09 (C-7), 151.53 (C-8), 138.42 (C-9), 113.74 (C-10), 131.54 (C-1′), 119.86 (C-2′), 148.98 (C-3′), 147.90 (C-4′), 114.56 (C-5′), 113.47 (C-6′), 74.54-70.70 (C-CH2Bn), 128.34-127.41 (CH-Ph), 137.73-136.82 (C-Ph), 171.99 (C-a), 33.87 (C-b), 29.51 (C-c), 29.18-22.43 (C-d), 13.46 (C-e)

FTIR (υ_{max} cm^{-1}): 3100-3000 (C-H stretch, aromatic), 2950-2850 (C-H stretch, aliphatic), 1731 (C=O stretch, ester), 1607 (C=C stretch, aromatic).



Compound [**8**]

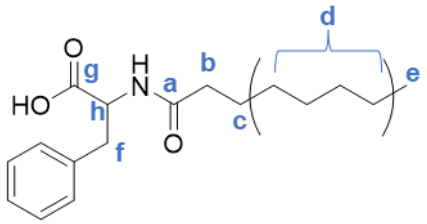
1 g of phenylalanine ethyl ester was dissolved in 50 ml of DCM. Caprylic acid (1 eq), BOP (1 eq) and triethylamine (3 eq) were added consecutively in the reaction's round bottom flask. The reaction took place overnight under calcium chloride guard through continuous agitation using a magnetic stirrer. The excess DCM was evaporated using Büchi rotavapor R-200 to obtain a viscous product. The obtained product was treated thrice with 50 ml 1M HCl and once with 30 ml saturated NaCl. The crude product was thus purified through slow wash with 1:1 cyclohexane and ethyl acetate (v/v) solvent mixture using a silica-bed fritted glass Pyrex Buchner funnel. The obtained filtrate was evaporated using Büchi rotavapor R-200 to obtain a yellow viscous compound [**8**]. The characterization of the compound is given below.

MW: 319.44 g/mol; Color: light yellow; Percentage yield: 81 %

^{1}H NMR (400 MHz, Acetone-d6) δ (ppm): 7.30-7.22 (5H, m, H^{Ph}), 4.74 (1H, t, H^{h}), 4.11 (2H, t, H^{i}), 3.13 (1H, dd, *J* 5.8 and 19.6 Hz, H^{f}), 2.99 (1H, dd, *J* 8.3 and 22.1 Hz, H^{f}), 2.20 (2H, m, H^{b}), 1.55 (2H, m, H^{c}), 1.38-1.24 (8H, m, H^{d}), 1,19 (3H, t, H^{j}) 0.90 (3H, t, H^{e})

^{13}C NMR (400 MHz, Acetone-d6) δ (ppm): δ (ppm): 173.80 (C-a), 35.53 (C-b), 35.49 (C-c), 31.59-22.40 (C-d), 13.48 (C-e), 37.52 (C-f), 171.56 (C-g), 53.55 (C-h), 60.51 (C-i), 13.55 (C-j), 137.32 (C-ph), 129.22-126.54 (CH-ph)

FTIR (υ_{max} cm^{-1}): 3400-3150 (N-H stretch, amine), 3100-3000 (C-H stretch, aromatic), 2950-2850 (C-H stretch, aliphatic), 1740 (C=O stretch, ester), 1649 (C=O stretch, amide)



Compound [**9**]

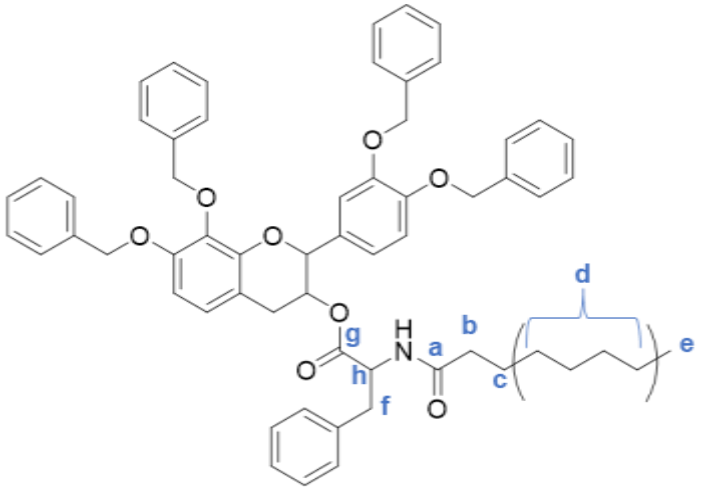
1 eq of compound [**8**] was placed in a round bottom flask. A solution of 9:1 of MeOH:H_2O containing 5 eq of dissolved NaOH was added to the flask. The reaction mixture was shaken well at room temperature for 4 hours. Under a cold bath, 5 eq of 1M HCl solution was added. The formed product was liquid, thus excess MeOH was evaporated in the rotavapor, then mixed with ethyl acetate to obtain the organic phase. The organic phase was dried by anhydrous $MgSO_4$ and evaporated in the rotavapor to obtain compound [**9**]. The characterization of the compound is given below.

MW: 291.38 g/mol; Color: white; MP: 125 °C; Percentage yield: 91 %

^{1}H NMR (400 MHz, Acetone-d6) δ (ppm): 7.29-7.21 (5H, m, H^{Ph}), 4.77 (1H, t, H^{h}), 3.32 (1H, dd, *J* 5.2 and 19.1 Hz, H^{f}), 3.01 (1H, dd, *J* 8.5 and 22.4 Hz, H^{f}), 2.22 (2H, m, H^{b}), 1.53 (2H, m, H^{c}), 1.38-1.19 (8H, m, H^{d}), 0.89 (3H, t, H^{e})

^{13}C NMR (400 MHz, Chloroform-d) δ (ppm): 175.21 (C-a), 36.41 (C-b), 34.10 (C-c), 31.65-22.59 (C-d), 14.05 (C-e), 37.32 (C-f), 174.19 (C-g), 53.41 (C-h), 136.02 (C-ph), 129.37-127.05 (CH-ph)

FTIR (υ_{max} cm^{-1}): 3350-3150 (N-H stretch, amine), 3100-3000 (C-H stretch, aromatic), 2950-2850 (C-H stretch, aliphatic), 1705 (C=O stretch, carboxylic acid), 1610 (C=O stretch, amide)



Compound [**10**]

1 g benzylated mesquitol was dissolved in 50 ml anhydrous DCM in a round-bottomed flask. Compound [**9**] (1 eq), BOP (1 eq) and triethylamine (3 eq) were added consecutively in the reaction's flask. The reaction took place for 48 hours under calcium chloride guard through continuous agitation using a magnetic stirrer. The excess DCM was evaporated using Büchi rotavapor R-200. The obtained product was treated thrice with 50 ml 1M HCl and once with 30 ml saturated NaCl. The crude product was thus purified via a classical column on silica gel using 8:2 cyclohexane and ethyl acetate solvent mixture. Visualization was done by the UV lamp and phosphomolybdic acid which revealed compound [**10**] at an Rf of 0.55 with an eluent system of 7:3 cyclohexane and ethyl acetate solvent mixture. The characterization of the compound is given below.

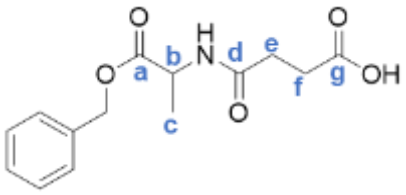
MW: 924.13 g/mol; Color: white; MP: 138-140 °C; Percentage yield: 51 %

^{1}H NMR (400 MHz, Chloroform-d) δ (ppm): 7.40-7.08 (25H, m, H^{Ph}), 6.92 (1H, s, $H^{N\underline{H}}$), 6.89-6.70 (3H, m, $H^{2', 5', 6'}$), 6.58-6.49 (2H, m, $H^{5, 6}$), 5.63 (1H, td, H^{3}), 5.16-4.95 (9H, m, H^{2}, $H^{C\underline{H}Bn}$), 2.72 (1H, dd, *J* 6.2 and 20.1 Hz, H^{4}), 2.58 (1H, dd, *J* 5.7 and 19.6 Hz, H^{4}), 4.72 (1H, t, H^{h}), 2.94 (1H, dd, *J* 4.1 and 20.8 Hz, H^{f}), 2.86 (1H, dd, *J* 5.3 and 21.7 Hz, H^{f}), 2.01 (2H, m, H^{b}), 1.47 (2H, m, H^{c}), 1.24-1.11 (8H, m, H^{d}), 0.79 (3H, t, H^{e})

^{13}C NMR (400 MHz, Chloroform-d) δ (ppm): δ (ppm): 77.69 (C-2), 71.24 (C-3), 29.17 31.67 (C-4), 107.53 (C-5), 123.76 (C-6), 148.94 (C-7), 151.61 (C-8), 137.81 (C-9), 112.80 (C-10), 131.08 (C-1′), 119.32 (C-2′), 148.91 (C-3′), 147.46 (C-4′), 114.85 (C-5′), 112.89 (C-6′), 75.06-71.28 (C-

CHBn), 129.28-127.08 (CH-Ph), 137.24-135.79 (C-Ph), 172.54 (C-a), 36.41 (C-b), 29.17 (C-c), 28.98-22.60 (C-d), 14.06 (C-e), 37.70 (C-f), 170.97 (C-g), 52.95 (C-h)

FTIR (u_{max} cm^{-1}): 3350-3200 (N-H stretch, amine), 3100-3000 (C-H stretch, aromatic), 2950-2850 (C-H stretch, aliphatic), 1726 (C=O stretch, ester), 1642 (C=O stretch, amide), 1607 (C=C stretch, aromatic).



Compound [**11**]

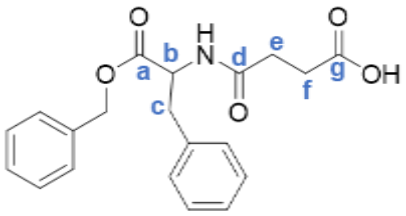
1 g benzylated alanine was dissolved in 50 ml anhydrous THF in a round-bottomed flask. 1 eq of succinic anhydride and 2 eq of triethylamine (TEA) were added to the solution and the reaction allowed to occur at 80 °C reflux system for 12 hours under continuous agitation. Excess THF and triethylamine that did not react overnight were removed through evaporation via the rotavapor. The obtained crude product was dissolved in DCM and washed three times with 50 ml 1M HCl and once with 30 ml saturated NaCl. Organic phase was dried using anhydrous $MgSO_4$ and then evaporated using the rotavapor to obtain compound [**11**]. The characterization of the compound is given below.

MW: 279.29 g./mol; Color: white; MP: 93 °C; Percentage yield: 88 %

^{1}H NMR (400 MHz, Chloroform-d) δ (ppm): 7.31-7.18 (5H, m, H^{Ph}), 6.41 (1H,s, H^{NH}), 5.13-5.06 (2H,m, H^{CHBn}), 4.56 (1H, q, H^{b}), 2.62 (2H, t, H^{e}), 2.44 (2H, t, H^{f}), 1.32 (3H, d, H^{c})

^{13}C NMR (400 MHz, Chloroform-d) δ (ppm): 176.978(C-a), 48.34 (C-b), 18.27 (C-c), 172.93 (C-d), 30.50 (C-e), 29.34 (C-f), 171.53 (C-g), 135.22 (C-Ph), 128.65-128.15 (CH-Ph), 67.29 (C-CHBn)

FTIR (υ_{max} cm^{-1}): 3350-3200 (N-H stretch, amine), 3100-3000 (C-H stretch, aromatic), 2950-2850 (C-H stretch, aliphatic), 1742 (C=O stretch, ester), 1694 (C=O stretch, carboxylic acid), 1645 (C=O stretch, amide)



Compound [**12**]

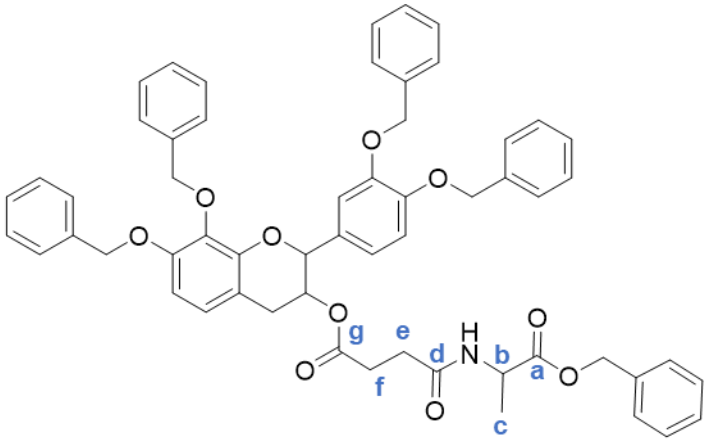
The same reaction for compound [**11**] was carried out again, however with benzylated phenylalanine (1 eq) in place of benzylated alanine to produce compound [**12**]. The characterization of the compound is given below.

MW: 355.38 g./mol; Color: white; MP: 118 °C; Percentage yield: 93 %

^{1}H NMR (400 MHz, Chloroform-d) δ (ppm): 7.29-6.90 (10H, m, H^{ph}), 6.16 (1H,s, H^{NH}), 5.10-4.87 (2H,m, H^{CHBn}), 4.84 (1H, t, H^{b}), 3.04 (2H, dd, H^{c}), 2.58 (2H, t, H^{e}), 2.4 ((2H, t, H^{f})

^{13}C NMR (400 MHz, Chloroform-d) δ (ppm): 176.98 (C-a), 53.31(C-b), 37.76(C-c), 171.40 (C-d), 30.48 (C-e), 29.24 (C-f), 171.32 (C-g), 135.53-134.95 (C-Ph), 129.33-127.12 (CH-Ph), 67.41 (C-CHBn)

FTIR (υ_{max} cm^{-1}): 3350-3200 (N-H stretch, amine), 3100-3000 (C-H stretch, aromatic), 2950-2850 (C-H stretch, aliphatic), 1728 (C=O stretch, ester), 1712 (C=O stretch, carboxylic acid), 1642 (C=O stretch, amide)



Compound [**13**]

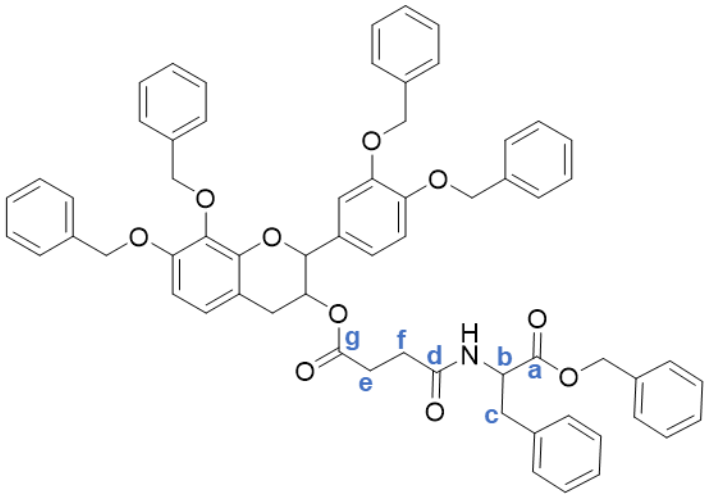
1 g benzylated mesquitol was dissolved in 50 ml anhydrous acetonitrile in a round-bottomed flask. DMAP (1.5 eq) was added very fast followed by DCCI (1.5 eq) and finally compound [**11**] (1 eq) under nitrogen environment. The reaction was then carried out at 80 °C reflux system for 12 hours. The reaction mixture was cooled down to room temperature and the formed product was filtered. The reaction flask was rinsed thoroughly with acetone to recover everything. The obtained filtrate was concentrated to dryness on the Büchi rotavapor R-200 to obtain a solid product. The product was dissolved in ethyl acetate then washed thrice with saturated NaCl. An organic phase was obtained that was dried with anhydrous $MgSO_4$. The obtained organic phase was concentrated in the rotavapor and a solid crude product formed. The crude product was then purified through a classical column on silica gel using 9:1 cyclohexane and ethyl acetate solvent mixture to obtain compound [1**3**]. Visualization was done by the UV lamp and phosphomolybdic acid which revealed the ester at an Rf of 0.53 with an eluent system of 7:3 cyclohexane and ethyl acetate. The characterization of compound is given below.

MW: 912.03 g/mol; Color: white; MP: 80 °C; Percentage yield: 54 %

^{1}H NMR (400 MHz, chloroform-d) δ (ppm): 7.38-7.11 (25H, m, H^{Ph}), 6.88-6.75 (3H, m, $H^{2', 5', 6'}$), 6.61-6.47 (2H, m, $H^{5, 6}$), 5.99 (1H, td, H^3), 5.18-4.96 (11H, m, H^2, $H^{C\underline{H}Bn}$), 2.39-2.29 (2H, dd, H^4), 4.52 (1H, q, H^b), 2.73 (2H, t, H^e), 2.51 (2H, t, H^f) 1.33 (3H, d, H^c)

^{13}C NMR (400 MHz, Acetone-d6) δ (ppm): 78.02 (C-2), 69.19 (C-3), 28.26 (C-4), 107.32 (C-5), 123.81 (C-6), 148.99 (C-7), 151.52 (C-8), 138.44 (C-9), 113.73 (C-10), 131.66 (C-1′), 119.68 (C-2′), 148.97 (C-3′), 147.82 (C-4′), 114.62 (C-5′), 113.28 (C-6′), 74.53-70.71 (C-CHBn), 128.41-127.49 (CH-Ph), 137.75-136.36 (C-Ph), 172.49 (C-a), 48.08 (C-b), 16.92 (C-c), 171.42 (C-d), 66.08 (C-e), 29.84 (C-f), 170.48 (C-g)

FTIR (u_{max} cm^{-1}): 3350-3200 (N-H stretch, amine), 3100-3000 (C-H stretch, aromatic), 2950-2850 (C-H stretch, aliphatic), 1733 (C=O stretch, ester), 1647, 1610 (C=O stretch, amides)



Compound [**14**]

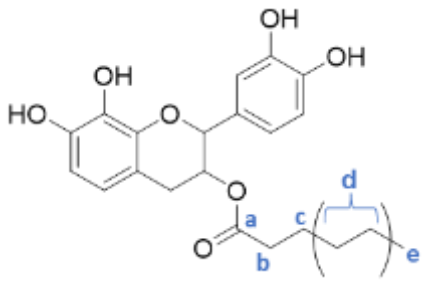
The same reaction for compound [**13**] was carried out again, however with compound [**12**] in place of compound [**11**]. After purification via column chromatography, visualization was done by the UV lamp and phosphomolybdic acid which revealed compound [**14**] at an Rf of 0.51 with an eluent system of 7:3 cyclohexane and ethyl acetate solvent mixture. The characterization of the compound is given below.

MW: 988.13 g/mol; Color: white; MP: 84 °C; Percentage yield: 60 %

^{1}H NMR (400 MHz, acetone-d6) δ (ppm): 7.36-7.03 (30H, m, H^{Ph}), 7.02-6.81 (3H, m, $H^{2', 5', 6'}$), 6.64-6.52 (2H, m, $H^{5, 6}$), 5.13 (1H, td, H^{3}), 5.02-4.91 (11H, m, H^{2}, H^{CHBn}), 2.77 (1H, dd, *J* 4.9 and 21.2 Hz, H^{4}), 2.64 (1H, dd, *J* 6.3 and 22.6 Hz, H^{4}), 4.62 (1H, q, H^{b}), 2.97 (1H, dd, *J* 6.1 and 19.9 Hz, H^{c}), 2.84 (1H, dd, *J* 7.7 and 21.6 Hz, H^{c}), 2.28 (4H, m, $H^{e, f}$)

^{13}C NMR (400 MHz, Acetone-d6) δ (ppm): 78.02 (C-2), 69.19 (C-3), 28.26 (C-4), 107.34 (C-5), 123.82 (C-6), 148.99 (C-7), 151.51 (C-8), 138.43 (C-9), 113.73 (C-10), 131.63 (C-1′), 119.69 (C-2′), 148.97 (C-3′), 147.82 (C-4′), 114.63 (C-5′), 113.28 (C-6′), 74.54-70.71 (C-CHBn), 129.27-126.62 (CH-Ph), 137.74-136.03 (C-Ph), 171.42 (C-a), 53.87 (C-b), 37.43 (C-c), 171.33 (C-d), 66.28 (C-e), 29.90 (C-f), 170.74 (C-g)

FTIR (υ_{max} cm^{-1}): 3350-3200 (N-H stretch, amine), 3100-3000 (C-H stretch, aromatic), 2950-2850 (C-H stretch, aliphatic), 1734 (C=O stretch, ester), 1648, 1610 (C=O stretch, amides)



Compound [**15**]

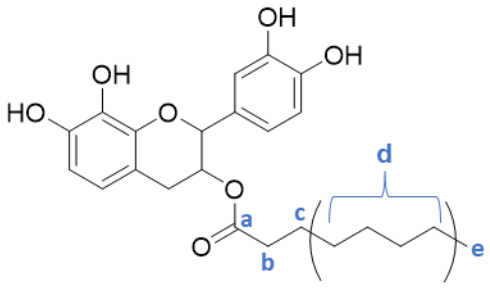
Under nitrogen environment, 1 g of compound [**3**] was dissolved in a 200 ml solution of a mixture of MeOH:EtOAc (1:1). Small quantities of palladium on carbon (10% basis) were added as a catalyst to the reaction mixture. Purging of the system was done using nitrogen gas followed by purging with hydrogen gas. This was followed by addition of hydrogen gas via the hydrogen reactor system. The reaction was carried out for 22 hours at a pressure of 40 bar. At the end of reaction, the Pd/C catalyst was filtered off on an acid celite-bed followed by rinsing with excess ethyl acetate followed by methanol. The filtrate was concentrated to dryness using a Büchi rotavapor R-200. The obtained product was purified through slow wash with 3:2 cyclohexane and ethyl acetate (v/v) solvent mixture using a silica-bed fritted glass Pyrex Buchner funnel. Visualization was done by the UV lamp and phosphomolybdic acid which revealed compound [**15**] at an Rf of 0.41 with an eluent system of 1:1 cyclohexane and ethyl acetate. The characterization of the compound is given below.

MW: 388 g/mol; Color: white; MP: 88 °C; Percentage yield: 88 %

^{1}H NMR (400 MHz, Acetone-d6) δ (ppm): 6.72-6.56 (3H, m, $H^{2', 5', 6'}$), 6.33-6.25 (2H, m, $H^{5, 6}$), 5.10 (1H, td, H^{3}), 4.88 (1H, d, H^{2}), 2.80 (1H, dd, *J* 5.0 and 21.1 Hz, H^{4}), 2.65 (1H, dd, *J* 6.9 and 22.9 Hz, H^{4}), 2.03 (2H, m, H^{b}), 1.30 (2H, m, H^{c}), 1.12-0.97 (4H, m, H^{d}), 0.69 (3H, t, H^{e})

^{13}C NMR (400 MHz, Chloroform-d) δ (ppm): 77.73 (C-2), 68.26 (C-3), 29.99 (C-4), 107.81 (C-5), 128.81 (C-6), 142.74 (C-7), 143.25 (C-8), 130.41 (C-9), 110.51 (C-10), 118.91 (C-1′), 118.59 (C-2′), 148.88 (C-3′), 148.38 (C-4′), 114.27 (C-5′), 112.44 (C-6′), 172.76 (C-a), 33.30 (C-b), 27.69 (C-c), 23.46-21.19 (C-d), 12.78 (C-e)

FTIR (u_{max} cm^{-1}): 3500-3100 (O-H stretch, alcohol), 2950-2850 (C-H stretch, aliphatic), 1703 (C=O stretch, ester), 1612 (C=C stretch, aromatic).



Compound [**16**]

The same reaction for compound [**15**] was carried out again, however with compound [**4**] in place of compound [**3**]. After purification, visualization was done that revealed compound [**16**] at an Rf of 0.43 with an eluent system of 1:1 cyclohexane and ethyl acetate solvent mixture. The characterization of the compound is given below.

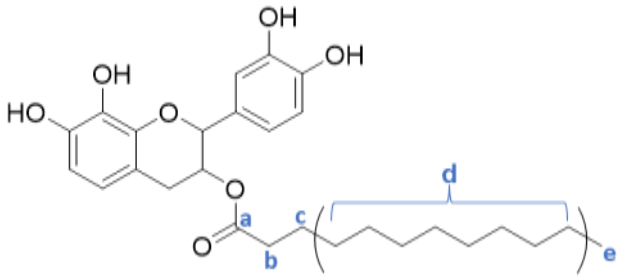
MW: 416.18 g/mol; Color: white; MP: 96 °C; Percentage yield: 89 %

^{1}H NMR (400 MHz, Acetone-d6) δ (ppm): 6.72-6.58 (3H, m, $H^{2', 5', 6'}$), 6.31-6.29 (2H, m, $H^{5, 6}$), 5.12 (1H, td, H^{3}), 4.90 (1H, d, H^{2}), 2.80 (1H, dd, *J* 4.9 and 21.2 Hz, H^{4}), 2.63 (1H, dd, *J* 6.9 and 23.1 Hz, H^{4}), 2.05 (2H, m, H^{b}), 1.31 (2H, m, H^{c}), 1.19-0.99 (8H, m, H^{d}), 0.74 (3H, t, H^{e})

^{13}C NMR (400 MHz, Acetone-d) δ (ppm): 78.39 (C-2), 68.13 (C-3), 31.46 (C-4), 108.45 (C-5), 129.96 (C-6), 144.93 (C-7), 145.11 (C-8), 132.66 (C-9), 110.91 (C-10), 118.98 (C-1′), 118.40 (C-

2′), 144.32 (C-3′), 142.18 (C-4′), 114.99 (C-5′), 113.54 (C-6′), 172.04 (C-a), 33.83 (C-b), 28.65 (C-c), 28.41-22.36 (C-d), 13.59 (C-e)

FTIR (u_{max} cm^{-1}): 3500-3100 (O-H stretch, alcohol), 2950-2850 (C-H stretch, aliphatic), 1706 (C=O stretch, ester), 1615 (C=C stretch, aromatic).



Compound [**17**]

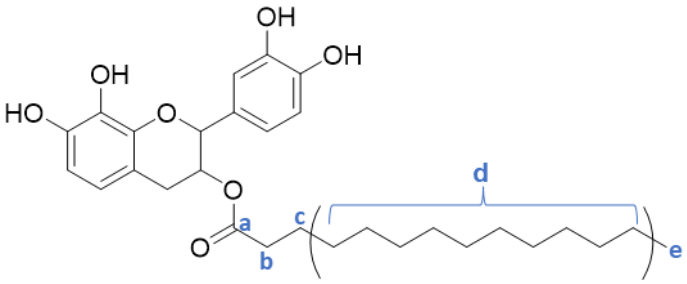
The same reaction for compound [**15**] was carried out again, however with compound [**5**] in place of compound [**3**]. After purification, visualization was done that revealed compound [**17**] at an Rf of 0.45 with an eluent system of 1:1 cyclohexane and ethyl acetate solvent mixture. The characterization of the compound is given below.

MW: 472.25 g/mol; Color: white; MP: 99 °C; Percentage yield: 92 %

^{1}H NMR (400 MHz, Acetone-d6) δ (ppm): 6.86-6.72 (3H, m, $H^{2', 5', 6'}$), 6.47-6.41 (2H, m, $H^{5, 6}$), 5.27 (1H, td, H^{3}), 5.04 (1H, d, H^{2}), 2.98 (1H, dd, *J* 5.0 and 21.1 Hz, H^{4}), 2.78 (1H, dd, *J* 6.8 and 22.8 Hz, H^{4}), 2.19 (2H, m, H^{b}), 1.45 (2H, m, H^{c}), 1.38-1.14 (16H, m, H^{d}), 0.91 (3H, t, H^{e})

^{13}C NMR (400 MHz, Acetone-d6) δ (ppm): 78.38 (C-2), 69.14 (C-3), 31.75 (C-4), 108.45 (C-5), 129.97 (C-6), 144.93 (C-7), 145.10 (C-8), 132.66 (C-9), 110.91 (C-10), 118.99 (C-1′), 118.40 (C-2′), 144.32 (C-3′), 142.17 (C-4′), 114.99 (C-5′), 113.53 (C-6′), 172.05 (C-a), 33.84 (C-b), 29.54 (C-c), 29.46-22.44 (C-d), 13.49 (C-e)

FTIR (u_{max} cm^{-1}): 3500-3100 (O-H stretch, alcohol), 2950-2850 (C-H stretch, aliphatic), 1738 (C=O stretch, ester), 1617 (C=C stretch, aromatic).



Compound [**18**]

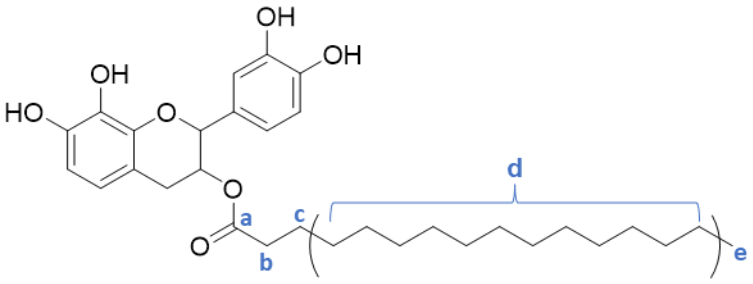
The same reaction for compound [**15**] was carried out again, however with compound [**6**] in place of compound [**3**]. After purification, visualization was done that revealed compound [**18**] at an Rf of 0.46 with an eluent system of 1:1 cyclohexane and ethyl acetate solvent mixture. The characterization of the compound is given below.

MW: 500.28 g/mol; Color: white; MP: 102 °C; Percentage yield: 90 %

^{1}H NMR (400 MHz, Chloroform-d) δ (ppm): 6.73-6.60 (3H, m, $H^{2', 5', 6'}$), 6.48-6.35 (2H, m, $H^{5, 6}$), 5.18 (1H, td, H^{3}), 4.84 (1H, d, H^{2}), 2.98 (1H, dd, *J* 4.9 and 21.3 Hz, H^{4}), 2.88 (1H, dd, *J* 6.9 and 23.1 Hz, H^{4}), 2.08 (2H, m, H^{b}), 1.35 (2H, m, H^{c}), 1.25-1.01 (20H, m, H^{d}), 0.81 (3H, t, H^{e})

^{13}C NMR (400 MHz, Chloroform-d) δ (ppm): 78.39 (C-2), 69.13 (C-3), 31.74 (C-4), 108.45 (C-5), 129.97 (C-6), 144.96 (C-7), 145.14 (C-8), 132.67 (C-9), 110.91 (C-10), 118.99 (C-1′), 118.44 (C-2′), 144.35 (C-3′), 142.19 (C-4′), 114.98 (C-5′), 113.53 (C-6′), 172.02 (C-a), 33.84 (C-b), 29.61 (C-c), 29.46-22.37 (C-d), 13.45 (C-e)

FTIR (υ_{max} cm^{-1}): 3500-3100 (O-H stretch, alcohol), 2950-2850 (C-H stretch, aliphatic), 1710 (C=O stretch, ester), 1610 (C=C stretch, aromatic).



Compound [**19**]

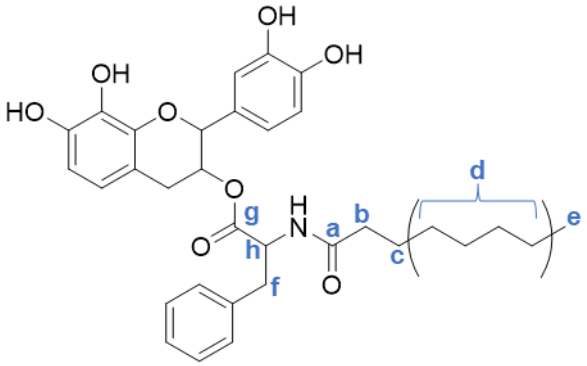
The same reaction for compound [**15**] was carried out again, however with compound [**7**] in place of compound [**3**]. After purification, visualization was done that revealed compound [**19**] at an Rf of 0.53 with an eluent system of 1:1 cyclohexane and ethyl acetate solvent mixture. The characterization of the compound is given below.

MW: 528.32 g/mol; Color: white; MP: 104 °C; Percentage yield: 96 %

^{1}H NMR (400 MHz, acetone-d6) δ (ppm): 6.74-6.67 (3H, m, $H^{2', 5', 6'}$), 6.33-6.24 (2H, m, $H^{5, 6}$), 5.13 (1H, td, H^{3}), 4.89 (1H, d, H^{2}), 2.81 (1H, dd, *J* 4.9 and 21.3 Hz, H^{4}), 2.66 (1H, dd, *J* 6.9 and 23.1 Hz, H^{4}), 2.04 (2H, m, H^{b}), 1.32 (2H, m, H^{c}), 1.22-0.99 (24H, m, H^{d}), 0.76 (3H, t, H^{e})

^{13}C NMR (400 MHz, acetone-d6) δ (ppm): 78.38 (C-2), 69.13 (C-3), 31.74 (C-4), 108.44 (C-5), 129.97 (C-6), 144.92 (C-7), 145.10 (C-8), 132.66 (C-9), 110.89 (C-10), 118.98 (C-1′), 118.39 (C-2′), 144.32 (C-3′), 142.17 (C-4′), 114.98 (C-5′), 113.53 (C-6′), 172.03 (C-a), 33.84 (C-b), 29.47 (C-c), 29.45-22.43 (C-d), 13.46 (C-e)

FTIR (υ_{max} cm^{-1}): 3500-3100 (O-H stretch, alcohol), 2950-2850 (C-H stretch, aliphatic), 1717 (C=O stretch, ester), 1612 (C=C stretch, aromatic)



Compound [**20**]

Under nitrogen environment, 1 g of compound [**10**] was dissolved in a 200 ml solution of mixture of MeOH: EtOAc (1:1). Small quantities of palladium on carbon (10% basis) were added as a catalyst to the reaction mixture. Purging of the system was done using nitrogen gas followed by purging with hydrogen gas. This was followed by addition of hydrogen gas via the hydrogen

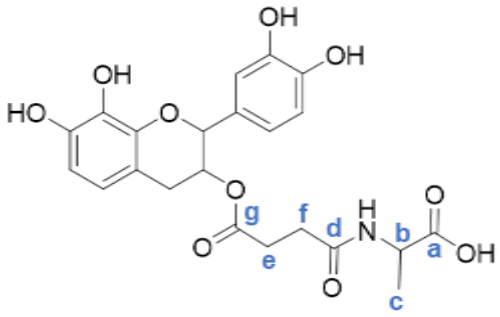
reactor system. The reaction was carried out for 22 hours at a pressure of 40 bar. At the end of reaction, the Pd/C catalyst was filtered off on an acid celite-bed followed by rinsing with excess ethyl acetate followed by methanol. The filtrate was concentrated to dryness using a Büchi rotavapor R-200 to obtain compound [**20**]. The characterization of the compound is given below.

MW: 563.25 g/mol; Color: white; MP: 86 °C; Percentage yield: 92 %

^{1}H NMR (400 MHz, Acetone-d6) δ (ppm): 7.18-7.04 (5H, m, H^{Ph}), 7.01-6.67 (3H, m, $H^{2', 5', 6'}$), 6.30-6.25 (2H, m, $H^{5, 6}$), 5.09 (1H, q, H^{3}), 4.93 (1H, d, H^{2}), 4.49 (1H, t, H^{h}), 2.83-2.51 (4H, m, $H^{4, f}$), 1.99 (2H, m, H^{b}), 1.37 (2H, m, H^{c}), 1.19-0.99 (8H, m, H^{d}), 0.73 (3H, t, H^{e})

^{13}C NMR (400 MHz, Acetone-d6) δ (ppm): 77.84 (C-2), 70.3 (C-3), 31.60 (C-4), 108.50 (C-5), 119.01 (C-6), 144.99 (C-7), 145.20 (C-8), 136.98 (C-9), 110.62 (C-10), 129.85 (C-1′), 118.10 (C-2′), 144.40 (C-3′), 142.05 (C-4′), 115.14 (C-5′), 113.55 (C-6′), 129.85-126.56 (CH-Ph), 132.69 (C-Ph), 172.59 (C-a), 35.42 (C-b), 28.89 (C-c), 28.38-22.40 (C-d), 13.46 (C-e), 37.26 (C-f), 170.66 (C-g), 53.89 (C-h)

FTIR (u_{max} cm^{-1}): 3500-3100 (O-H stretch, alcohol), 2950-2850 (C-H stretch, aliphatic), 1736 (C=O stretch, ester), 1631 (C=O stretch, amides), 1610 (C=C stretch, aromatic)



Compound [**21**]

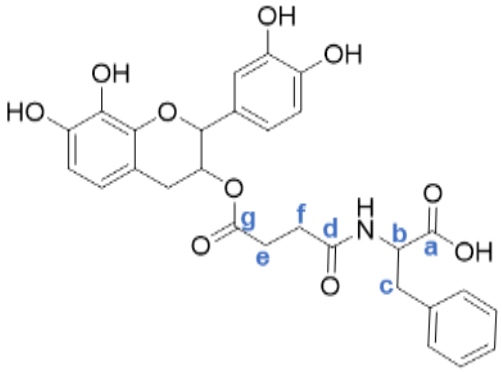
The same reaction for compound [**20**] was carried out again, however with compound [**13**] in place of compound [**10**] to produce compound [**21**]. The characterization of the compound is given below.

MW: 461.13 g/mol; Color: white; MP: 98 °C; Percentage yield: 84 %

^{1}H NMR (400 MHz, Methanol-d4) δ (ppm): 6.69-6.56 (3H, m, $H^{2', 5', 6'}$), 6.31-6.26 (2H, m, $H^{5, 6}$), 5.18 (1H, q, H^{3}), 5.06 (1H, d, H^{2}), 2.77 (1, dd, *J* 4.7 and 21.0 Hz, H^{4}), 2.65 (1, dd, *J* 5.6 and 21.9 Hz, H^{4}), 4.24 (1H, q, H^{b}), 2.38 (4H, m, $H^{e, f}$), 1.26 (3H, d, H^{c})

^{13}C NMR (400 MHz, Methanol-d4) δ (ppm): 77.79 (C-2), 69.97 (C-3), 27.02 (C-4), 108.14 (C-5), 118.76 (C-6), 144.93 (C-7), 144.98 (C-8), 132.54 (C-9), 110.72 (C-10), 129.95 (C-1′), 117.50 (C-2′), 143.97 (C-3′), 142.08 (C-4′), 114.87 (C-5′), 112.89 (C-6′), 172.10 (C-a), 48.44 (C-b), 16.25 (C-c), 174.77 (C-d), 29.05 (C-e), 29.65 (C-f), 172.52 (C-g)

FTIR (υ_{max} cm^{-1}): 3500-3100 (O-H stretch, alcohol and carboxylic acid), 2950-2850 (C-H stretch, aliphatic), 1728 (C=O stretch, ester), 1649, 1631 (C=O stretch, amides), 1612 (C=C stretch, aromatic)



Compound [**22**]

The same reaction for compound [**20**] was carried out again, however with compound [**14**] in place of compound [**10**] to produce compound [**22**]. The characterization of the compound is given below.

MW: 537.16 g/mol; Color: white; MP: 92 °C; Percentage yield: 88 %

^{1}H NMR (400 MHz, Acetone-d6) δ (ppm): 7.18-7.02 (5H, m, H^{Ph}), 6.72-6.55 (3H, m, $H^{2', 5', 6'}$), 6.32-6.24 (2H, m, $H^{5, 6}$), 5.08 (1H, q, H^{3}), 4.59 (1H, d, H^{2}), 2.78 (1H, dd, *J* 4.9 and 21.1 Hz, H^{4}), 2.64 (1H, dd, *J* 6.5 and 22.6 Hz, H^{4}), 4.59 (1H, q, H^{b}), 3.05 (1H, dd, *J* 5.3 and 19.2 Hz, H^{c}), 2.88 (1H, dd, *J* 7.9 and 21.8 Hz, H^{c}), 2.28 (4H, m, $H^{e, f}$)

^{13}C NMR (400 MHz, Acetone-d6) δ (ppm): 78.05 (C-2), 69.55 (C-3), 27.99 (C-4), 108.44 (C-5), 119.04 (C-6), 144.91 (C-7), 145.05 (C-8), 137.29 (C-9), 110.90 (C-10), 130.03 (C-1′), 118.19 (C-2′), 144.29 (C-3′), 142.11 (C-4′), 115.08 (C-5′), 113.48 (C-6′), 132.64 (C-Ph), 129.31-126.54 (CH-Ph), 172.15 (C-a), 53.47 (C-b), 37.26 (C-c), 171.43 (C-d), 29.18 (C-e), 29.99 (C-f), 170.94 (C-g)

FTIR (υ_{max} cm^{-1}): 3500-3100 (O-H stretch, alcohol and carboxylic acid), 2950-2850 (C-H stretch, aliphatic), 1726 (C=O stretch, ester), 1651, 1626 (C=O stretch, amides), 1609 (C=C stretch, aromatic)

2.7 Physicochemical and biological properties

There are various physicochemical and biological properties carried out on mesquitol and its derivatives in this project. They include antioxidant properties, antibacterial properties, gelling properties, antifungal properties and wood durability tests.

2.7.1 Antioxidant properties

Antioxidant properties was tested using the DPPH (2,2-diphenyl 1-picryldhydrazyl) Radical Scavenging Activity (RSA) method. The anti-radical activity of the samples (pure mesquitol, crude mesquitol, catechin and hemi-syntheized mesquitols) were measured by UV-visible spectroscopy at an absorbance of 517 nm using the DPPH technique. This method measured the antioxidant ability of the samples to reduce the purple DPPH radical to yellow DPPHH. 200 ppm stock solution of each sample were prepared in methanol and the DPPH solution was prepared at a concentration of 10^{-4} M. The IC50 value was calculated using the formula below:

$$\mathrm{IC50} = \frac{(y - b)}{a}$$

The equation is obtained from the linear equation of y = ax + b. In the above equation, a is the directing coefficient whereas b is the y-intercept. The experiment was done in triplicates.

2.7.2 Antibacterial properties

The antibacterial activity of the samples (pure mesquitol, crude mesquitol and hemi-synthesized mesquitols) was measured using the resazurin reduction test. In this technique, blue color

changes to fluorescent pink when metabolism occurs from bacterial respiratory chain cells. The cells metabolic activity was measured through reading the fluorescence over a period of time. The bacterial strains of *Escherichia coli* were cultured overnight on solid LB (Luria-Bertani) medium at 35 °C. This was followed by taking some of the colonies and placing them in a nutrient broth, which was incubated at 35 °C till an optical density of 0.4 was attained. The antibacterial tests were carried out in a 96-well microplate whereby the concentrated samples in DMSO, the inoculum, and the resazurin were introduced. A negative control test was carried out with ethoxyethanol on the samples plate. A positive control test containing DMSO was carried out to help in verifying the bacterial growth in the absence of samples.

2.7.3 Gelling properties

40 mg of samples (pure mesquitol, crude mesquitol and hemi-synthesized mesquitols) was placed in a glass haemolysis tube and topped up to 1 g with different solvents (water, olive oil and ethyl oleate). The mixture was heated in an ultrasonic bath at 80 °C until everything was solubilized. The solution was set aside overnight. Observation was done in the morning to assess whether the compounds gelled or not.

2.7.4 Antifungal properties

2.7.4.1 Microorganism

One white rot fungus, namely *Trametes versicolor* (TV), and one brown rot fungus, namely *Poria placenta* (PP), were used for the antifungal activity test.

2.7.4.2 Samples

Samples used were crude mesquitol extract, pure mesquitol, catechin, tebuconazole and compound **[17]**. Additionally, mixtures of tebuconazole and different other samples were used for antifungal activity to help in assessing synergy.

2.7.4.3 Fungal growth tests

The procedure was performed according to the method of Bopenga Bopenga et al. (2020). The procedure was somewhat revised from Chang et al. (1999). Different concentrations of samples from 0.01 ppm to 10 ppm for tebuconazole and 100 ppm to 10000 ppm for other samples were

solubilized in a minimum amount solvent (approximately 2 mL acetone or water) and then added to 120 mL of autoclaved cooled malt extract-agar solution. The malt extract-agar was prepared by mixing 4% malt extract and 2% nutrient agar in distilled water. Then, 20 mL of media from each concentration was transferred into 6 Petri dishes for carrying out the experiment in triplicates for the TV and PP. Mature fungi were then inoculated to the media by cutting a small portion and placing it at the center of the Petri dish. 6 more Petri dishes acting as control tests were similarly inoculated with the two fungi, but without the samples or extractives. The experiment was carried out at a temperature of 22 °C with a relative humidity of 70% under incubation and the fungal mycelial growth was monitored daily by measuring the control distance of colony and extractive distance of the colony until the control Petri dishes are completely covered by mycelium (Bopenga Bopenga et al., 2020; Chang et al., 1999).

First fungal growth test involved crude mesquitol, pure mesquitol and catechin. Second growth test involved a tebuconazole sample, which was tested on the fungi to determine the lowest concentration at which tebuconazole has a minimal effect on TV and PP. Third fungal growth test involved a mixture of the samples with lower concentrations of tebuconazole (0.1 ppm and 0.5 ppm) to assess their synergistic response against the two fungi. The fourth fungal growth test involved equal mixtures of 100 ppm, 500 ppm and 1000 ppm of catechin and pure mesquitol to assess their synergistic response against the two fungi.

The ability of the samples to inhibit the growth of the fungi was calculated using the antifungal index (AI) formula below.

$$\text{AI}\ (\%) = \frac{(\text{DC} - \text{DE})}{\text{DC}} \text{X}\ 100$$

Where, DE is the extractives distance (cm) and DC is the control distance (cm) of the colony. The AI (%) results rely typically on the DC.

2.7.5 Wood durability tests

The wood durability study involved the wood impregnation test, leaching test and wood decay test. The wood impregnation, leaching and decay tests were carried out according to the European standard NF EN 113-1 procedure, whereby the treatment was done on beech wood (*Fagus sylvatica L.*) (NF EN 113-1, 2020). Hydrogel used for this activity was prepared from a

reaction of glycerol carbamate and dodecyl amine according to the procedure of Obounou-Akong et al. (2015).

2.7.5.1 Formulations and treatments

The parameters used for the test were:

Fungi: *Trametes versicolor* (white rot) and *Poria placenta* (brown rot)
Wood: Beech (*Fagus sylvatica L*.)
Wood dimension: 26 x 15 x 6 mm^3
Expected duration: 12 weeks
Samples per treatment: 36 (24 treated + 12 non-treated)
Quantity of total samples: 558 (360 treated + 180 non-treated + 18 untreated control/blank)

The key for the test is:

- 0.1 T = 0.1 ppm tebuconazole
- 0.5 T = 0.5 ppm tebuconazole
- K-1000 = 1000 ppm catechin
- CM-1000= 1000 ppm crude mesquitol
- CM-5000 = 5000 ppm crude mesquitol
- PM-1000 = 1000 ppm pure mesquitol
- PM-5000 = 5000 ppm pure mesquitol
- H = 3% hydrogel
- C12-1000 = 1000 ppm compound **[17]**
- N = non-treated samples
- C = untreated control/blank samples

The formulations and treatments for the test are described as in table 3 below.

Table 3: Wood durability test formulations and treatments

S/No.	Nomenclature	Description
1.	CM-1000	Treatment of wood using 1,000 ppm crude mesquitol with and without leaching test
2.	PM-1000	Treatment of wood using 1,000 ppm pure mesquitol with and without leaching test
3.	K-1000	Treatment of wood using 1000 ppm catechin with and without leaching test
4.	CM-5000	Treatment of wood using 5000 ppm crude mesquitol with and without leaching test
5.	PM-5000	Treatment of wood using 5000 ppm pure mesquitol with and without leaching test
6.	HPM-5000	Treatment of wood using 3% hydrogel and 5000 ppm pure mesquitol with and without leaching test
7.	0.1 T	Treatment of wood using 0.1 ppm tebuconazole with and without leaching test
8.	0.5 T	Treatment of wood using 0.5 ppm tebuconazole with and without leaching test
9.	0.1 TCM-1000	Treatment of wood using 0.1 ppm tebuconazole and 1000 ppm crude mesquitol with and without leaching test
10.	0.1 TPM-1000	Treatment of wood using 0.1 ppm tebuconazole and 1000 ppm pure mesquitol with and without leaching test
11.	0.1 TK-1000	Treatment of wood using 0.1 ppm tebuconazole and 1000 ppm catechin with and without leaching test
12.	0.5 TCM-1000	Treatment of wood using 0.5 ppm tebuconazole and 1000 ppm crude mesquitol with and without leaching test
13.	0.5 TPM-1000	Treatment of wood using 0.5 ppm tebuconazole and 1000 ppm catechin with and without leaching test
14.	0.5 TCM-5000	Treatment of wood using 0.5 ppm tebuconazole and 5000 pm crude mesquitol with and without leaching test
15.	C12-1000	Treatment of wood using 1000 ppm compound 16 with and without leaching test

2.7.5.2 Wood impregnation

The 15 treatments were investigated for the beech wood. All the blocks of wood samples used were first dried in the oven at 103 °C for 48 hours before measuring the initial mass of the wood samples. The dried wood blocks were put in a beaker with the impregnation solutions per treatment then placed inside a desiccator and subjected to a vacuum pressure for 30 minutes. The wood blocks were then impregnated through suction with the various formulations for 2 hours under vacuum as shown in figure 24. This was followed by further 4 hours impregnation outside the desiccator in the atmosphere. Glass beads were put in the top of the wood blocks in the beaker to allow the wood blocks to be completely submerged in the solutions during the impregnation process. For each treatment, 12 wood samples were impregnated. After the impregnation, the wood blocks were removed from the impregnation solutions, dried with an absorbent paper and then dried in the oven at 103 °C for 48 hours. The final impregnation test mass was taken after 48 hours.



Fig 24: Wood impregnation set-up

The weight percentage gain (WPG) after impregnation was thus calculated as:

$$\text{WPG } (\%) = \frac{(\text{m1} - \text{m0})}{\text{m0}} \text{ x } 100$$

Where, m0 is the initial mass before impregnation and m1 is the final mass after impregnation

2.7.5.3 Wood leaching

In the leaching process, for every treatment, 12 blocks from the impregnation tests were leached by immersing the sample blocks in 1200 ml distilled water and subjecting them to different leaching periods through continuous mechanical agitation. The first leaching process was done for 1 hour, 2 hours and then 4 hours with the water being changed after every 1, 2 and 4 hours respectively. The wood blocks were removed and left with no water for 16 hours. This was followed by the second leaching process that was done for 8 hours, 16 hours and finally 48 hours with water being changed after every 8, 16 and 48 hours respectively. All the leached wood samples were removed from the leaching bottles, dried with absorbent paper and then over the oven at 103 °C for 48 hours. The mass of the leached wood samples was measured.

The weight percentage loss after leaching (WPLAL) was calculated as:

$$\text{WPLAL}\ (\%) = \frac{(\text{m1} - \text{m2})}{\text{m1}}\ \text{x}\ 100$$

Where, m1 is the final mass after impregnation and m2 is the final mass after leaching

2.7.5.4 Wood decay

TV and PP fungi were inoculated on Petri dishes containing a sterile malt-agar culture media. The fungi were then incubated at a temperature of 22 °C at a relative humidity of 70 % till their fungal mycelium completely grew to cover the entire surface of the Petri dishes. For every treatment, 3 culture media were used with each containing 3 beech wood samples. For the culture media, there was 1 non-treated sample and 2 treated samples. Experiment was carried out in triplicates culture media for both TV and PP fungi. This test made use of both leached wood samples and unleached wood samples. Three triplicates of the control were similarly prepared by putting 3 un-treated wood samples on the mycelia.

The wood samples were removed from the media after 12 weeks, and were softly cleaned with a brush to help in removing the mycelia. This was followed by drying in the oven at 103 °C for 48 h. The fungicidal effectiveness of the wood treatment was calculated using percentage weight loss (WL):

$$\mathrm{WL}\ (\%) = \frac{(\mathrm{m2} - \mathrm{m3})}{\mathrm{m2}}\ \mathrm{x}\ 100$$

Where, m2 is the final mass after leaching and m3 is the final mass after wood decay. For the wood samples that did not go through the leaching process as well as for the control wood samples, their final mass after impregnation (m1) and initial mass before impregnation (m0) were used, respectively, as a substitute to m2.

CHAPTER 3: CHEMICAL MODIFICATIONS OF MESQUITOL

3.1 Bibliographic review of chemical modifications of flavonoids

Different classes of flavonoids can undergo various reactions that chemically modify their structures to form different derivatives. The formed derivatives are assumed to be less sensitive and have better formulation properties as well as better bioactivity than the corresponding flavonoids. According to Arbenz and Avérous (2015), chemical modification of flavonoids, using catechin (flavan-3-ol) as an example, can be carried through three different ways: heterocycle reactivity that involves ring opening reaction in C-ring leading to chemical structure rearrangement; reactivity of the nucleophilic sites, which is due to the presence of OH in the aromatic rings; and reactions at the phenolic and aliphatic OH. The summary of the reactions is shown in figure 25 below (Arbenz & Avérous, 2015).

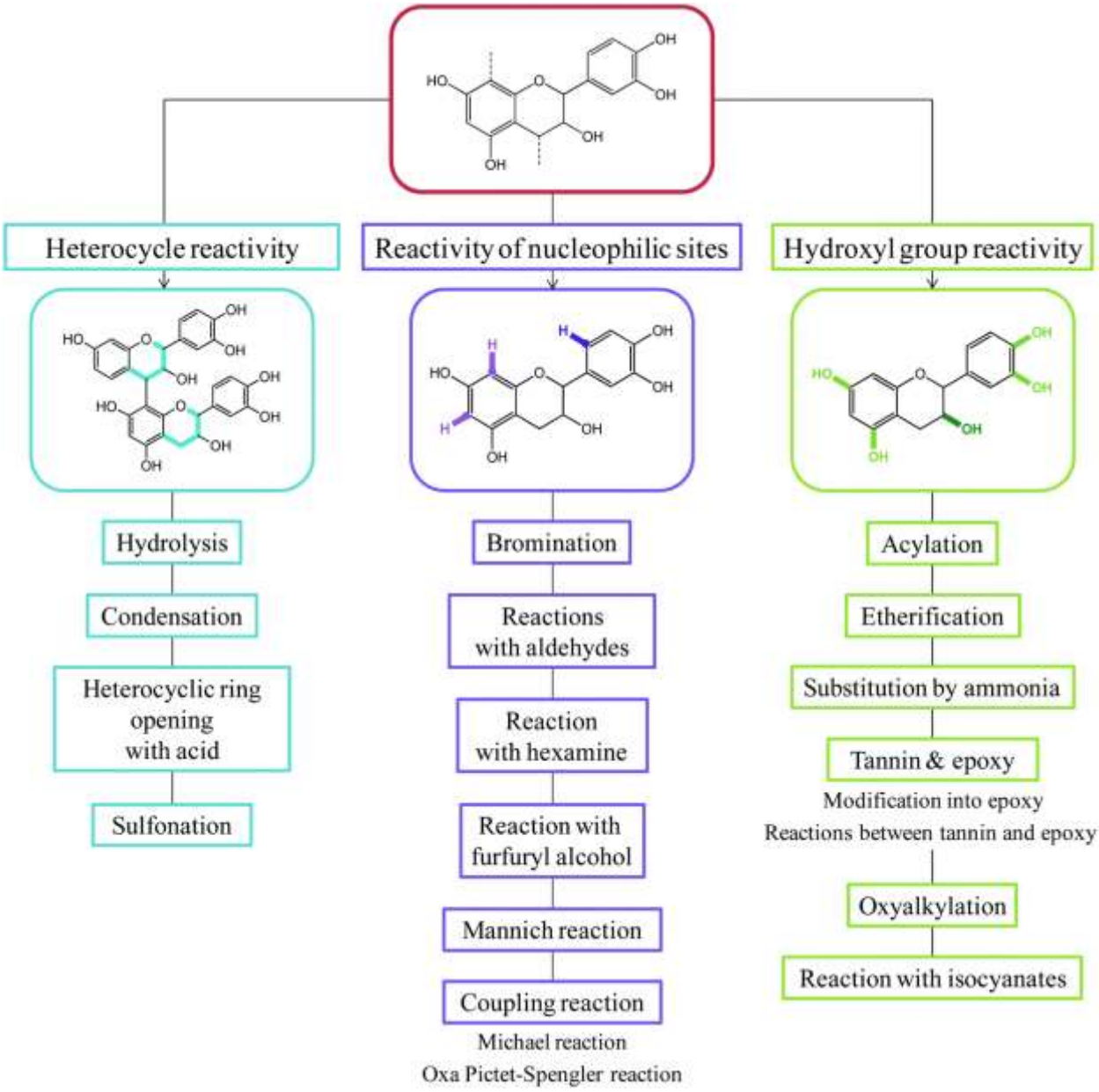


Fig 25: Reactivity of flavonoids using catechin, a flavan-3-ol, as an example (Arbenz & Avérous, 2015)

The most common reactions of flavonoids involve the phenolic and aliphatic hydroxyl groups of the flavonoids structure, which are key for their antioxidant and many other biological properties (Kumar & Pandey, 2013). These reactions involve acylation, etherification, substitution by ammonia, modification into epoxy, reactions with epoxy groups, oxyalkylation and reaction with isocyanides (figure 26).

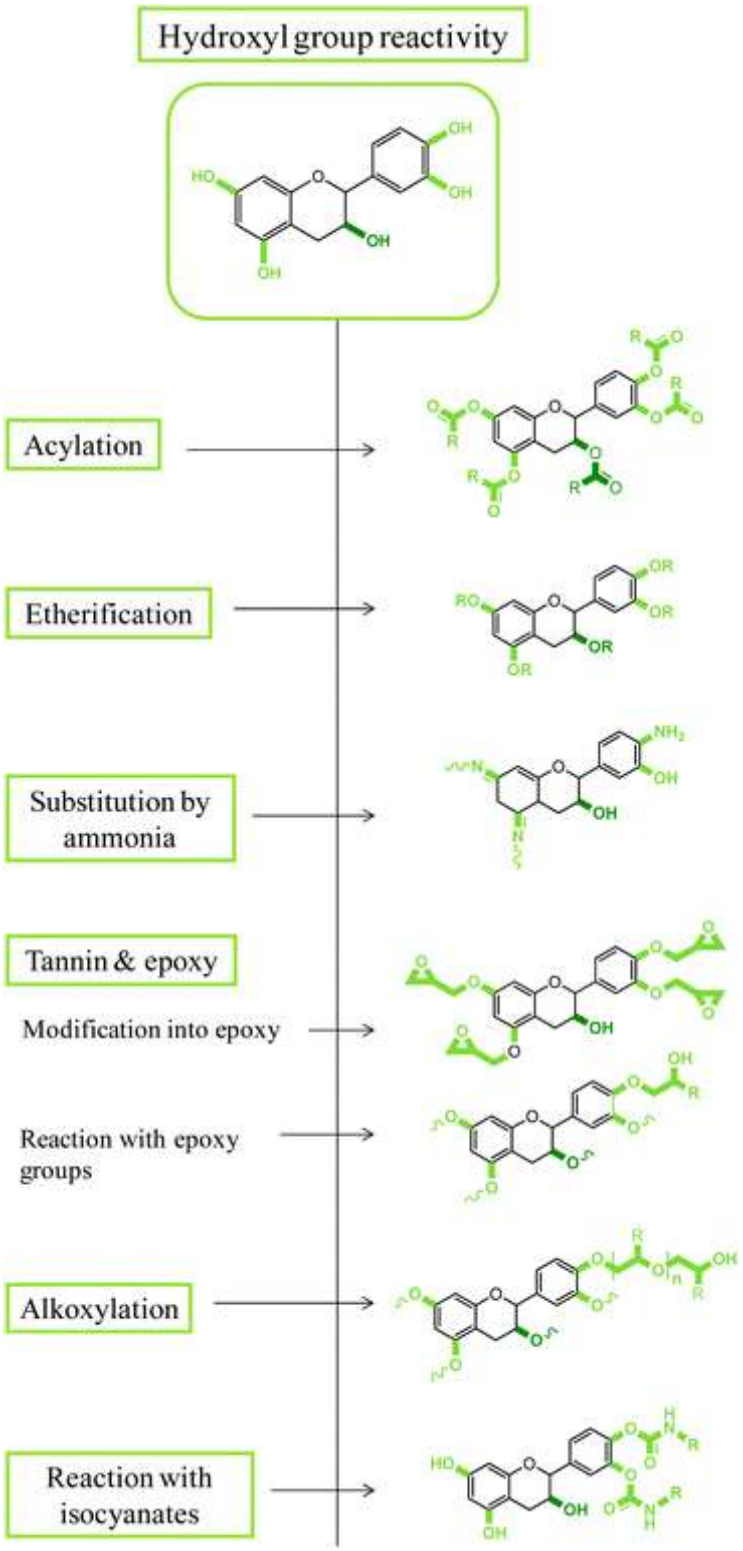


Fig 26: Chemical reactions with phenolic hydroxyl and aliphatic hydroxyl groups (Arbenz & Avérous, 2015)

Apart from carrying out modification of flavonoids chemically as described above, modification can also be carried out enzymatically. The enzymatic technique can be used for acylation reactions and it is particularly very selective (chemoselective, regioselective or stereoselective). It takes place in mild reaction conditions, thus it avoids many steps needed by the classical chemical techniques (De Araújo et al., 2016). Different hydrolytic enzymes such as lipases, proteases and esterases are highly recognized as influential biocatalysts because they possess wide availability and are cheap. Amongst the three mentioned hydrolases, the most effective as well as well-studied biocatalysts for flavonoids acylation reactions is lipases. Naturally, lipases help in catalyzing the hydrolysis reaction of ester bonds at the hydrophilic-hydrophobic interface. However, under specific conditions, more particularly when water is absent, lipases are capable of reversing the reaction, and this results to esterification, leading to the formation of glycerides from glycerol and fatty acids. It is this synthesis activity catalyzed by lipases that has been effectively utilized in the manufacture of flavonoid esters so as to enhance their biological and physicochemical properties (Kyriakou et al., 2012; Rajendran et al., 2009). Enzymatic acylation of flavonoids can be carried through direct esterification of flavonoids or transesterification process as shown in figure 27 below.

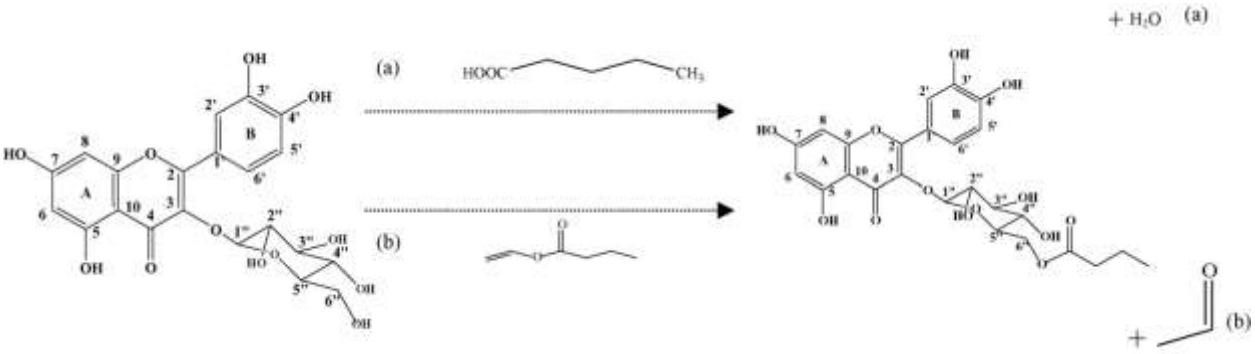


Fig 27: Enzymatic acylation of flavonoids : (a) esterification, (b) transesterification (Chebil et al., 2006)

According to De Araújo et al. (2016), the most excellent biocatalyst lipase that has high regioselectivity and enantioselectivity is *Candida antarctica* lipase B (CALB). CALB has been effectively used to carry out acylation of hesperidin, a flavanone, using decanoic acid (Araújo et al., 2011). In another study, CALB has been regioselectivity used to acylate isoquercetin and rutin by molecular modeling method (E. B. De Oliveira et al., 2009). Enzymatic acylation method selectivity and conversation rate of flavonoids are affected by different factors, which include

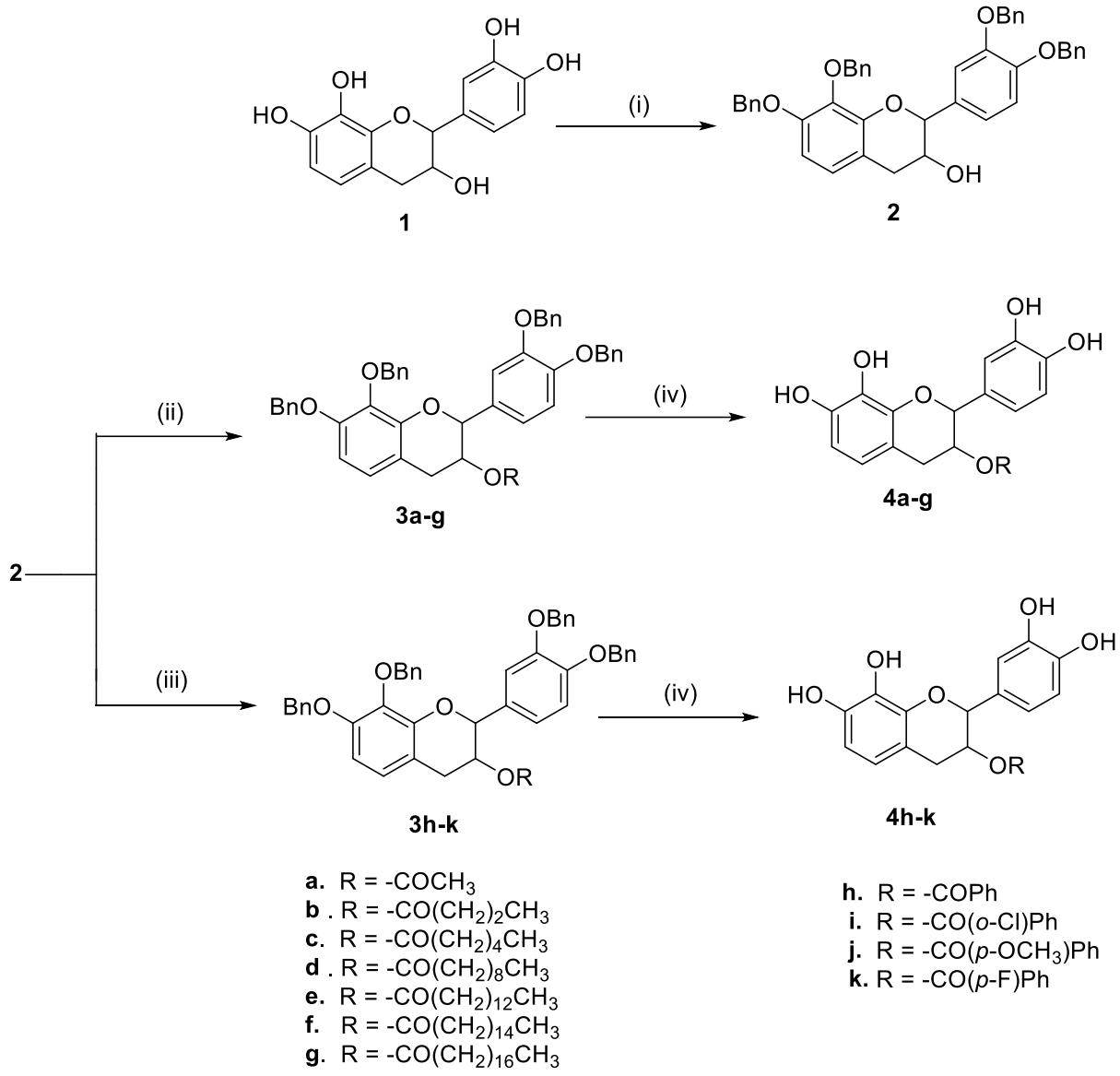
chain length and nature of the acyl donors, temperature and the water content and composition of the reaction medium (De Araújo et al., 2016; Razak & Annuar, 2015).

3.2 Bibliographic review of chemical modifications of mesquitol

In this particular research study, acylation was the main reaction used in the chemical modification of the mesquitol. Acylation is a type of reaction involving the introduction of an acyl group (RCO-) into a given compound. This is a common reaction in organic chemistry that leads to formation of amides and ketones. However, in plants, flavonoids acylation occurs via either aromatic acylation or aliphatic acylation (Essa et al., 2023). Mostly, the flavonoids acylation via aliphatic acylation route leads to formation esters in flavan-3-ol molecules through nucleophilic acyl substitution reactions. Most flavan-3-ols, such as catechin and mesquitol, have very good antioxidant properties due to the presence of a catechol moieties in their structures. These moieties permit the compounds to willingly donate either hydrogen atoms or electrons to help in neutralizing free radicals, therefore making them act as antioxidants (Anitha et al., 2020; Kumar & Pandey, 2013; Luo et al., 2022). To preserve such mentioned property, mesquitol often undergo chemical modification on the aliphatic OH position. Thus, it can undergo selective acylation, which involves protection of the phenolic hydroxyl groups (with a benzyl, acyl or silyl group) prior to acylating the aliphatic OH. After acylation reaction, the formed molecule undergoes deprotection process on the phenolic OH to produce the expected acylated flavan-3-ol (S. C. Chepkwony, 2019; Green, 1999; Jagadeeshwar Rao et al., 2003; Roberts & Caserio, 1977).

Chemical modification of mesquitol and other flavan-3-ols with different acyl donors on the aliphatic OH position can lead to lipophilic or amphiphilic derivative with good pharmacological properties. Y. Zhong and Shahidi (2011) studied the antioxidant effect of the functionalized product of epigallocatechin gallate with long chain fatty acids. They discovered that the derivatized epigallocatechin gallate compound had greater antioxidant properties with enhanced lipophilicity (Y. Zhong & Shahidi, 2011). The acyl donors include fatty acids, amides, acyl chlorides and acid anhydrides. Chemical modification of mesquitol involves the use of different coupling reagents which include DCC/DMAP, EDC/HOBT, BOP/Et_3N. Different studies carried out acylation reactions with the mentioned coupling reagents. Jagadeeshwar Rao et al. (2003) showed that it is possible to chemically modify mesquitol through esterification by protecting the phenolic OH with benzyl groups, reacting the aliphatic OH with different acyl donors to form an

ester using DCC/DMAP, then deprotect the phenolic OH using 10% Pd/C (Jagadeeshwar Rao et al., 2003) as shown in scheme 3 below.



Scheme 3: Functionalization reactions of mesquitol [Reagents and conditions: (i) BnBr, K_2CO_3, acetone, reflux; (ii) corresponding aliphatic acid, DCC, DMAP, DCM; (iii) corresponding aromatic acid chloride, Et_3N, DCM; (iv) H_2, 10% Pd/C, MeOH] (S. C. Chepkwony, 2019; Jagadeeshwar Rao et al., 2003)

3.3: Obtention of pure mesquitol

The heartwood part of *Prosopis juliflora* plant was collected from Baringo County, Kenya, air dried under the shade and then ground into fine powders to increase the contact surface between the wood and the solvent allowing a better extraction of interesting chemical compounds.

In this particular research, two different extraction methods were studied: Soxhlet and maceration extractions. The two extraction methods involved two serial extraction solvents, namely cyclohexane and acetone. Cyclohexane was first used to extract the lipophilic compounds, thus helped in eliminating apolar compounds from the plant. Acetone was used to extract mesquitol that has been known to be present in the heartwood part of the plant in large quantities. From table 4 below, it can be noted that Soxhlet acetone extracts had more yields that maceration acetone extracts. Thus, it is a good method for getting more extractives than maceration.

Table 4: Percentage extraction yields

Solvents	**Soxhlet extraction % Yield**	**Maceration extraction % Yield**
Cyclohexane	0.26 ± 0.046	0.27 ± 0.045
Acetone	9.89 ± 0.442	5.86 ± 0.264

To obtain pure mesquitol, it was necessary to purify the obtained mixture from Soxhlet and macerated extracts. To do this, several solvent mixtures of solvents were used before the best solvent mixture for purification was chosen. These solvent mixtures included diethyl ether alone, diethyl ether and cyclohexane, ethyl acetate and methanol, and cyclohexane and ethyl acetate. The best solvent mixture according to TLC analysis of the crude product and purification on silica gel was 30% cyclohexane and 70% ethyl acetate. Purification of mesquitol was thus done through wash with a solvent mixture composed of 30% cyclohexane and 70% ethyl acetate, using a silica-bed fritted Pyrex Buchner funnel under low pressure. The produced yield was 62.7 ± 0.47% for Soxhlet and 66.3 ± 1.53% for maceration extraction method. Purification can also be done to obtain very pure mesquitol through deprotection reaction of benzylated crude extract. The pure mesquitol obtained by these methods were characterized using FTIR, ^{1}H and ^{13}C NMR and MP. This mesquitol was used in several properties' tests, such as wood durability

evaluation, antifungal, antibacterial, gelling and antioxidant activities, and similarly for hemi-synthesis reactions of mesquitol leading to chemical modification of mesquitol.

3.4 Hemi-synthesis reactions of mesquitol

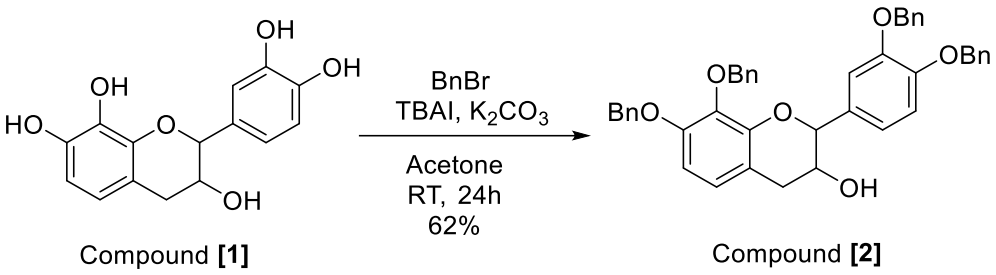
Hemi-synthesis reactions of mesquitol were carried out to help in chemically modifying mesquitol to different mesquitol derivatives. Commonly, mesquitol, being a polyphenol, is soluble in water but sparingly soluble in lipophilic solvents. Thus, mesquitol becomes less effective in their important applications in any lipophilic systems such as oils and fats, manufacture of lipid-based foods and preparation of cosmetic formulation (S. C. Chepkwony, 2019; Cruz et al., 2015; Ran et al., 2024; Y. Zhong et al., 2025). Mesquitol are known to be very good antioxidants, however, this solubility factor limits their applications in various fields, thus, the need to chemically modify their structure to help in improving their lipophilicity for diverse industrial applications. The chemical modification process involves 3 steps: protection of the phenolic hydroxyls using benzyl groups, followed by the esterification of the aliphatic hydroxyl group and finally the deprotection of the phenols to obtain different mesquitol derivatives.

3.4.1 Chemical modification of mesquitol using carboxylic acids

Compound **[1]**, also known as mesquitol, was the starting material for chemical modifications using carboxylic acids. This process was carried out through Steglich esterification reactions, which allowed the conjugation to fatty acids at the aliphatic hydroxyl group of the compound to promote hydrophobicity. Five different fatty acids were used for the chemical modification. They include caproic acid, caprylic acid, lauric acid, myristic acid and palmitic acid.

3.4.1.1 Protection of the phenolic groups

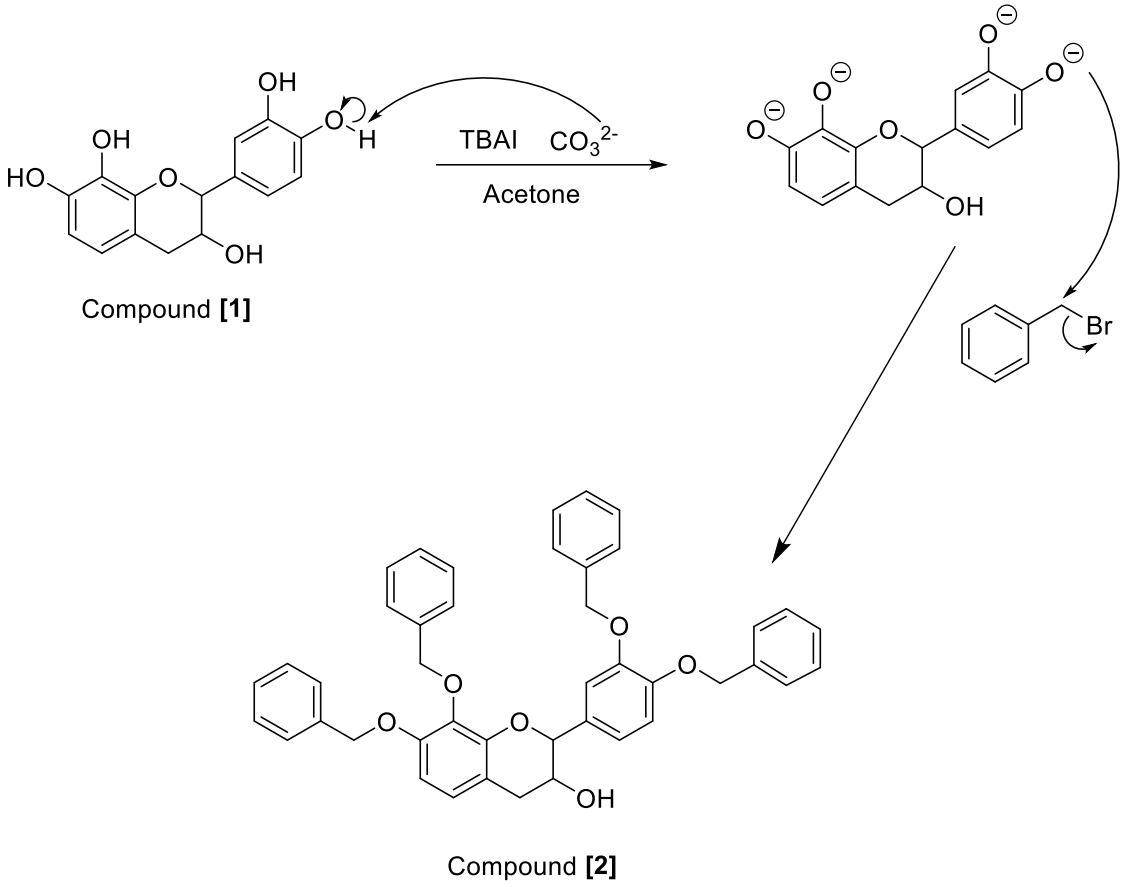
Benzylation reaction was the best available option for protection of mesquitol. Benzylation is specific to only the phenolic OH groups thus this reaction favors use of both pure and crude extract to get the benzylated mesquitol as shown in scheme 4 below.



Compound **[1]** Compound **[2]**

Scheme 4: Benzylation reaction of mesquitol (Bn = benzyl group)

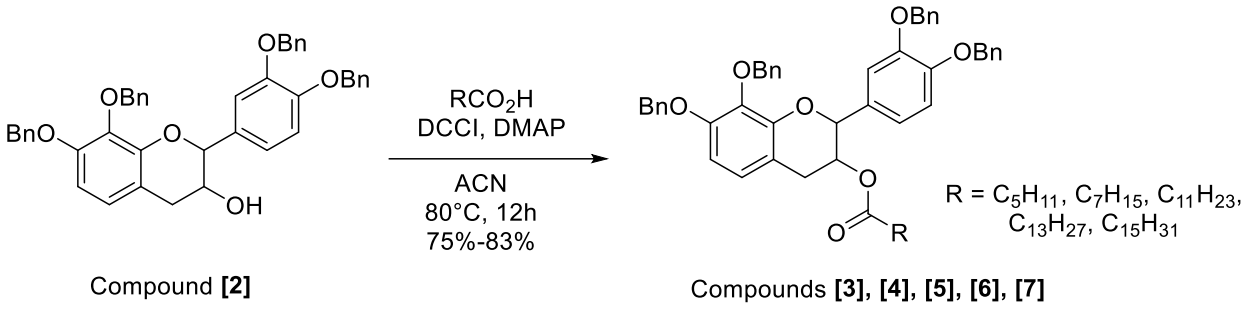
The phenolic hydrogens are considered more acidic compared to the alcoholic hydrogen. This is because the phenolic hydrogens are stabilized by resonance effect. Their acidity is also increased by the intramolecular hydrogen bonding in the phenolic hydroxyl groups' catechol structure. This promotes their ability to become nucleophiles under basic conditions and consequently increases the ability of the phenolic OHs being ionized to produce phenoxide ion. Benzyl bromide reacted with the formed phenoxide anions in a SN2 reaction produced a benzylated compound **[2]** as shown in scheme 5 reaction mechanism. In the absence of a phase transfer catalyst, mostly the reactants are soluble in aqueous phase but not soluble in the organic phase. Therefore, TBAI was used in this reaction as the phase transfer catalyst that helps in solubilizing K_2CO_3 salt into acetone organic phase. TBAI does this by promoting the migration of the anionic nucleophile, which is an alkoxide produced by K_2CO_3 from the solid phase to the organic phase so as to allow it to react with BnBr. The iodide ion (I^-) obtained from TBAI is also considered a good nucleophile and thus it can take part in a substitution reaction that leads to formation a very reactive intermediate. TBAI helps to convert a less reactive BnBr (benzyl bromide) to a more reactive BnI (benzyl iodide) in situ, thus it facilitates faster and better yields in a benzylation reaction. Benzylated mesquitol produced in this step can also be deprotected to obtain a pure mesquitol.



Scheme 5: Reaction mechanism for benzylation reaction

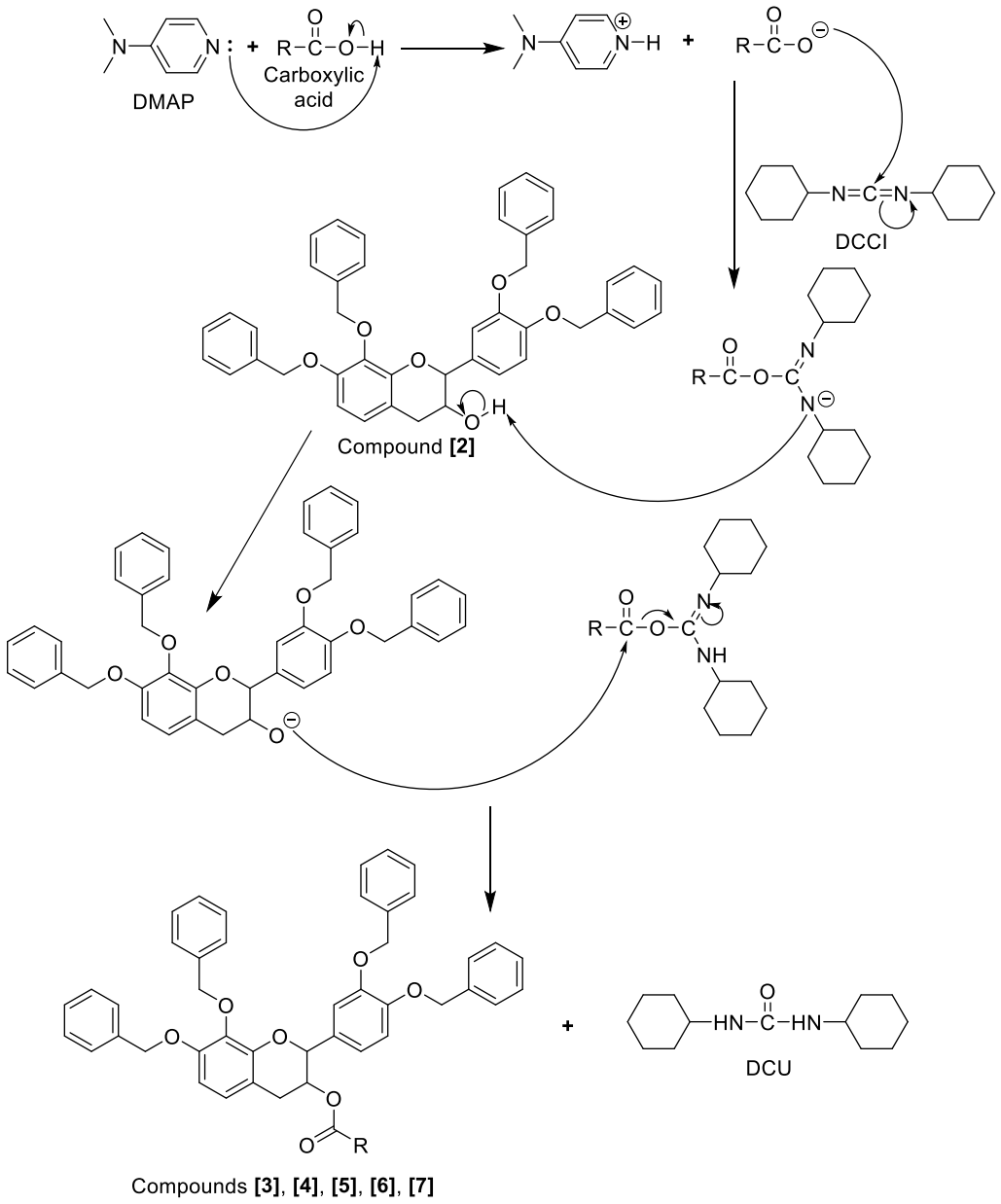
3.4.1.2 Esterification reaction

Steglich esterification process was used to carry out esterification reaction of benzylated mesquitol using fatty acids as shown in scheme 6 below.



Scheme 6: Esterification of benzylated mesquitol using DCCI/DMAP

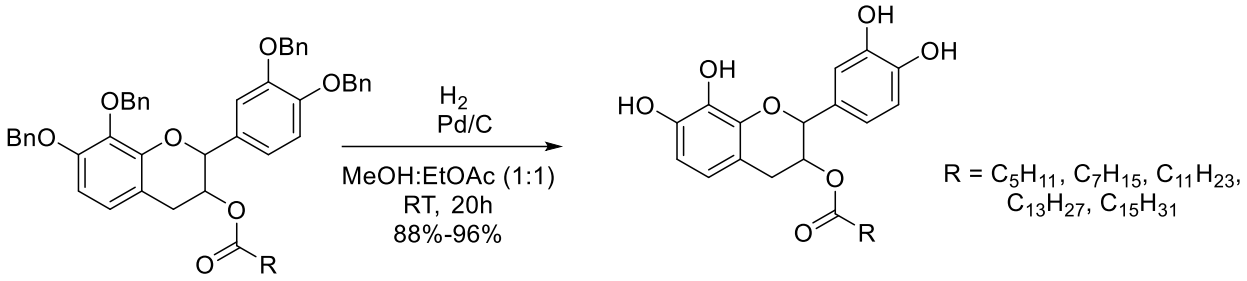
Steglich esterification makes use of DCCI as its coupling reagent. For this reaction, compound **[2]** was reacted with the five carboxylic acids in the presence of DCCI/DMAP. DMAP is a nucleophilic catalyst, thus an effectively known acyl transfer agent as seen in the reaction mechanism in scheme 7. The reaction resulted to the formation of five acylated benzylated mesquitol compounds **[3]**, **[4]**, **[5]**, **[6]** and **[7]**. The reaction had the percentage yields vary by increasing from 75% to 83% as the length of the fatty acid increased.



Scheme 7: Reaction mechanism for the esterification of benzylated mesquitol using DCCI/DMAP

3.4.1.3 Deprotection reaction

Deprotection of benzylated mesquitol esters consisting of compounds **[3]**, **[4]**, **[5]**, **[6]**, and **[7]** was carried out through hydrogenation process as shown in scheme 8.

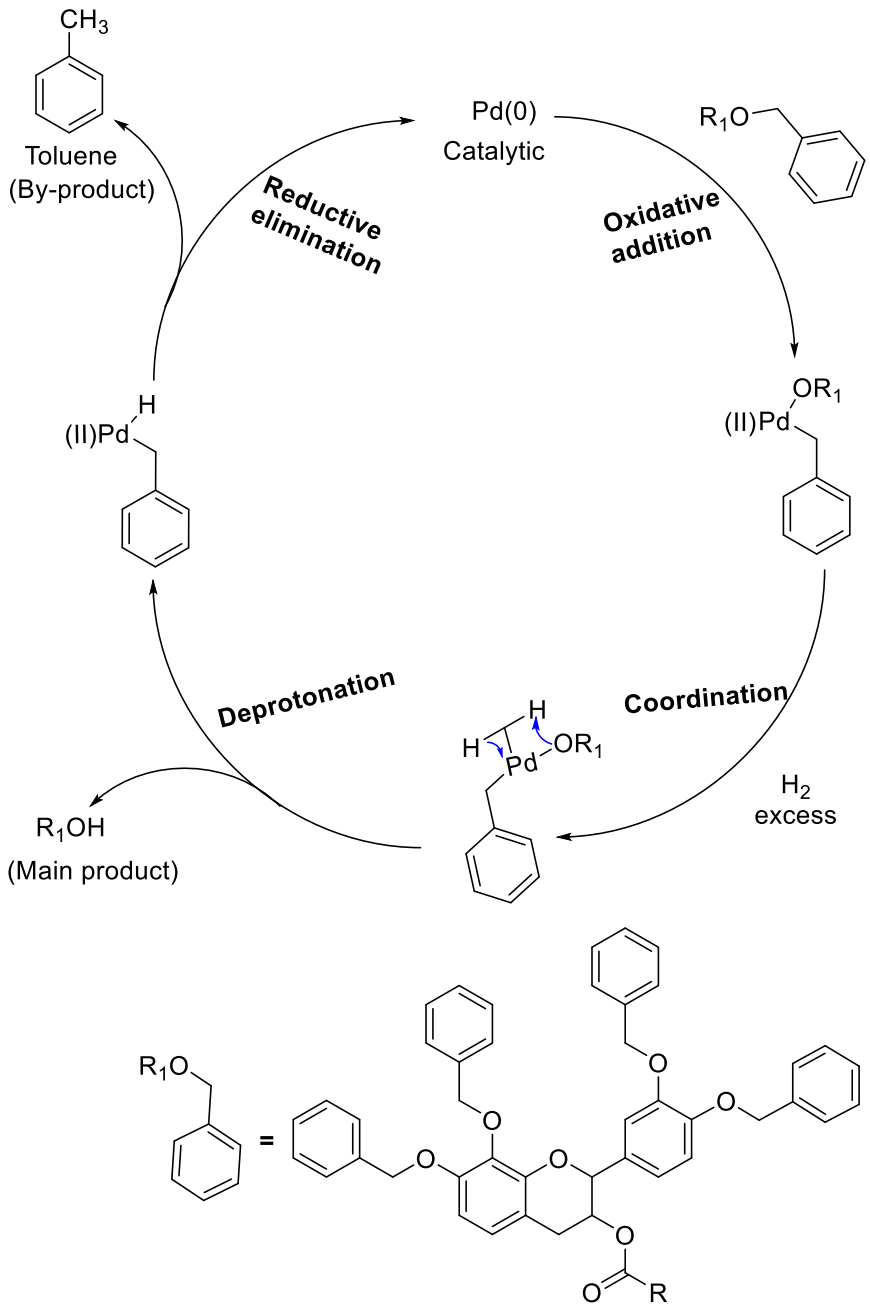


Compounds **[3]**, **[4]**, **[5]**, **[6], [7]**

Compounds **[15]**, **[16]**, **[17]**, **[18], [19]**

Scheme 8: Hydrogenation of benzylated mesquitol esters **[3]**, **[4]**, **[5]**, **[6]**, **[7]**

Deprotection reaction basically involved the removal of the benzyl groups from the flavan-3-ol esters. This was carried out through hydrogenation process in the presence of palladium on carbon catalyst. The reaction mechanism for the deprotection process is shown in scheme 9 below.



Scheme 9: Reaction mechanism for hydrogenation of benzylated mesquitol esters

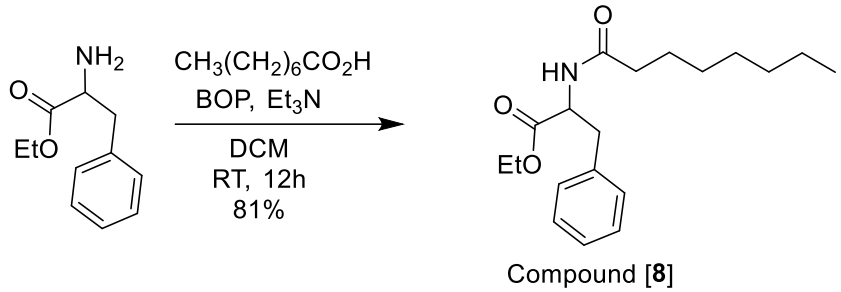
3.4.2 Chemical modification of mesquitol using amides

Compound **[1]**, was still, the starting material for chemical modifications using amides. This hemi-synthesis reactions also followed the three steps for modification. There are two sets of amides used in this chemical modification, which include:

i. Amide formed from a reaction of an amino acid and a fatty acid
ii. Amides formed from reactions of amino acids and acid anhydride

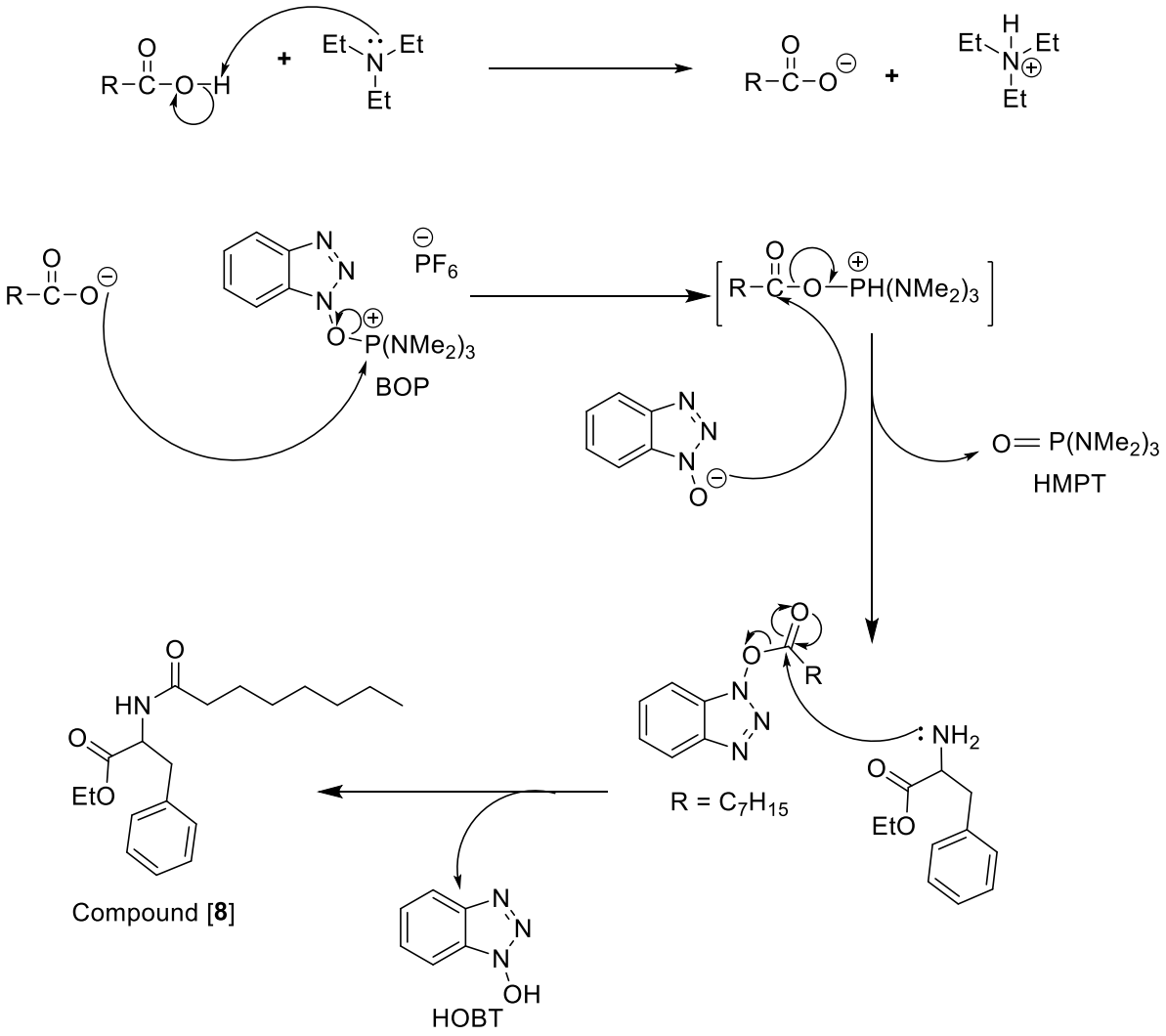
3.4.2.1 Formation of an amide from a reaction of an amino acid and a fatty acid

One protected amino acid (phenylalanine ethyl ester) was reacted with caprylic acid in the presence of BOP and TEA as shown in scheme 10 to produce compound **[8]**.



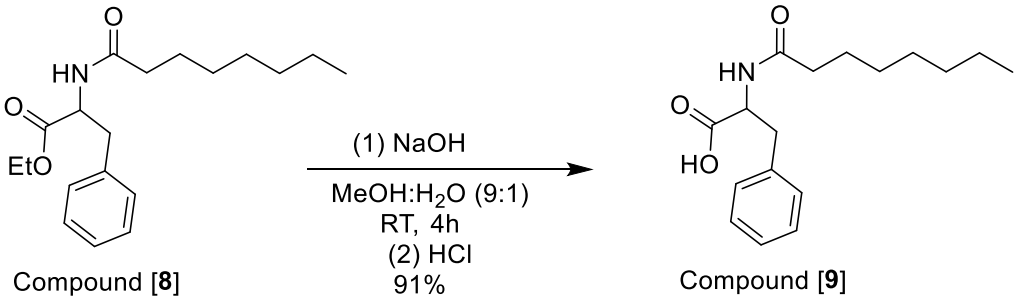
Scheme 10: Reaction of phenylalanine and caprylic acid

BOP is used in the reaction as common coupling reagent for formation of amides from amines and carboxylic acids. BOP helps in activating the carboxylic acid by producing an active ester intermediate that is used for reaction with the amino acid to produce the ethyl protected amide as explained in scheme 11. In this BOP-mediated process, TEA is used as a non-nucleophilic base to help in scavenging for an acid from fatty acid and helps in neutralizing any acidic byproducts, thus it prevents the byproducts from interfering with the above reaction and thus facilitating a faster rate of reaction.



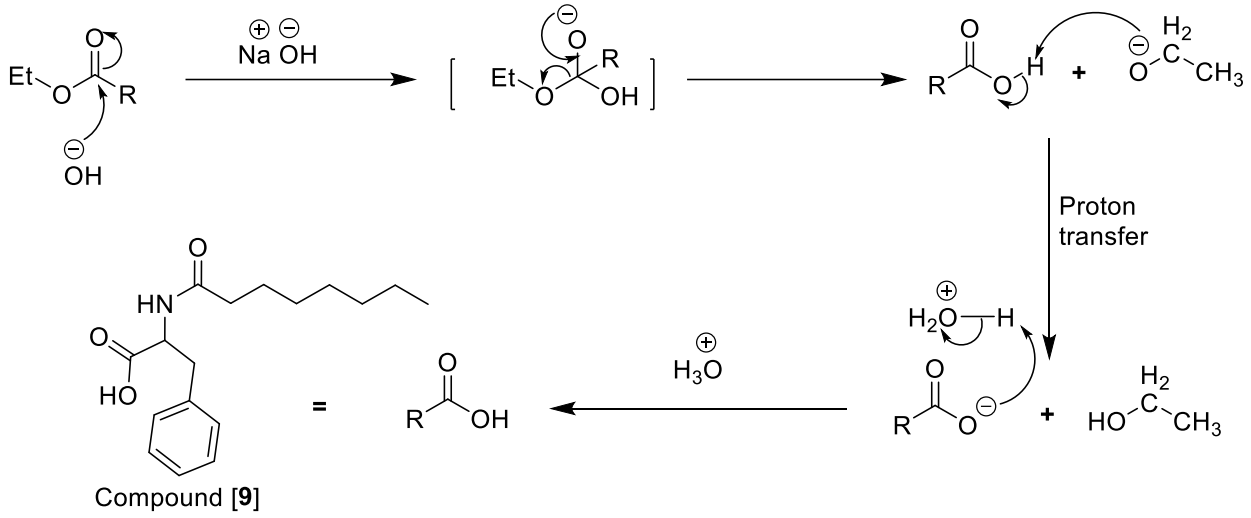
Scheme 11: Reaction mechanism for reaction of phenyl alanine and caprylic acid

Compound **[8]** was then saponified in the presence of NaOH in MeOH:H_2O (9:1) and HCl to produce a carboxylic acid salt and an alcohol. The carboxylic acid salt is further protonated by HCl to form the corresponding free carboxylic acid as shown in scheme **12**.



Scheme 12: Saponification reaction of compound **[8]**

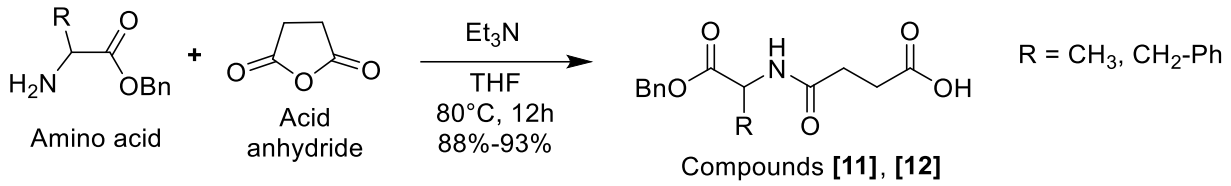
Considering the reaction as elaborated in scheme 13, compound **[8]**, which is an ester, reacts with NaOH, a strong base, in a process known as alkaline hydrolysis to form the sodium salt of the carboxylic acid and alcohol. The strong acid, HCl, is then added afterwards to reacts with the sodium salt of the carboxylic acid, thus replacing the sodium ion and substituting with a proton to produce the free desired carboxylic acid as compound **[9]**.



Scheme 13: Reaction mechanism for saponification reaction of compound **[8]**

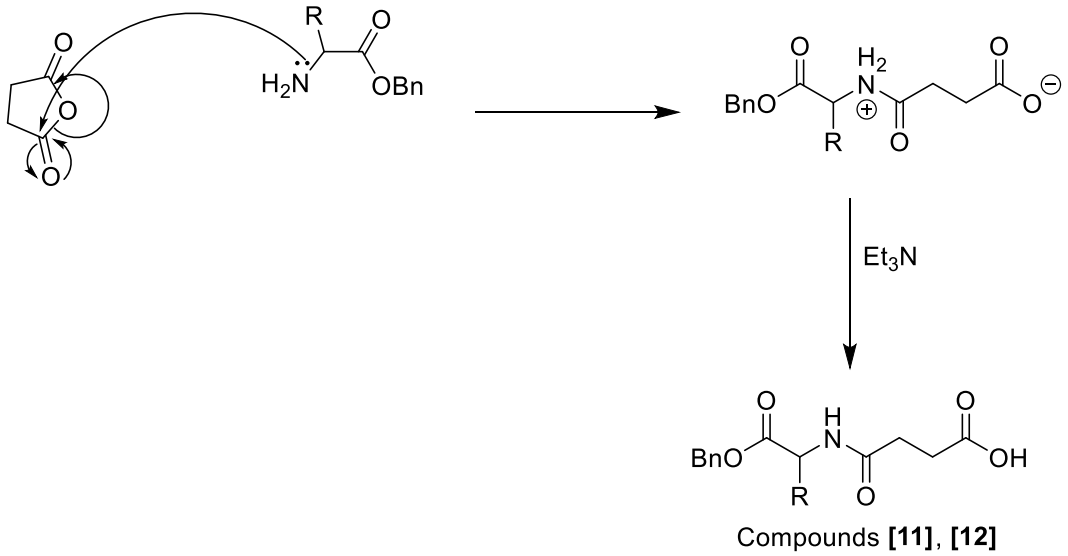
3.4.2.2 Formation of amides from reactions of amino acids and acid anhydride

Two protected amino acids namely, benzylated phenylalanine and benzylated alanine, were used for the amidation reaction with succinic anhydride in the presence of triethylamine (TEA) as shown in scheme 14.



Scheme 14: Reaction of amino acids with succinic anhydride

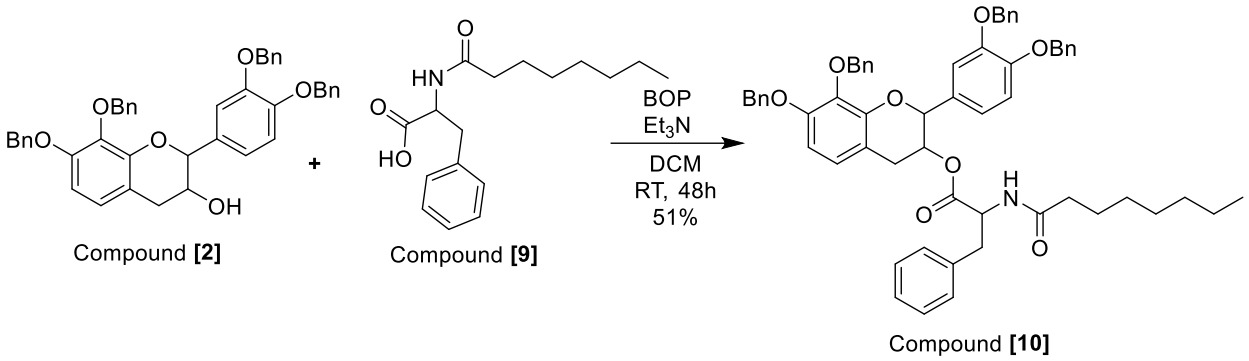
In the above reaction, the amine groups of benzylated amino acids act as a nucleophile, thus they attack either of the carbonyl carbons of the cyclic acid anhydride leading to the opening of the anhydride ring. This resulted into a new C-N amide bond as seen in scheme 15 reaction mechanism. The next step involves proton transfers, which occur under the assistance of TEA that acts as a base by removing the proton from the formed amide and using it to neutralize the formed carboxylate end. This reaction forms two products namely, N-(benzylated alaninyl) succinamic acid and N-(benzylated phenylalaninyl)succinamic acid referred to as compounds **[11]** and **[12]** respectively.



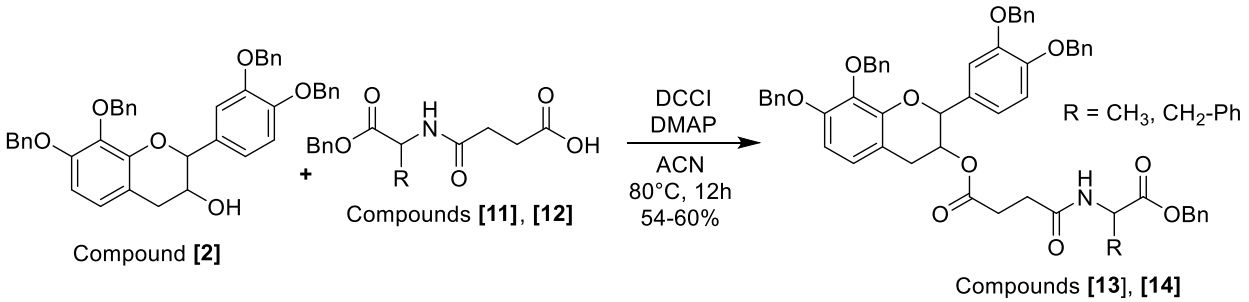
Scheme 15: Reaction mechanism for reaction of amino acids with succinic anhydride

3.4.2.3 Esterification process

Compound **[2]**, which is the benzylated mesquitol, was used for esterification process at the aliphatic OH position. The two sets of amides were esterified using different reagents as shown in scheme 16 and scheme 17.



Scheme 16: Esterification process of benzylated mesquitol with amide from phenylalanine and caprylic acid

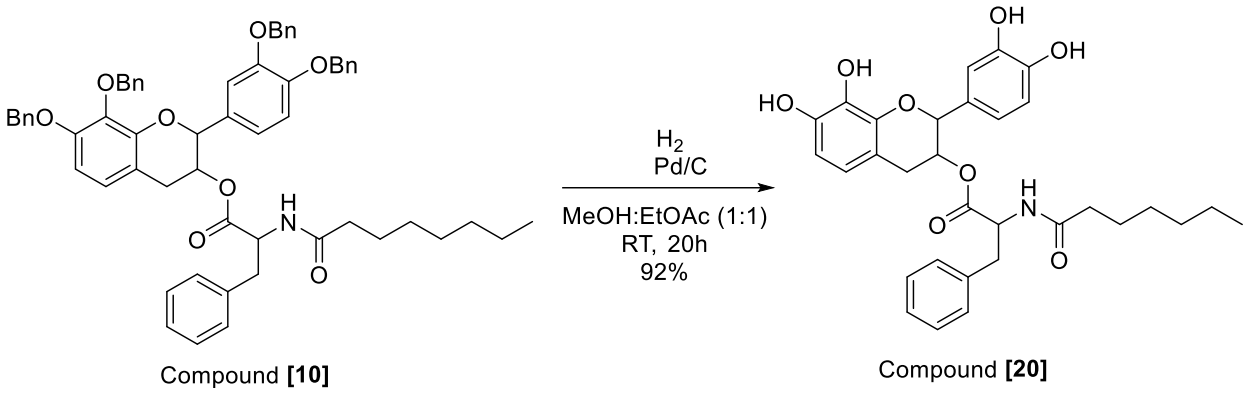


Scheme 17: Esterification process of benzylated mesquitol with amide from amino acids and succinic anhydride

BOP and TEA are used as reagents for esterification for compound **[9]** in scheme 16 and they follow the same reaction mechanism as scheme 11. On the other hand, esterification of compounds **[11]** and **[12]** was carried through Steglich esterification method that use DCCI/DMAP reagents. The mechanism for the reaction is the related to the mechanism elaborated in scheme 7. Carboxylic acid in scheme 7 is replaced by the amides in compounds **[11]** and **[12]**.

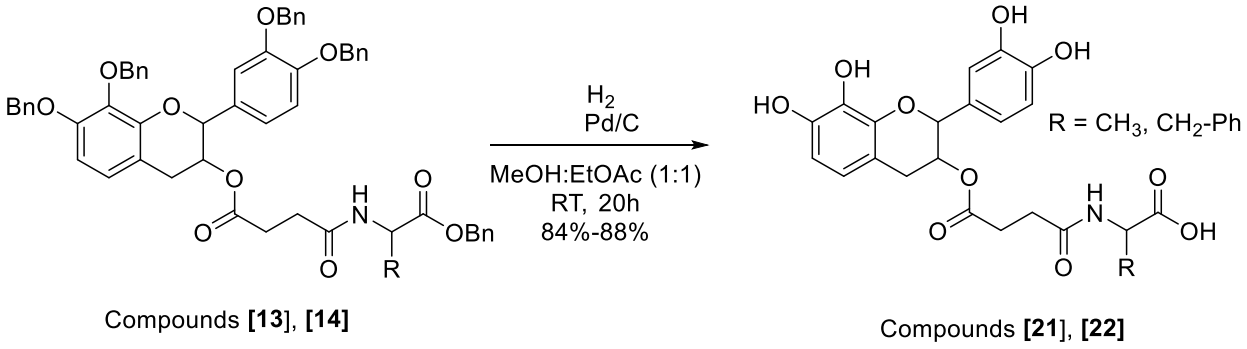
3.4.2.4 Deprotection reaction

Deprotection of benzylated mesquitol esters consisting of compounds **[10]**, **[13]** and **[14]** was carried out through hydrogenation process to produce compounds **[20]**, **[21]** and **[22]** as shown in scheme 18 and 19. The reaction mechanism for the deprotection reaction is as explained in scheme 9.



Compound **[10]** Compound **[20]**

Scheme 18: Hydrogenation of benzylated mesquitol ester **[10]**



Compounds **[13]**, **[14]** Compounds **[21]**, **[22]**

Scheme 19: Hydrogenation of benzylated mesquitol esters **[13]**, **[14]**

CHAPTER 4: PHYSICOCHEMICAL AND BIOLOGICAL PROPERTIES OF MESQUITOL AND ITS DERIVATIVES

4.1 Bibliographic review of physicochemical and biological properties of mesquitol and its derivatives

4.1.1 Pharmacological properties of flavonoids

Flavonoids are a collection of plant-derived molecules that possess various pharmacological properties, which include antioxidant and complexation, anti-inflammatory, antibacterial, antifungal, antiviral, cardiovascular and anticancer properties among others (Hasnat et al., 2024; Ullah et al., 2020).

4.1.1.1 Antioxidant and complexation properties

Antioxidants are compounds that help in protecting the cells from any damage that is brought about by free radicals or reactive oxygen species (ROS), or unstable compounds released by the metabolism of the body as well as contact to different environmental factors. The all-inclusive mode of action of flavonoids comprise of quenching free radical elements, overpowering the enzymes linked with the generation of free radical, promotion of internal antioxidant enzymes, and chelating metal (Gulcin, 2025; Halliwell, 1996). Different studies have shown various flavonoids with good antioxidant properties, such as mesquitol, catechin, quercetin, myricetin, luteolin, eriodictyol and naringenin amongst others (Aaby et al., 2004; Chagas et al., 2022; Chanput et al., 2016; Sirmah et al., 2011). The antioxidant properties of flavonoids are believed to arise their chemical structure that has the C6-C3-C6 ring system. To be specific, the antioxidant activities rely on the position and number of the hydroxyl groups in the chemical structure. In many flavonoids, a number of free radicals are reduced through proton transfer via the homolytic cleavage of the 3´-OH and 4´-OH catechol group available the structures of flavonoids. Nevertheless, even though the catechol moiety is considered to be a key factor for antioxidant properties, others flavonoids that do not have catechol groups likewise show outstanding radical scavenging abilities because of their capacity to transfer hydrogen atoms, or the concurrent hydrogen or electron transfer or consecutive electron transfer with loss of a proton (R. F. V. De Souza & De Giovani, 2004; Samsonowicz et al., 2017).

Complexation properties is the ability of flavonoids to form complexes with a metal ion, whereby the flavonoid becomes the ligand, thus it binds to the metal ion (Ren et al., 2008). Metal-flavonoid complexes have been studied to have increased antioxidant activities thus they offer a resourceful approach for the potential new drugs development, which can be used for the

treatment of different neurodegenerative illnesses, particularly Parkinson's disease, Alzheimer's disease and amyotrophic lateral sclerosis (Rodríguez-Arce & Saldías, 2021). Flavonoids are admirable ligands for developing metal complexes. Mostly, OH-substituted flavonoids, such as flavonols, have advantageous characteristics in their structure for coordinating many metal ions. Flavonols possess three possible coordination sites: First, between the 3-OH group and 4-C=O group found in the C-ring; second, between the 5-OH group found in the A-ring and 4-C=O group found in the C-ring; and third, between the 3´-OH group and 4´-OH group found in the B-ring as shown in figure 28.

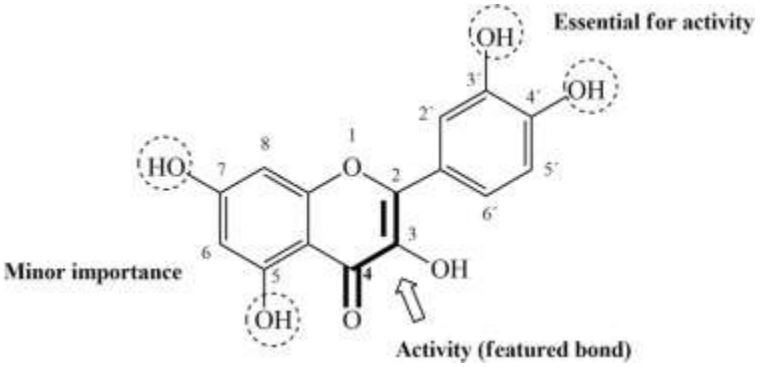


Fig 28: Flavonol structure with potential antioxidant metal-flavonoid coordinating sites (Rodríguez-Arce & Saldías, 2021)

Flavonoids are considered as weak acids that can go through deprotonation reaction, which powerfully enhances their coordination to the metal ions. To be specific, the acidity of the 3-OH protons in the flavonol structure is the highest; consequently, this explains why 3-OH group and 4-C=O group are the first sites to be affected in the process of complexation. In metal-flavonoid complexes having 3´-OH group and 4´-OH group, their catechol moiety mainly acts as a second binding site for metal ion. In many cases, the 5-OH group does not participate in complexation due to its lower steric hindrance and proton acidity brought about by the first metal-flavonoid complexation (Bukhari et al., 2009; Kasprzak et al., 2015). In a study by Pekal et al., it was reported that there the Cu(II)-quercetin complex had increased radical scavenging activity thus showing a lower redox potential values when compared to those of free quercetin (Pękal et al., 2011). Such metal-flavonoid complexes possess improved free radical scavenging activities in ABTS+ and DPPH assays. However, increase in the antioxidant activities rely on the type of flavonoid used and central metal ion (Khater et al., 2019; Rodríguez-Arce & Saldías, 2021).

4.1.1.2 Anti-inflammatory and anti-allergic properties

Flavonoids also possess anti-inflammatory and anti-allergic properties. From different studies, quercetin, luteolin, kaempferol, galangin and myricetin are a few flavonoids known to have anti-inflammatory and anti-allergic activities (Ayoub et al., 2021; Hwang et al., 2019; Jang et al., 2017; H. N. Lee et al., 2017; Molitorisova et al., 2021; Rakha et al., 2022; Theoharides et al., 2017; Vo et al., 2020). This explains why many plant-based extracts containing quercetin are currently being utilized as the key component in different possible anti-allergic drugs (Irie et al., 2016; Rogerio et al., 2010). Many flavonoids are known to be good anti-inflammatory agents through targeting various mechanisms taking part in inflammation as well as its development, through inhibiting regulatory enzymes and the transcription factors (Rakha et al., 2022). Flavonoids have noteworthy anti-allergic effect by preventing histamine release, trying to overturn the production of some inflammatory cytokines such as IL-4 and IL-13, and they also control the immune cells activity, which are involved in such allergic reactions (Kawai et al., 2007; Tanaka & Takahashi, 2013).

4.1.1.3 Antimicrobial properties

Flavonoids possess very good antimicrobial activities (antibacterial, antifungal and antiviral properties). Flavonoids display antibacterial activity through disruption of cell processes of bacteria. Some can interfere with the performance the cell membrane of bacteria, resulting to cellular contents leakages and ultimate death of cell (Herlina et al., 2025). Other flavonoids can disrupt the synthesis process of proteins, DNA and RNA, and yet these are very essential activities for the survival as well as replication of bacteria (Rodríguez et al., 2023; Yan et al., 2024). In other cases, flavonoids act by inhibiting energy production pathways of the bacteria, like ATP synthase that is very vital for growth and survival of bacteria (Cushnie & Lamb, 2005; H. Zhou et al., 2023). From different studies, flavonoids, such as genistein, luteolin, daidzein, and apigenin have been shown to interfere with ATP synthase production, therefore disrupting bacterial energy production (Herlina et al., 2025). (-)-Epigallocatechin gallate and sophoraflavone G are capable of interfering with the cytoplasmic membrane function of a bacteria (Cushnie & Lamb, 2005). CID12261165 flavonoid has displayed a noteworthy antibacterial property when tested against quinolone-resistant *Staphylococcus aureus* strains (Morimoto et al., 2023). In another study, phloretin is able to limit toxin production and thus hinder biofilm formation in *Escherichia coli* (Gallegos et al., 2016). In addition to the good

antibacterial properties displayed by different flavonoids, it has been noted that lipophilicity can similarly affect their activities, particularly against the gram-positive bacteria, such as *E. coli* (Yan et al., 2024).

Flavonoids have got good antifungal activities, mostly against a fungal pathogen known as *Candida albicans.* They act as antifungal agents inhibiting the fungal growth through plasma membrane disruption, introducing mitochondrial dysfunction, as well as disallowing cell division, cell wall formation, protein synthesis and RNA/DNA synthesis. Lichochalcone-A and quercetin have exhibited efficacy against *C. albicans* when tested in animal models, whereby lichochalcone A was used topically and quercetin via vaginal gavage (J.-H. Lee et al., 2024; Nguyen et al., 2021). Plant flavonoids are really helpful in lowering the rate of formation of free radicals in wood and therefore, they act as inhibitors of the activities different phytopathogenic fungi that affect different wood species (Pusztahelyi et al., 2015; Ribera & Zuñiga, 2012). They are natural anti-oxidants, which means they play a key role in reducing the imbalances and oxidative stress that occur in the wood cell redox reactions. These redox reactions are linked with reactive oxygen species. Lots of the wood-degrading fungi use reactive oxygen species (ROS) to help loosen the cell walls of plants, thus causing different wood cell polymer defects. ROS are considered as a subset of free radicals that contain oxygen. ROS being categorized under oxidants therefore requires antioxidants to solve its problems. This mechanism for the degradation of wood is considered relevant to one type of phytopathogenic fungi called brown rot fungi (Castaño et al., 2018; Hammel et al., 2002). Other types of phytopathogenic fungi, such as white rot fungi act by producing different enzymes to assist them in breaking down the complex wood polymers, namely lignin, cellulose and hemicellulose. Flavonoids, thus, act on the white rot fungi through enzyme inhibition method. They target specific enzymes that break down the plant cell wall (Kijpornyongpan et al., 2022; Sigoillot et al., 2012). In other cases, flavonoids can directly degrade the fungi cell wall or indirectly interfere with the fungi cell membrane, thus weakening its cell structure or causing cell damage and eventually fungal death (Das et al., 2024; Jin, 2019).

Flavonoids also show very good antiviral activities. They act by interfering with several viral replication stages. Flavonoids can act by blocking virus entry into cells, act by disrupting viral replication progressions, and at times modulate the immune response of the host to lower viral load. From different studies, numerous flavonoids, namely quercetin, kaempferol, apigenin, genistein and cyanidin have exhibited efficacy against diverse groups of viruses, which include

the ones of veterinary importance as well as human pathogens, such as influenza, herpes simplex virus and coronaviruses (Badshah et al., 2021; Da Fonseca et al., 2025; Evers et al., 2005; Kaul et al., 2021; Vicente et al., 2023).

4.1.1.4 Anticancer properties

Flavonoids display promising anticancer effects by interfering with several mechanisms, which promote progression and development of cancers. They have exhibited potential in prevention of cancer, cancer cell growth inhibition, and promotion of the efficiency of conventional treatments of cancer. Flavonoids, because of their good antioxidant activities, can help in neutralizing dangerous free radicals, which can cause DNA damage and thus contributing to the development of cancer (Mir et al., 2024). Flavonoids can inhibit enzymes taking part in carcinogen activation or promote some enzymes that can help in detoxifying carcinogens, consequently preventing initiation of cancer (N. Sharma et al., 2011). Other flavonoids can induce programmed cell death in cancer cells, a process called apoptosis, thus eradicating them from the body (Silva-Pinto et al., 2025). Different researches have shown that luteolin can help in reducing the self-renewal ability of the stem cells of cancer whereas apigenin can prevent the activity of cancer stem cell and promote the success of chemotherapy (Slika et al., 2022; Ullah et al., 2020). Another study has also shown that epigallocatechin-3-gallate, which is available in green tea can lower the toxicity of radiation therapy and possibly prevent the growth of cancer cells (Farhan et al., 2023). Many research works have displayed very good results in the laboratory as well as the animal models, but still lots clinical trials are required in confirming the anticancer properties of flavonoids in human beings. This is an active research area (Ansari & Akhtar, 2019; Ullah et al., 2020).

4.1.2 Applications of flavonoids

4.1.2.1 Cosmetics

Flavonoids are known for their potential as surfactants and gelators, mainly in applications such as cosmetics. This is because of their exceptional amphiphilic properties, whereby they possess both fat-soluble parts and water-soluble part. The amphiphilic properties permit them to stabilize emulsions and form gels, thus making them useful in cosmetic application (Barman & Banjare, 2024; Liga & Paul, 2025). Flavonoids, in other cases, are utilized in cosmetics for their soothing and antioxidant properties, to help in protecting as well as maintaining the health of skin (Tran &

Le, 2022). They act by neutralizing free radicals that are capable of damaging skin cells. Through neutralizing free radicals and supporting production of collagen, flavonoids can help in lowering the appearance of fine lines and wrinkles. Flavonoids help in maintaining the overall quality of the skin through solving various concerns such as uneven tone, dryness and dullness (Arct et al., 2002; Epstein, 2009; Sousa et al., 2025). The most commonly used flavonoids in cosmetic application are rutin, hesperidin, quercetin, catechins and epigallocatechin gallate (Arct et al., 2002; Dave Mehta et al., 2023). One main challenge of flavonoids in cosmetics application is their relatively low bioavailability. This means that the human body might not absorb as well as use them efficiently, thus this can hinder them from being effective in attaining desired cosmetic (J. E. De Souza et al., 2015; Yuan et al., 2024). Still, more researches are continuing to enhance the effectiveness and delivery of flavonoids in different cosmetic formulations.

4.1.2.2 Pharmaceutical applications

Flavonoids are known to exhibit many pharmaceutical applications because of their diverse biological properties. They have antioxidant, anticancer, antibacterial, antifungal, antiviral, cardioprotective and anti-inflammatory properties. Thus, such properties have allowed them to be investigated for their possible application in treatment of many conditions such as cardiovascular diseases disease and cancer among others (Dave Mehta et al., 2023; Hasnat et al., 2024; Ullah et al., 2020). The ability of flavonoids to form gels and act as surfactants promotes improved bioavailability, solubility and controlled release of medications, particularly the ones with poor water solubility. This supports their application in drug delivery systems. Studies on flavonoid-based nanogels have shown their application in cancer therapy (Liga & Paul, 2025). To improve the pharmaceutical application properties of flavonoids, they can undergo functionalization. Functionalized flavonoids are well recognized for their solubilization, transportation, dispersion and suspension proficiencies. Because of their self-assembling character, they are able to be effectively utilized as surfactants. These flavonoid-based surfactants are helpful in stabilizing of suspensions or emulsions, thus resulting to better drug efficacy and delivery (Ni et al., 2017; Tripathy, 2022).

4.2 Physicochemical and biological properties of mesquitol and its derivatives

Several physicochemical and biological properties were tested on compound **[1]** and its derivatives. The results are shown and discussed below.

4.3.1 Antioxidant properties

DPPH assay method was used for carrying out the antioxidant properties test. The assay measures the free radical scavenging ability of samples used in the experiment by observing the change of color from deep purple to pale yellow as the stable DPPH radical undergoes reduction by the antioxidants. The antioxidant activity of mesquitol and its derivatives was investigated very precisely through measurement of the IC_{50} value that is considered to be the antioxidant concentration that scavenges 50% of DPPH. The table 5 below shows the IC50 values for the project samples. For comparison purposes, IC_{50} value was also determined for catechin, a reference compound known to have good antioxidant activities.

Table 5: IC_{50} values of catechin, mesquitol and its derivatives

Product	IC_{50} value (µg/mL)	IC_{50} value (µM)
Crude mesquitol (CM)	2.73	9.40
Pure mesquitol (PM)	1.15	3.97
Catechin	2.80	9.64
C6	2.46	6.34
C8	2.23	5.37
C12	2.56	5.42
C14	2.73	5.46
C16	2.69	5.08
Phe-C8	2.92	5.18
Ala-SA	2.55	5.54
Phe-SA	3.04	5.66

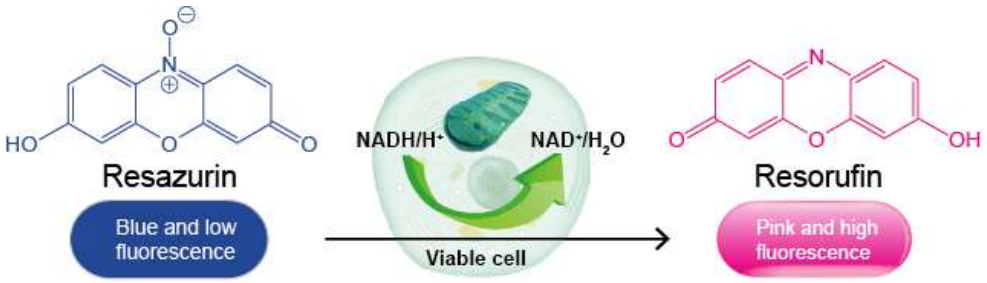
CM = Crude mesquitol, PM = Pure mesquitol, C6 = caproic acid ester (compound **[15]**), C8 = caprylic acid ester (compound **[16]**), C12 = lauric acid ester (compound **[17]**), C14 = myristic acid ester (compound **[18]**), C16 = palmitic acid ester (compound **[19]**), Phe-C8 = phenylalanine-octanoic acid ester (compound **[20]**), Ala-C8 = alanine-octanoic acid ester (compound **[21]**), Phe-SA = phenylalanine-succinic anhydride ester (compound **[22]**)

The inhibitory concentration at 50% DPPH (IC_{50}) was determined for mesquitol and its derivatives as well as catechin by reporting the percentage RSA as a function of antioxidant concentration. This free radical scavenging method involves mechanisms in which antioxidant compounds can inhibit the microorganisms' enzymatic activities and lipid peroxidation. Thus, the

DPPH assay is utilized for extensive screening of antioxidants from natural products and plant extractives (Lobo et al., 2010; Raza & John, 2007; Sithisarn et al., 2007). The results in table 5 show that pure mesquitol has got better antioxidant activities at 3.97 µM as compared to the reference compound, catechin, at 9.64 µM. Catechin antioxidant activities are comparable to the crude mesquitol activities at 9.40 µM. Mesquitol has got two catechol moieties as compared to catechin that has one catechol moiety in their structure. Catechol moieties are very important structural features that leads to potential antioxidant activities. This ability results from the capacity of the catechol group to be able to donate an electron or a hydrogen atom to help in neutralizing free radicals, thus protecting any present biological macromolecules from oxidative damage (Anitha et al., 2020; Guardado et al., 2012; Kumar & Pandey, 2013; Luo et al., 2022; Silva et al., 2002). Considering the bimodular products of mesquitol and fatty acids and the trimodular products of mesquitol, fatty acids and amino acids as well as mesquitol, amino acids and succinic anhydride, all of them have their antioxidant IC_{50} values at approximately 5 µM, which is a good antioxidant activity compared to the reference compound value. Thus, derivatization of mesquitol has still preserved the semi-synthesized products' antioxidant properties.

4.3.2 Antibacterial properties

Antibacterial test was done using resazurin assay method where the blue color under low fluorescence changes to pink color under high fluorescence when metabolism occurs from bacterial respiratory chain cells as illustrated in scheme 20 (Novikova et al., 2023).



Scheme 20: Resazurin cell viability assay

Escherichia coli (*E. coli*) was the chosen microorganism for the antibacterial test. This is because it is a common bacterium that is effortlessly cultured, as well as fully-understood microorganism. It is familiar foodborne pathogen causing serious diseases. Thus, it was used as

a reliable indicator for the efficacy test of potential new antibacterial compounds from mesquitol and its bimodular and trimodular compounds. From the resazurin assay results, the % inhibition of *E. coli* for the different samples used are as shown in figure 29 and figure 30.

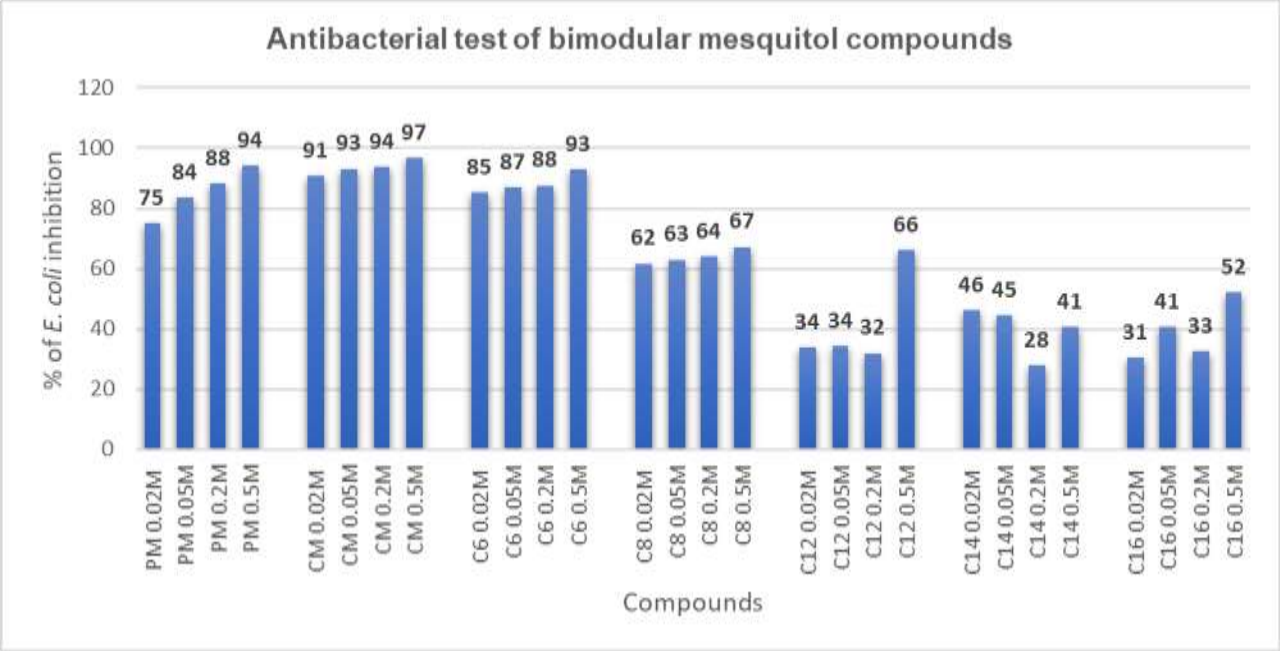


Fig 29: Antibacterial test of bimodular mesquitol compounds

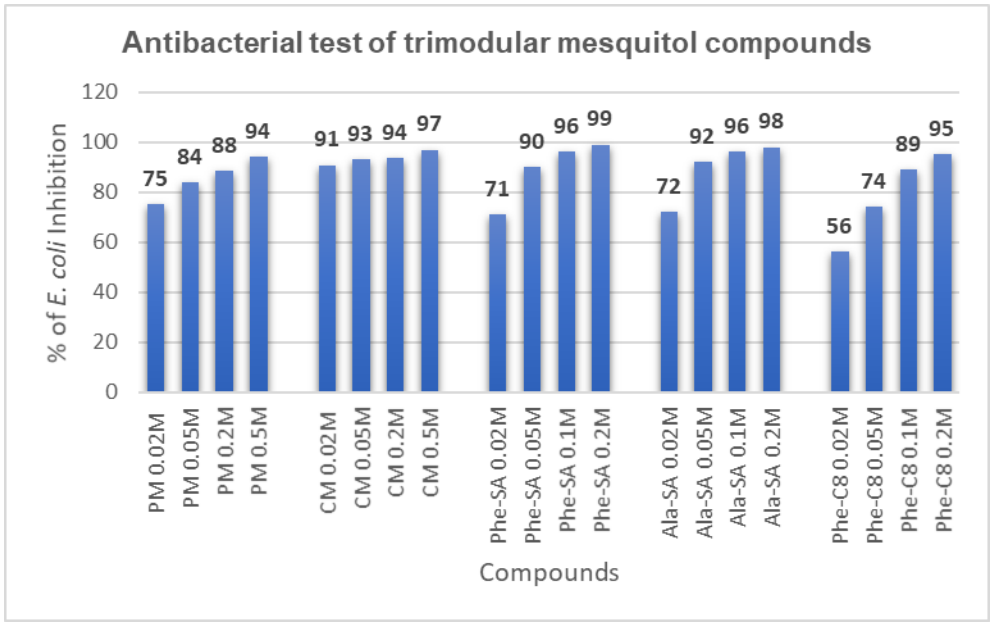


Fig 30: Antibacterial test of trimodular mesquitol compounds

The antibacterial results show that CM has got better antibacterial activity against *E. coli* than PM. For the Bimodular compounds, C6 and C8 have better results than C12, C14 and C16. C6 ester has still better results than PM for 0.02M and 0.05M concentrations. For the Trimodular compounds, all of them had very good results as the concentration increased from 0.02 M to 0.2 M. The hydrophilic trimodular compounds formed from SA had better results than hydrophobic trimodular compounds formed from C8. Thus, generally, derivatization of mesquitol has improved the antibacterial activities of mesquitol for the trimodular mesquitol compounds. These flavonoids which had good percentage inhibition of *E. coli* growth are targeting various cellular functions of the bacteria for a better antibacterial activity. They act on bacteria by damaging the cell membrane, inhibiting the synthesis of nucleic acids that are critical for survival and replication of bacteria, inhibiting synthesis of cell wall and interfering with energy metabolism (Gallegos et al., 2016; Liu et al., 2025). C6 ester and trimodular mesquitol compounds with better antibacterial activities on *E. coli* can thus play a key role in the development of antibacterial drugs (antibiotics), which are utilized in the treatment of bacterial infections related to the bacteria.

4.3.3 Gelling properties

Mesquitol and its derivatives were tested on their ability to act as gelling agents. Gelling agents have the ability to form gels when solubilized in a liquid phase as a colloidal mixture by forming a weakly cohesive internal structure. These agents possess gelling properties. The gelling properties of pure mesquitol, crude mesquitol and hemi-synthesized products are as shown in table 6 below and figure 31.

Table 6: Gelling properties of crude mesquitol, pure mesquitol and hemi-synthesized products at 4% in water, olive oil and ethyl oleate

Compounds	Water	Olive oil	Ethyl oleate
CM	P	P	P
PM	S	P	P
C6	P	S	S
C8	P	S	S
C12	OG	S	S
C14	OG	S	S
C16	OG	S	S
Phe-C8	P	P	P
Ala-SA	S	P	P
Phe-SA	P	P	P

P: precipitate, S: solution, OG: opaque gel



Fig 31: Opaque white gel for C12 ester (compound **[17]**) at 4%

Pure and crude mesquitol were not able to form gels in amongst the three chosen solvent (water, olive oil and ethyl oleate). Nevertheless, with improvement of hydrophobicity and increase in fatty acid chain length in the hemi-synthesized mesquitol, the ability to form gels existed from C12 (figure 31), C14 and C16 mesquitol esters at 4%. Further test of gelling properties at 2% showed that C14 and C16 still had good gelling properties as they formed

white opaque gels. C12, C14 and C16 gels are supramolecular gels that are formed from self-assembling actions of Low Molecular Weight Hydrogelators (LMWHs) via non-covalent interactions. They are "stimulus responsive" and reversible under the action of stimuli, such as change in PH, change in temperature, mechanical agitation, UV action, ultrasound effect or chemical changes. C12, C14 and C16 gelling properties are useful in cosmetics and pharmaceutical companies since they can help in controlling stability, texture, and drug delivery. In cosmetics, these gels can help in maintaining the structural integrity of formulations, thus hindering ingredient separation. In pharmaceuticals, such gels promote drug delivery through increasing the drug contact time with the absorption sites such as eyes, skin, nasal passages, etc (Kawee-ai, 2025; Liga & Paul, 2025; B. Sharma et al., 2018).

CHAPTER 5: ANTIFUNGAL PROPERTIES, SYNERGISTIC INTERACTIONS AND WOOD DURABILITY STUDY OF MESQUITOL, CATECHIN AND TEBUCONAZOLE

5.1 Bibliographic review of wood preservation and synergistic interaction of flavonoids

5.1.1 Wood preservation

Wood normally has many uses, which include renewable source of energy, furniture making, construction and paper production. This is because of its good qualities such as light weight, attractive aesthetics, processability, sustainability, easy machinability and good mechanical properties (Jones, 2017; Khademibami & Bobadilha, 2022; Olorunnisola, 2018). Different species of wood need to be maintained to increase their durability, nevertheless under unfavorable conditions, these woods are easily destroyed. It has been studied that the free OH groups in wood can absorb water thus this increases the moisture content making them susceptible to biotic attack. The biotic attack include attack by microorganisms, such as fungi, bacteria and insects (Fassina Brocco et al., 2025). The wood cell wall comprises of bio-synthesized cellulose fibrils fixed in lignin and hemicellulose matrix. Because of the definite chemical structure of wood, its unprotected application in an environment with high moisture content leads to cell wall biodegradation by fungi, insects and bacteria that further causes wood degradation. Thus, affected special species of wood that are considered non-durable should be treated or preserved to increase their durability. Wood durability can be enhanced via wood protection techniques that comprise of wood preservatives and modification systems (Khademibami & Bobadilha, 2022; Ramage et al., 2017; Thomasson et al., 2015; Zabel & Morrell, 2020).

Wood protection ought to be very safe to user, cost-effective, efficient and permanent. It should not be able to corrode any metal or even degrade the components of wood (Khademibami & Bobadilha, 2022). The low wood durability restricts its usage particularly in outdoor fields. Making durable wood timber through modification of cell wall and chemical treatment is a common means of protecting wood from biotic attack (Rowell, 2006). Anhydrides, acid chlorides, carboxylic acids, isocyanides, aldehydes, epoxides and graft polymerization methods have been utilized for improvement of the wood properties. These are chemical treatments, which aim at making covalent bonding between the cell wall polymers and related chemical used or forming cross-links. The applied chemicals react with the OH groups as well as the phenyl groups of the polymers of cell wall, thus leading to the OH groups blockage thus decrease in hygroscopicity. Mostly, chemical modification produces a permanent molecular structure change and results to dimensional stability and good durability of wood without loss of strength when compared to wood treatment via thermal modification. This method of

modifications helps in increasing wood hydrophobicity thus lowering humidity and water uptake (Y. Li et al., 2012; Ramage et al., 2017). Chemical modification of wood has got some environmental concerns linked to the chemicals used in the process (Costa et al., 2023).

Wood protection can be done through the use of various wood preservatives. The main purpose of wood preservatives is to decrease the number of wood products replacements and lower the rate of repairs of wood. Different preservatives used in protection of wood include oil borne preservatives, water borne preservatives, wood coatings and natural wood preservatives (Khademibami & Bobadilha, 2022). Oil borne preservatives, such as creosote and pentachlorophenol (PCP), have been used widely for protection of timber bridges and poles in Europe and North America. However, their usage has been restricted due to their disposal concerns to the environment. Any current research on oil borne preservatives should focus on best ways to manage their disposal, carry out recycling and also remediation of preserved woods. Water borne preservatives have been in the market since 1950s and they are safe, cost effective and provide a very clean surface of the treated wood. They can be used in utility poles, timber and residential lumber. They include arsenical, non-arsenical copper, as well as non-metal preservatives like borates. The disadvantages of these wood borne preservatives is their potential for timber to swell because of moisture content, thus requires re-drying after treatment, and leachability issue of the treatment ingredients with time. The other method for wood preservation is through coating, which involves painting the surface of wood material to supply a physical barrier against degradation and weathering (Ramage et al., 2017). Even though wood coatings are not planned to protect wood from wood decay, in some of their formulations there are biocidal components that can inhibit growth of fungi (Khademibami & Bobadilha, 2022).

Another wood preservation method is filling the micro-voids and nano-voids of cell walls that helps in limiting the entry of humidity into the wood cell walls. Thus, probable fungi attack can be controlled, since fungi can only grow in the wood at specific levels of humidity. However, sometimes air entrapment or incomplete wetting during the process can result to residual pores and voids, and this can lower the interfacial strength of the wood material and lead to more weak points (Ermeydan et al., 2014; Mehdikhani et al., 2019). There is also wood preservation using organic pesticides, which include triazoles, such as tebuconazole and propiconazole. However, these synthetic preservatives are considered toxic and highly affected by leaching (Dong, 2024; Khademibami & Bobadilha, 2022).

Most of the above-mentioned preservatives have been noted to be harmful to the environment and human health, and thus it explains why currently many studies have considered the use of natural wood preservatives, as a viable alternative for wood preservation. There is a continuing need of different non-toxic wood preservatives to help in improving wood protection technology by replacing different synthetic as well as inorganic compounds with natural wood preservatives. The use of environmentally friendly preservative has lately appeared to be a common suitable option for many wood preservation industries as it promotes greener chemistry. Natural wood preservatives are plant extractives that contain polyphenolic compounds, such as flavonoid, that are obtained mainly from the heartwood part of the plant (González-Laredo et al., 2015; Joseleau et al., 2022; Khademibami & Bobadilha, 2022). It has been noted that the natural protection of wood against wood degrading organisms, such as *Trametes versicolor* and *Poria placenta*, is mainly attributed to the type and quantity of plant extractives used, particularly the ones that are present in the heartwood part of the plant (Fassina Brocco et al., 2025). These wood preservatives are eco-friendly and help in improving wood properties such as water repellency, thus they lower the rate of leaching (Keržič et al., 2024).

Natural wood preservatives contain flavonoids that are known to significantly affect the quality and characteristic of wood, particularly durability and color of the wood (Kirker et al., 2024; Nascimento et al., 2013). Flavonoids are basically natural antioxidants through their ability to scavenge for free radicals. It is this property that hugely leads to their natural wood preservation and wood durability through protecting the wood against fungal attack. Flavonoids can help in donating hydrogen atoms and/or electrons that are used in neutralizing harmful free radicals and ROS, thus stopping oxidative damage to tissues and cells. Flavonoids can also act by binding to metal ions (such as copper and iron) that help in catalyzing the release of free radicals, therefore hindering metal-induced oxidative stress. In other cases, flavonoids can hinder the enzymatic activity that produce the reactive species, further lowering the production of ROS (Anouhe et al., 2018; Pietarinen et al., 2006). According to Schultz and Nicholas (2002), the combination of organic biocide with antioxidants or metal chelating compounds improves protection against fungi and other microorganism. For example, combining tebuconazole and flavonoids, such as mesquitol or catechin, can promote the development of wood preservative substances, which are environmentally friendly (Goodell et al., 2003; Schultz & Nicholas, 2002).

5.1.2 Synergistic interactions of flavonoids

Flavonoids display synergistic interactions with many other compounds, thus promoting their biological activities. Such interactions can result to better antioxidant properties, enhanced antimicrobial activities, as well as improved therapeutic benefits in treatment of cancer and other illnesses. Flavonoids interact in three different ways, namely synergistically, antagonistically or additively. Synergistic interaction implies that the combined effect of the phytochemicals involved is greater than the summation of their individual effect. Antagonistic interaction implies that the combined effect of the phytochemicals is less compared to the sum of their individual effects. Additive interactions means that the combined effect is equal to the sum of the individual effects (Bittick, 2017; Chaachouay, 2025; Vaou et al., 2022). Considering the three ways, it has been studied that plant flavonoids can interact synergistically with various antibiotics or fungicides to improve their activities. Since flavonoids have got good antioxidant properties that improves their antimicrobial activities, they can thus be used to decrease the quantity of harmful synthetic biocides like tebuconazole through synergy when combined together to be used as wood preservatives (Ons et al., 2020).

A study on a combination of flavonoids with aminoglycoside antibiotics or with β-lactam antibiotics shows that synergy promotes lower antibiotic dosages, thus possibly lowering the risk of development of antibiotic resistance (Donadio et al., 2021; Liu et al., 2025). In other studies, flavonoids from the plant extracts can be mixed with synthetic fungicides to improve their functionality. This synergy can principally be used in fighting drug-resistant fungal strains or lowering the complete toxicity of treatments (Da Silva et al., 2014; Redondo-Blanco et al., 2020). There are different mechanisms of flavonoid synergism with synthetic fungicides. They include targeting different pathways of fungi, promoting uptake of fungicide, overcoming resistance and lowering of toxicity levels. A number of flavonoids can act by disrupting cell membrane whereas synthetic fungicides such as azoles act by targeting ergosterol synthesis. A combination of these diverse approaches can result to an enhanced and comprehensive range of antifungal activities (Cebeci et al., 2024; Rodríguez et al., 2023; K. Wang et al., 2023). Thus, flavonoids can hypothetically increase the penetrability of fungal cell membranes, permitting for more uptake of the synthetic fungicide.

Some fungal strains have been studied to become resistant to synthetic fungicides, however the introduction of flavonoids helps them bypass such resistance mechanisms, thus possibly

reinstating the fungicide efficacy(Dantas et al., 2025; Seleem et al., 2017). By making use of a combination of plant flavonoid extracts with synthetic fungicides, such as tebuconazole, it can be possible to lower the overall dose of the required synthetic fungicide, therefore reducing possible side effects (Matrose et al., 2021). From different studies, it has been noted that flavonoids, such as catechin, epigallocatechin gallate and quercetin, when mixed with fluconazole, a synthetic fungicide, can enhance morphological changes and apoptosis in fluconazole-resistant *Candida tropicalis* strains (Da Silva et al., 2014). Eugenol, a flavonoid, has also displayed good synergistic effects with itraconazole, a fungicide, when tested against a dermatophyte, known as *Trichophyton mentagrophytes*, thus possibly lowering the essential itraconazole dosage (Malakootikhah et al., 2025; Rajčević et al., 2022). Studies have similarly exhibited that mixing various plant extracts can result to very good synergistic antifungal properties (Donkor et al., 2023) (Nahar et al., 2024; Rodríguez et al., 2023).

5.2 Antifungal properties of mesquitol, catechin and tebuconazole

Antifungal properties are the capacities of certain compounds, such as mesquitol and its derivatives, to inhibit fungal growth and control fungal pathogens, by showing their efficacy against them. The fungi used in the study were TV and PP fungi. In this research, the growth inhibition test of the mentioned fungi was carried out on crude mesquitol (CM), pure mesquitol (PM), catechin (K), compound **[17]**, tebuconazole (T) and synergistic activities assessed on mixtures of tebuconazole with different extractives and mixtures of catechin with pure mesquitol.

5.2.1 Crude mesquitol, pure mesquitol, catechin and compound [17] growth inhibition of fungi

CM, PM, C12 and K had good antifungal activity as the concentration increased from 100 ppm to 10000 ppm. This can be seen as their percentage antifungal index increased from a lower concentration to a higher concentration (figure 32, figure 33, figure 34 and figure 35).

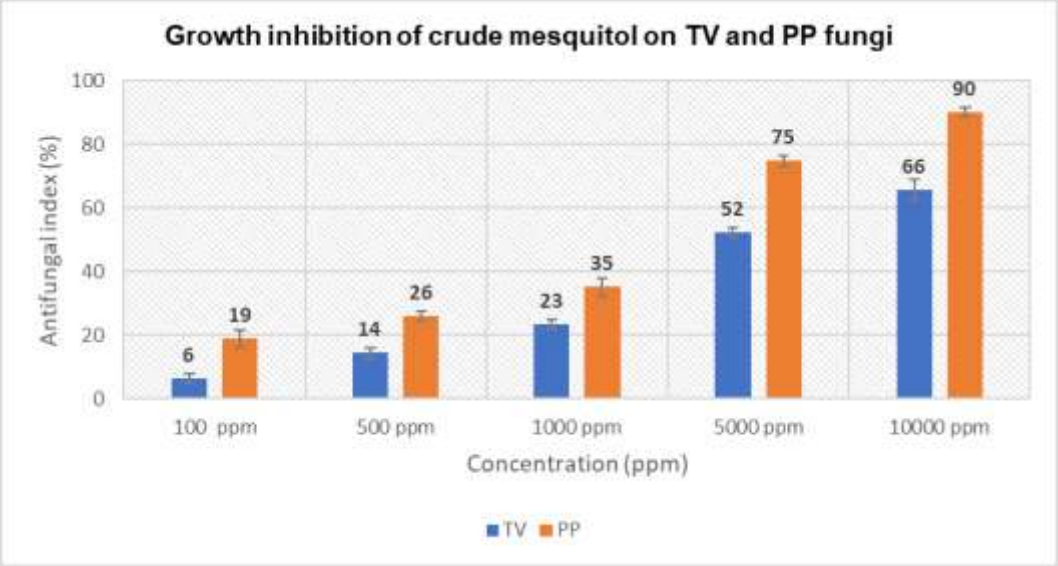


Fig 32: Fungal growth inhibition test of crude mesquitol

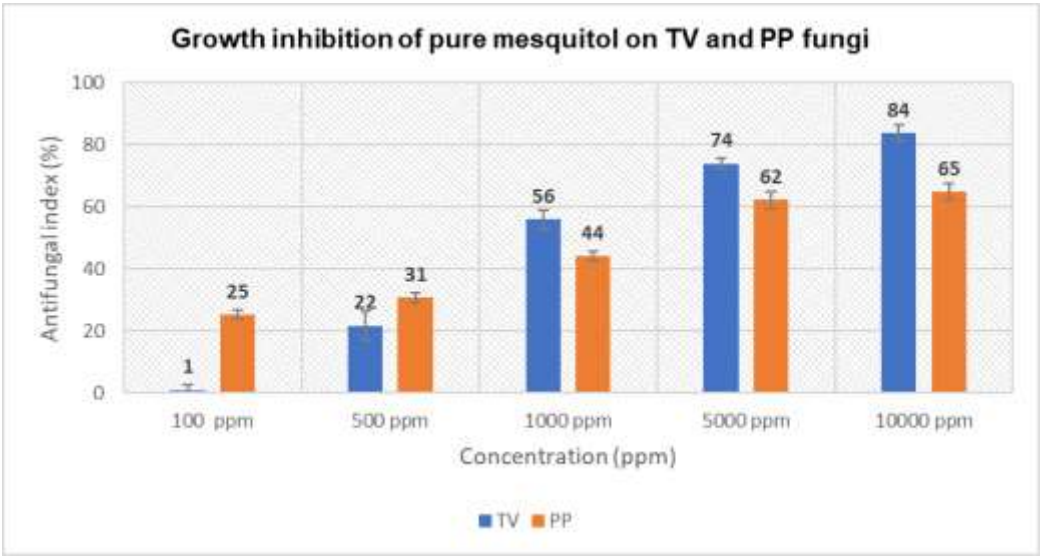


Fig 33: Fungal growth inhibition test of pure mesquitol

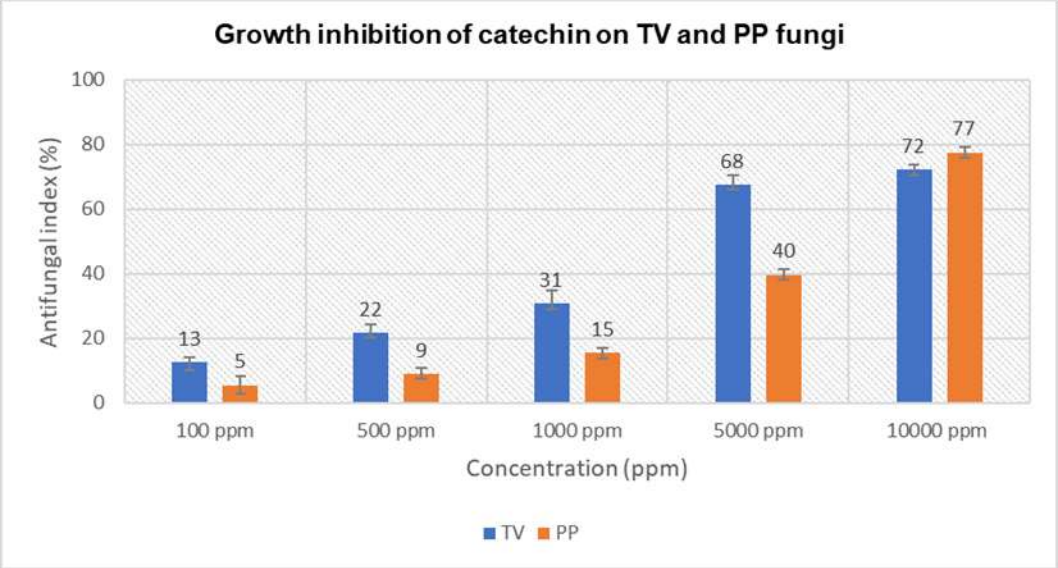


Fig 34: Fungal growth inhibition test of catechin

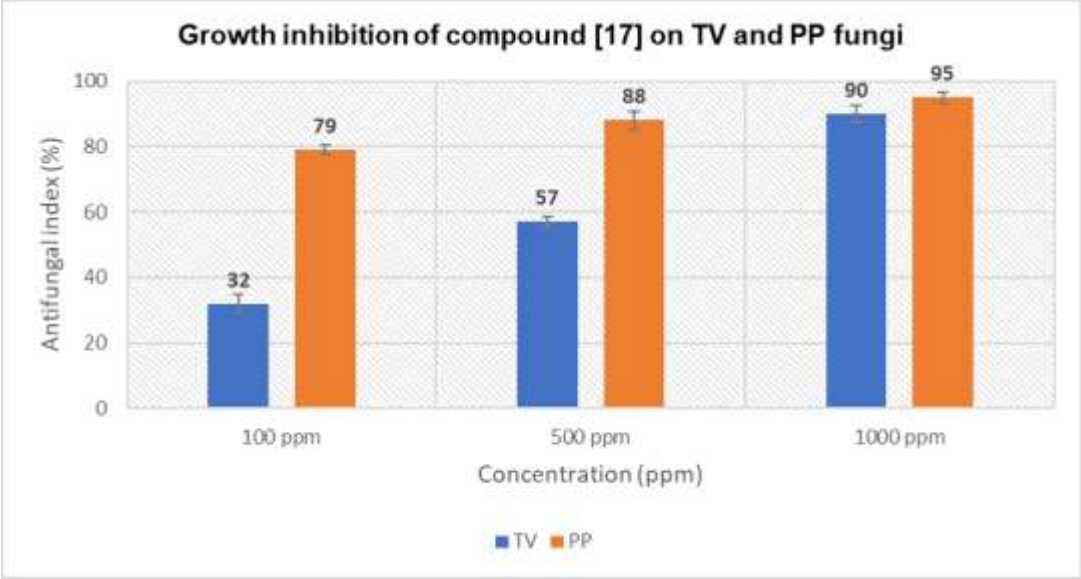


Fig 35: Fungal growth inhibition test of compound **[17]**

The results in figures 32-35 show that antifungal activity of the samples increased as the concentrations increased. Taking into account the type of fungus used and concentration, PM is more effective for TV fungi while CM is more efficient for PP fungi. Catechin is very efficient for TV up to 5000 ppm, but at 10000 ppm, catechin becomes very effective for PP fungi. Catechin is a well-known flavan-3-ol, which has good antifungal activity; hence, it is used a reference natural fungicide. Compound **[17]** had better growth inhibition results compared to the results of

pure mesquitol at the same concentrations of 100 ppm, 500 ppm and 1000 ppm. According to the graphs in figures 32-34, the efficiency of the compounds relies on concentration and it requires nearly 5000 ppm of compounds to obtain 50% fungal growth inhibition.

5.2.2 Tebuconazole growth inhibition of fungi

Tebuconazole is referred to as triazole fungicide that is known for its protective, eradicant and curative effect on fungi affecting wood materials and plants. Mosty phytopathogenic fungi are primarily managed and controlled by recognized synthetic fungicides like tebuconazole. Tebuconazole is considered as a reference synthetic fungicide for the study. Several concentrations of tebuconazole were tested on the two fungi, TV and PP. Tebuconazole concentrations from 1000 ppm to 10 ppm had had 100% growth inhibition on the two fungi. However, at less than 10 ppm concentrations, the rate of growth inhibition reduced till 0.01 ppm, as seen in figure 36. To assess ability of tebuconazole forming synergy with other samples, its two concentrations (0.1 ppm and 0.5 ppm) were selected from the produced results.

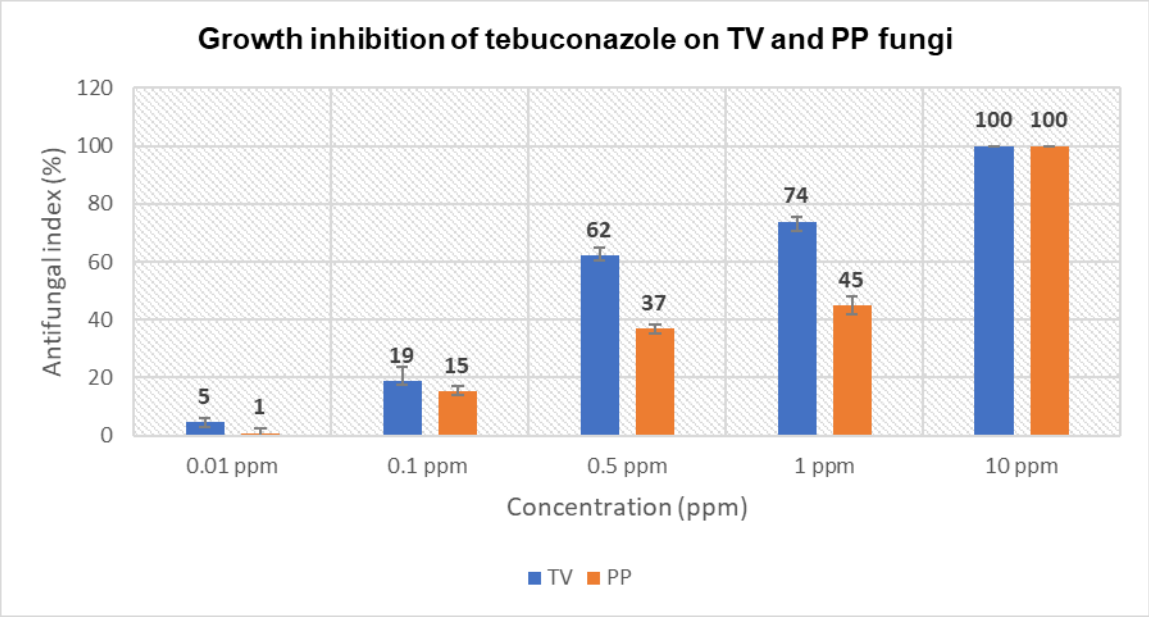


Fig 36: Fungal growth inhibition test of tebuconazole

5.2.3 Growth inhibition of mixtures of 0.1 ppm/0.5 ppm tebuconazole with 1000 ppm/5000 ppm samples on TV and PP fungi

Figure 37, below, displays the growth inhibition test results from combining 0.1 ppm and 0.5 ppm tebuconazole with 1000 ppm and 5000 ppm of the samples.

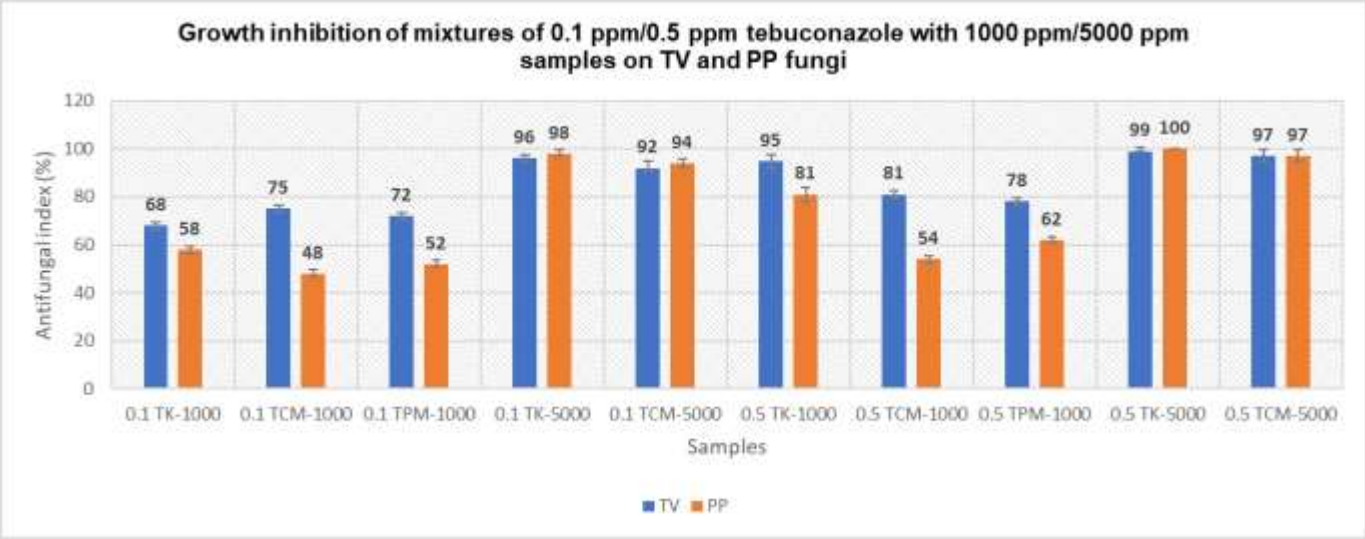


Fig 37: Fungal growth inhibition test of mixtures of 0.1 ppm/0.5 ppm tebuconazole with 1000 ppm/5000 ppm samples on TV and PP fungi [0.1 T = 0.1 ppm tebuconazole, 0.5 T = 0.5 ppm tebuconazole, K-1000 = 1000 ppm catechin, K-5000 = 5000 ppm catechin, CM-1000 = 1000 ppm crude mesquitol, CM-5000 = 5000 ppm crude mesquitol, PM-1000 = 1000 ppm pure mesquitol]

Synthetic fungicides like tebuconazole are considered very toxic to the users and thus they have harmful effects to the human health and environments. According to figure 37, combination of lower concentrations of the tebuconazole with natural fungicides from extractives can help solve the effect of synthetic fungicides. The mixed combinations of 0.1 ppm/0.5 ppm tebuconazole with catechin, crude mesquitol and pure mesquitol at different concentrations of 1000 ppm and 5000 ppm had much better growth inhibition activities as compared to their individual activities. It has been studied that plant extractives contain natural flavonoids that interact synergistically with the synthetic fungicides to form a new fungicide which are much safer and has low toxicity (Gérardin et al., 2023; Joseleau et al., 2022; Kirker et al., 2024).

5.2.4 Growth inhibition of equal mixtures of 100 ppm, 500 ppm and 1000 ppm catechin and pure mesquitol on TV and PP fungi

Figure 38, below, displays the growth inhibition test results from equal combination of 100 ppm, 500 ppm and 1000 ppm of catechin and pure mesquitol on the two fungi.

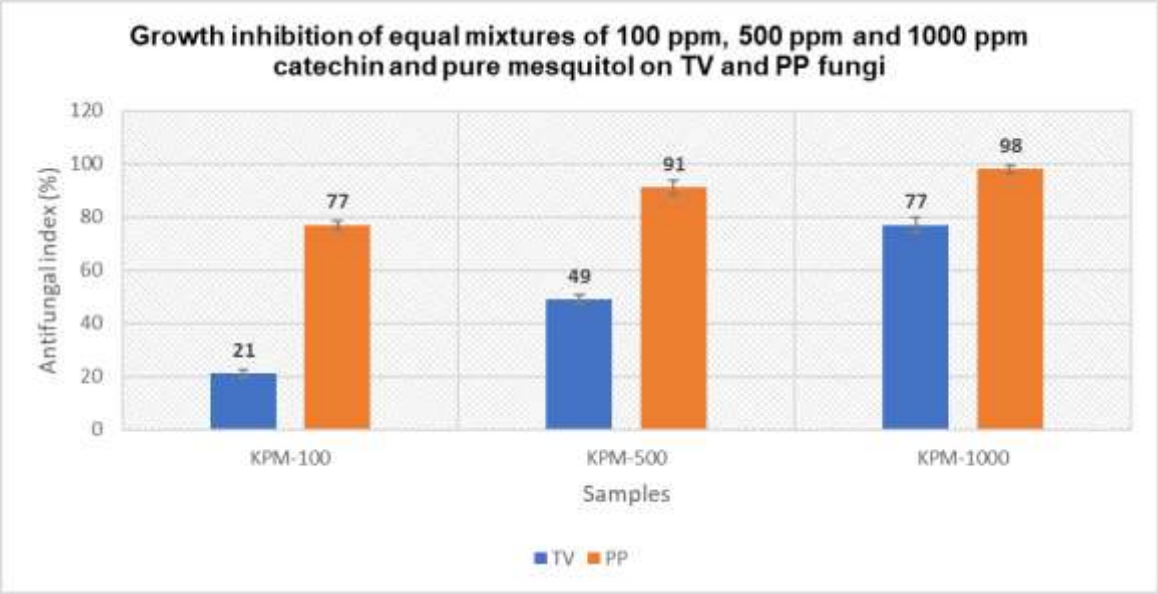


Fig 38: Fungal growth inhibition test of equal mixtures of 100 ppm, 500 ppm and 1000 ppm catechin and pure mesquitol on TV and PP fungi [KPM-100 = an equal mixture of 100 ppm catechin and 100 ppm pure mesquitol (1:1), KPM-500 = an equal mixture of 500 ppm catechin and 500 ppm pure mesquitol (1:1), KPM-1000 = an equal mixture of 100 ppm catechin and 100 ppm pure mesquitol (1:1)]

Plant extractives, such as catechin and mesquitol, are considered as natural fungicides. According to figure 38, combination of the two samples as concentration increases from 100 pm up to 1000 ppm improves the fungal growth inhibition, thus better antifungal activity as compared to the use of their individual extractives for the test. Using a mixture of natural fungicides can improve their efficacy through existence of multiple of modes of action, improved cellular stress to the microorganisms and enhanced bioavailability (Augostine & Avery, 2022; Vaou et al., 2022).

5.3 Synergistic, antagonistic and additive effect

According to Colby (1967), synergistic, additive and antagonistic responses for combinations of fungicides can be calculated through the use of an expected response formula. It is clarified in the paper that when the observed response, also known as the experimental response, is greater than expected response, then the final combination response of the fungicides is synergistic; nevertheless, when the observed response is less than the expected response, then the combination is considered as antagonistic. Additionally, if by any chance, the observed

response and the expected response are found to be equal, then the combination response is additive (Colby, 1967). The expected response formula is given as:

$$E = X + Y - \left(\frac{XY}{100}\right)$$

whereby E = expected response, X and Y = antifungal index of samples X and Y respectively.

For this study, tables 7 and 8, below, show the synergistic/additive/antagonistic responses, as calculated using Colby expected response formula. In the results from the two tables, the values of the observed responses and expected responses for the 0.5 TPM-1000 combination for TV fungi and 0.5 TCM-1000 and 0.5 TPM-1000 combinations for PP fungi are considered to be approximately equal; thus, their effect is more additive. There is one antagonistic combination between a lower concentration of 100 ppm catechin and pure mesquitol for TV fungi. All other combinations are synergistic.

Table 7: Synergistic, additive and antagonistic responses of varied combinations of tebuconazole, catechin, crude mesquitol and pure mesquitol on TV fungi

X sample	Y sample	Combination	E	O	Comparison	Sy/Ad/An effect
0.1 ppm T	1000 ppm K	0.1 TK-1000	44	68	O > E	Sy effect
0.1 ppm T	1000 ppm CM	0.1 TCM-1000	38	75	O > E	Sy effect
0.1 ppm T	1000 ppm PM	0.1 TPM-1000	64	72	O > E	Sy effect
0.5 ppm T	1000 ppm K	0.5 TK-1000	74	95	O > E	Sy effect
0.5 ppm T	1000 ppm CM	0.5 TCM-1000	71	81	O > E	Sy effect
0.5 ppm T	1000 ppm PM	0.5 TPM-1000	83	78	E ≈ O	Ad effect
0.1 ppm T	5000 ppm K	0.1 TK-5000	74	96	O > E	Sy effect
0.1 ppm T	5000 ppm CM	0.1 TCM-5000	61	92	O > E	Sy effect
0.5 ppm T	5000 ppm K	0.5 TK-5000	88	99	O > E	Sy effect
0.5 ppm T	5000 ppm CM	0.5 TCM-5000	82	97	O > E	Sy effect
100 ppm K	100 ppm PM	KPM-100	14	21	O>E	Sy effect
500 ppm K	500 ppm PM	KPM-500	39	49	O>E	Sy effect
1000 ppm K	1000 ppm PM	KPM-1000	70	77	O>E	Sy effect

Key: E = Expected response, O = Observed response, Sy = Synergistic, Ad = Additive, An = Antagonistic

Table 8: Synergistic, additive and antagonistic responses of varied combinations of tebuconazole, catechin, crude mesquitol and pure mesquitol on PP fungi

X sample	Y sample	Combination	E	O	Comparison	Sy/Ad/An effect
0.1 ppm T	1000 ppm K	0.1 TK-1000	28	58	O > E	Sy effect
0.1 ppm T	1000 ppm CM	0.1 TCM-1000	45	48	O > E	Sy effect
0.1 ppm T	1000 ppm PM	0.1 TPM-1000	52	52	E = O	Ad effect
0.5 ppm T	1000 ppm K	0.5 TK-1000	46	81	O > E	Sy effect
0.5 ppm T	1000 ppm CM	0.5 TCM-1000	59	54	E ≈ O	Ad effect
0.5 ppm T	1000 ppm PM	0.5 TPM-1000	65	62	E ≈ O	Ad effect
0.1 ppm T	5000 ppm K	0.1 TK-5000	49	98	O > E	Sy effect
0.1 ppm T	5000 ppm CM	0.1 TCM-5000	79	94	O > E	Sy effect
0.5 ppm T	5000 ppm K	0.5 TK-5000	62	100	O > E	Sy effect
0.5 ppm T	5000 ppm CM	0.5 TCM-5000	84	97	O > E	Sy effect
100 ppm K	100 ppm PM	KPM-100	29	77	O>E	Sy effect
500 ppm K	500 ppm PM	KPM-500	37	91	O>E	Sy effect
1000 ppm K	1000 ppm PM	KPM-1000	52	98	O>E	Sy effect

Key: E = Expected response, O = Observed response, Sy = Synergistic, Ad = Additive, An = Antagonistic

Tables 7 and 8 show that there exist various synergistic combinations of tebuconazole (synthetic fungicide) with catechin, crude mesquitol and pure mesquitol (natural fungicides) and just a few additive combinations. There also exists more synergistic combinations between natural fungicides, catechin and pure mesquitol, at varying concentrations of 100 ppm-1000 ppm. This is in agreement with literature studies that show that combination of the secondary metabolites, having polyphenols, with the fungicides produce a synergistic response with improved antifungal effect and less toxic products to the users (Gisi, 1996; Joseleau et al., 2022; Simonetti et al., 2020; Zhang et al., 2025). The combined mixtures of fungicide possess a broad spectrum of antifungal activities and therefore they are considered to offer better disease control when compared to their individual components. Such mixtures are similarly capable of lowering the risk of the fungi developing resistance, thus they can ultimately be used in provision of effective and lasting wood protectants (Gisi, 1996; Kirker et al., 2024).

5.4 Wood durability study

It is well known that wood is vulnerable to degradation by different microorganisms, such as white-rot and brown-rot fungi. Not several studies have explained the mechanisms of biochemical degradation of wood. A few papers explain peroxidases such as manganese peroxidase and lignin peroxidase in white rot fungi are the main cause of degradation of wood, as the enzymes help in breaking down lignin, which is known as the rigid polymer that is used in structuring wood (Mäkelä et al., 2021; Qi et al., 2023). In brown-rot fungi, hydroxyl radicals (OH) are known to be the main factors contributing to the wood degradation. These radicals are released by the fungi via processes such as the Fenton reaction, and they attack the wood, mainly cellulose as well as hemicellulose part of the wood, thus destroying its structure to permit the fungus to eat the wood (Hammel et al., 2002; Kaneko et al., 2005; Tornberg & Olsson, 2002). The phenolic antioxidants, obtained from mesquitol and its hemi-synthesized products, therefore act as hydrogen donor by blocking any chain oxidation reactions through functioning as the radicals' "trap." Phenolic antioxidants help in protecting the wood through releasing hydrogen atoms that further neutralize the free radicals coming from the fungi. This process disrupts the capacity of the fungi to carry out wood degradation. The phenolic compound changes to phenoxy radical, which is stable and less reactive. The phenoxy radical can thus terminate the free radical chain reaction, which might otherwise destroy the structure of the wood (S. C. Chepkwony, 2019; Gulcin, 2025; H. Fini et al., 2022).

The capability of mesquitol, catechin, tebuconazole and mixture of the samples to protect beech wood against the two fungi (TV and PP) were investigated. This study involved the wood impregnation test, leaching test and wood decay tests. The advantage of using the 15 treatments for this study is that they exhibit very good antioxidant properties, unlike the other widely used fungicides, thus they are likely to have increased biological properties through synergy with other fungicides. Figures 39, 40, 41 and 42 below shows the results for wood impregnation, leaching and wood decay tests.

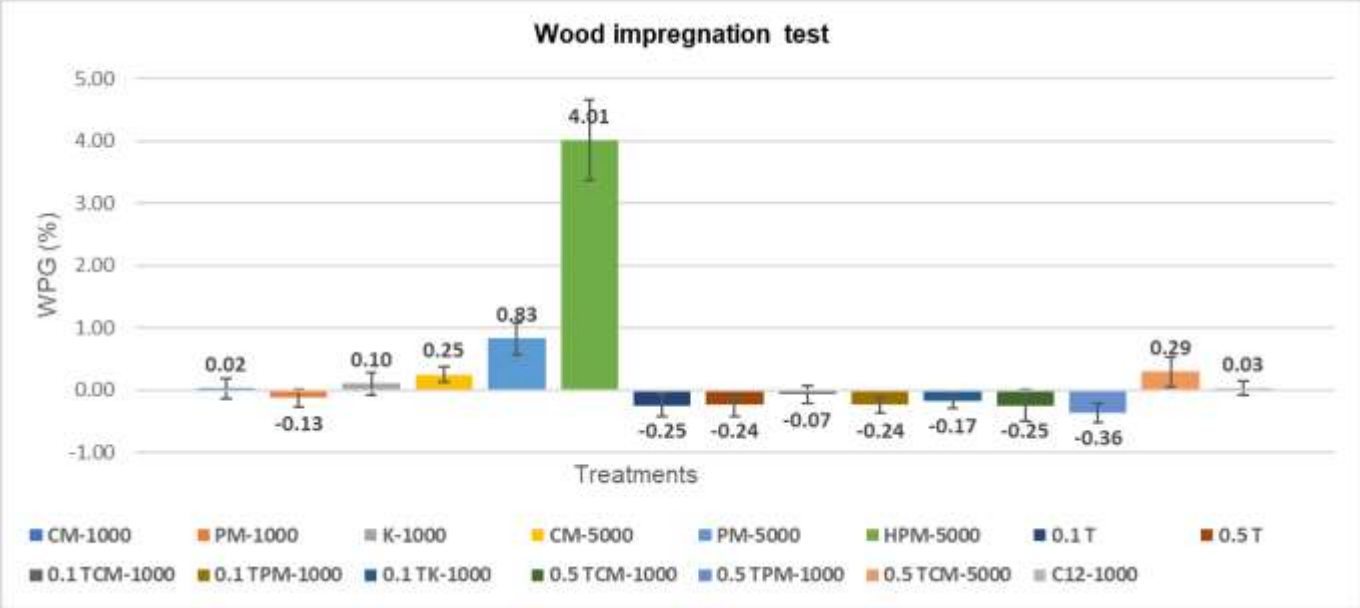


Fig 39: WPG (%) of the 15 treatments for wood impregnation test

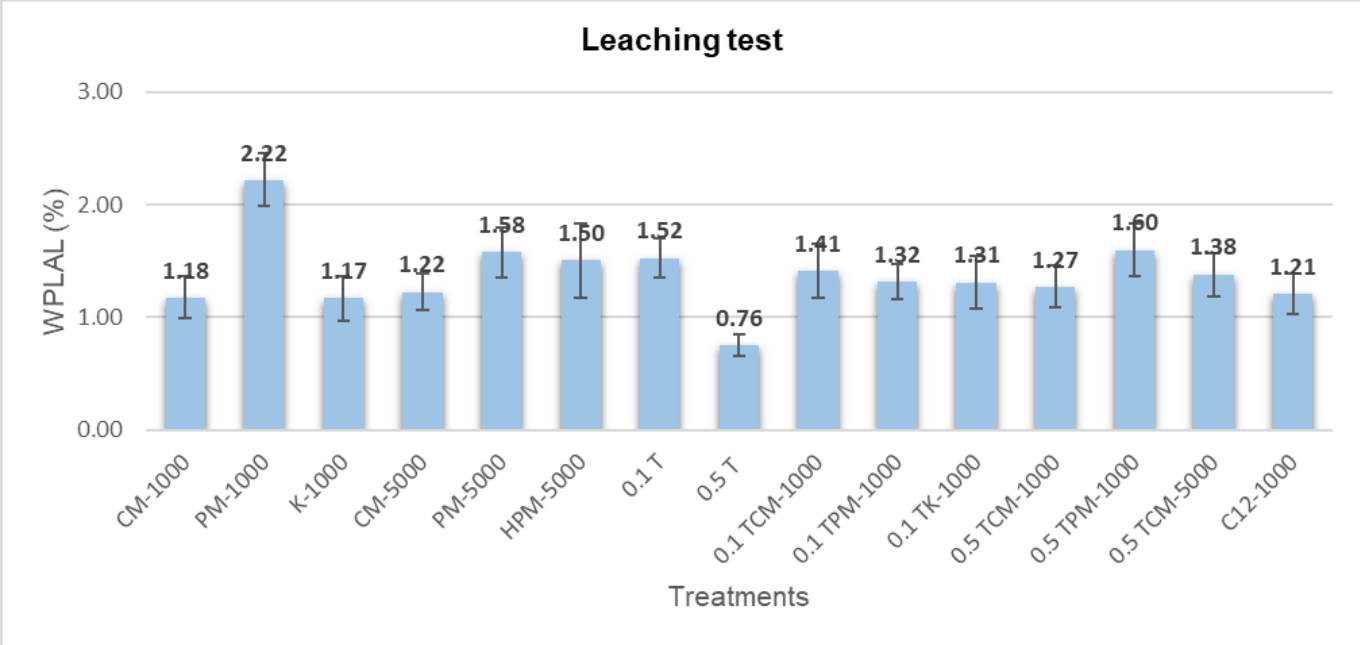


Fig 40: WPLAL (%) of the 15 treatments for leaching test

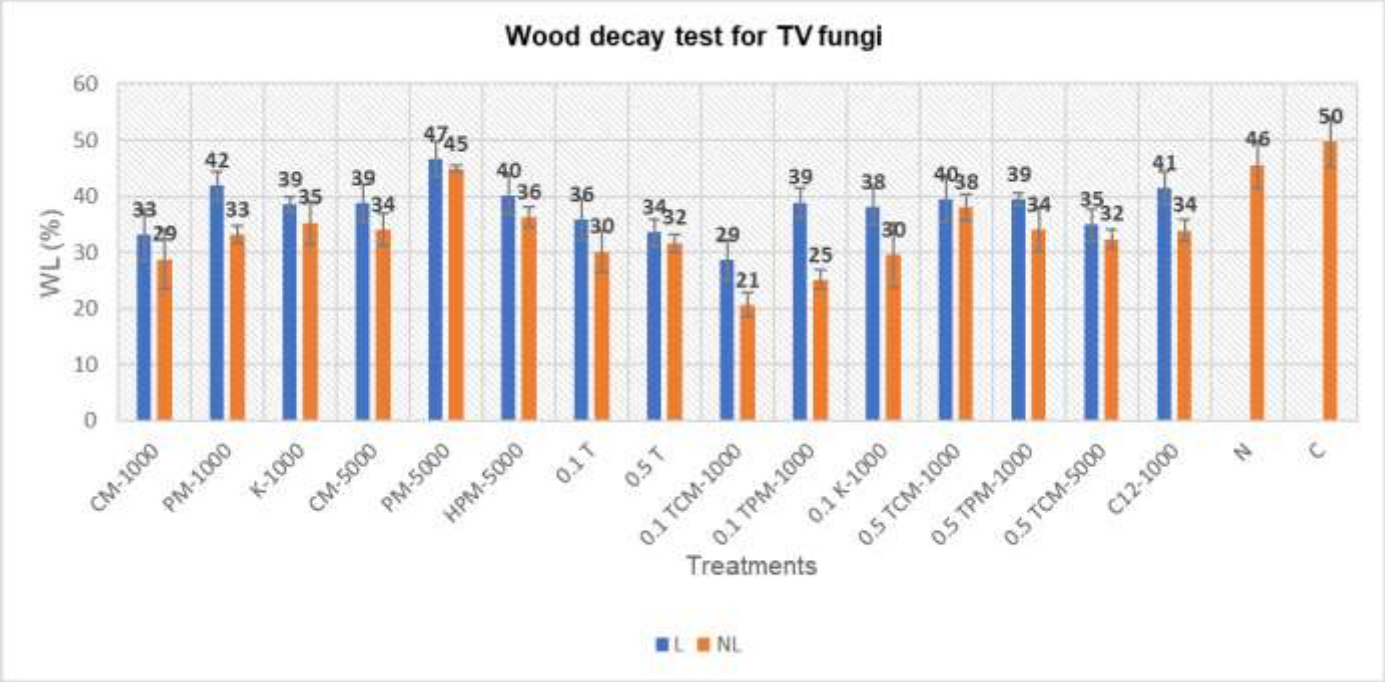


Fig 41: WL (%) of the wood decay test for TV fungi

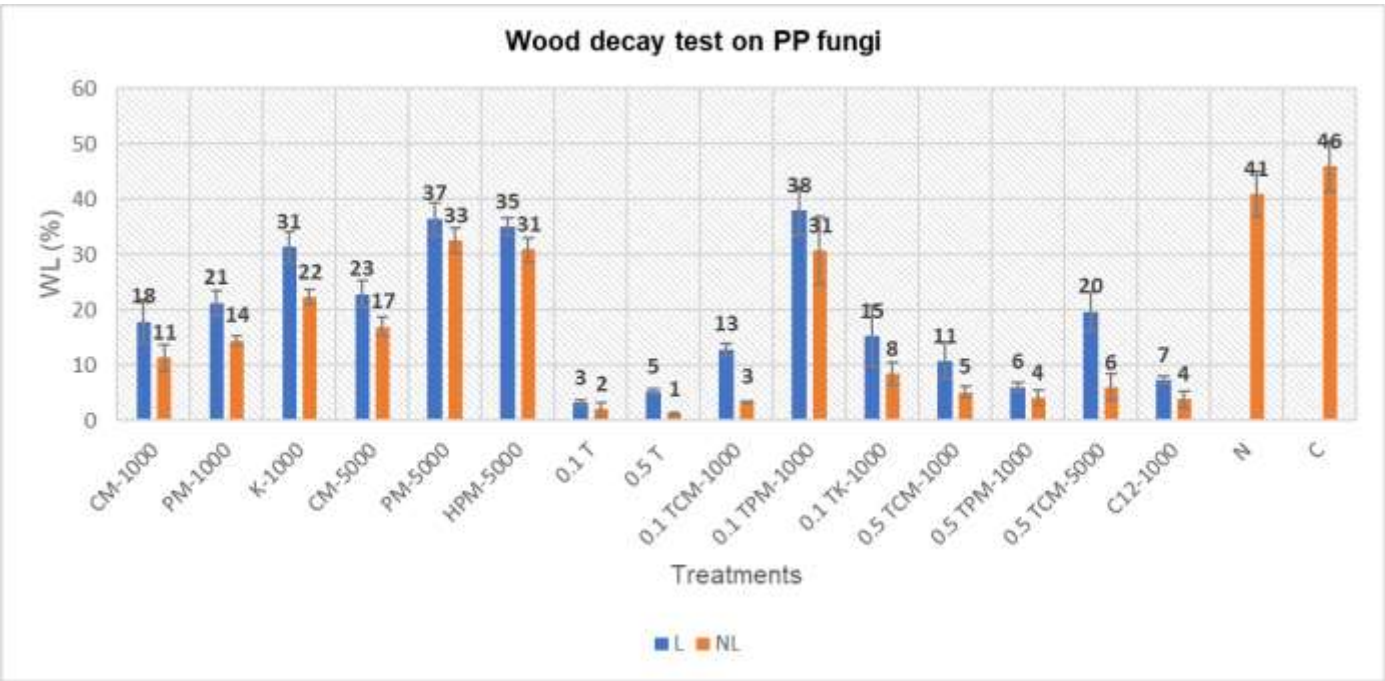


Fig 42: WL (%) of the wood decay test for PP fungi

There was significant WPG (%) for HPM-1000 in figure 39 compared to the rest of the treatments. This is because the 3% hydrogel provided enhancement in the properties of beech wood through creating strength to the wood. The hydrogels work through infiltration of the structure of the wood to help in filling cell walls and pores, thus providing a means of cross-linking the two in provision of support (Obounou-Akong et al., 2015; Z. Wang et al., 2024). There were better impregnation results for CM-5000, PM-5000 and 0.5 TCM-5000 compared to the rest. Considering figure 40, leaching was highest in PM-1000 compared to the other treatments. 0.1 T and 0.5 T treatments were also affected by leaching, though it does not reflect the actual leaching results of tebuconazole because of the solubility effect of tebuconazole in water during preparation of the concentrations. C12-1000 is a derivatized ester of mesquitol from lauric acid, thus it is lipophilic. This makes it more resistance to leaching compared to a hydrophilic mesquitol. That is why it has WPLAL (%) at 1.21 compared to that of mesquitol at 2.22.

No significant WL (%) was observed for beech wood blocks treated with 0.1 T and 0.5 T samples for PP fungi in figure 42. However, there was a significant WL (%) beech wood blocks treated with 0.1 T and 0.5 T samples for TV fungi in figure 41. In both figures, the leached wood blocks were highly affected by the fungi compared to the non-leached samples. The WL (%) got from mesquitol and its derivative showed that were not capable of effectively preventing wood degradation against the TV. The use of the mixtures with tebuconazole allowed very minimal weight loss differences due to the solubility issue of tebuconazole during the preparation stage. Nevertheless, the treatments were effective for the PP fungi as observed in figure 41. A few treatments had synergy for PP fungi according to Tallarida (2001). A synergistic mixed treatment occurs when the isoeffective concentration of the mixture is meaningfully lower than the ones of the individual components (Caesar & Cech, 2019; Tallarida, 2001). The derivatized mesquitol (C12-1000) had less wood degradation compared to PM-1000 and PM-5000 for the PP fungi. Overall, the 15 treatments on beech wood had better activity on PP fungi than TV fungi.

CONCLUSION ET RECOMMANDATIONS

1 Conclusion

Cette étude a utilisé deux techniques principales d'extraction du mesquitol, à savoir la méthode Soxhlet et la méthode de macération. La méthode Soxhlet a produit un rendement plus élevé de mesquitol, à 9,89 ± 0,442 %, par rapport à la méthode de macération, à 5,86 ± 0,264 % pour les extraits d'acétone. Les deux extraits contenaient du mesquitol brut qui a été purifié avec succès pour obtenir une quantité importante de mesquitol, soit 62,7 ± 0,47 % pour la méthode Soxhlet et 66,3 ± 1,53 % pour la méthode de macération. Le mesquitol produit à partir de ces deux méthodes a servi de matière première principale pour les réactions de semi-synthèse qui ont permis d'obtenir divers dérivés du mesquitol.

La modification chimique du mesquitol a conduit à l'introduction de différents groupes fonctionnels par la formation du composé **[15]**, du composé **[16]**, du composé **[17]**, du composé **[18]** et du composé **[19]** à partir de l'estérification avec des acides carboxyliques de différentes longueurs de chaîne, du composé **[20]** à partir d'une réaction avec un amide formé à partir d'un acide aminé (phénylalanine) et d'un acide carboxylique (acide caprylique), et des composés **[21]** et **[22]** à partir d'une réaction avec un amide formé à partir d'anhydride succinique et d'acides aminés (phénylalanine et alanine, respectivement).

Des tests physicochimiques et biologiques ont montré que le mesquitol et ses dérivés possèdent de très bonnes propriétés antioxydantes d'environ 5 µM. La fonctionnalisation du mesquitol a préservé ses propriétés antioxydantes tout en améliorant les propriétés antibactériennes des composés trimodulaires, à savoir le composé **[20]**, le composé **[21]** et le composé **[22]**. La fonctionnalisation du mesquitol a également amélioré les propriétés gélifiantes des composés bimodulaires, à savoir le composé **[17]**, le composé **[18]** et le composé **[19]** dans l'eau, ainsi que les propriétés antifongiques du composé **[17]**. La combinaison de 0,1 ppm et 0,5 ppm de tébuconazole, un fongicide synthétique, avec différents extraits à 1000 ppm et 5000 ppm (mesquitol, catéchine) a montré qu'il existe une synergie dans leurs activités d'inhibition de la croissance contre les champignons TV et PP. Il existait une synergie entre une combinaison de mesquitol et de catéchine à 100 ppm, 500 ppm et 1000 ppm. Ces résultats suggèrent que ces combinaisons peuvent être utilisées pour préparer de meilleurs produits de préservation du bois. Le test de durabilité du bois a montré que la plupart des traitements sont lessivables à l'eau, mais que la lipophilie des échantillons traités, comme dans le cas du C12-1000, réduit le lessivage des échantillons de bois de hêtre. L'utilisation des

mélanges avec du tébuconazole a permis de réduire au minimum les différences de perte de poids. La faible solubilité du tébuconazole a affecté les résultats de durabilité du bois, ce qui a entraîné des résultats moins synergiques dans diverses combinaisons. En résumé, les 15 traitements sur le bois de hêtre se sont avérés plus efficaces pour les champignons PP que pour les champignons TV.

2 Recommandations

Il est nécessaire de mener davantage d'études sur les dérivés du mesquitol provenant de l'OH phénolique, plutôt que de l'OH aliphatique. Cela permettra de comparer les propriétés physicochimiques et biologiques des composés issus des deux séries de dérivés. Étant donné que les propriétés antioxydantes proviennent des fragments catéchol du mesquitol, la modification chimique du mesquitol au niveau des positions OH phénoliques pourrait soit améliorer, soit réduire les propriétés antifongiques des dérivés.

D'autres études sur la dérivatisation du mesquitol par introduction de différents groupes fonctionnels, autres que l'acide carboxylique, les acides aminés et les anhydrides d'acides, pourraient également être menées.

L'étude recommande de réaliser des tests de durabilité du bois sur tous les autres composés bimodulaires et trimodulaires à base de mesquitoline afin d'évaluer leurs capacités de préservation du bois. Il conviendrait également de mener davantage d'études visant à améliorer les résultats en matière de durabilité du bois en résolvant le problème de solubilité du tébuconazole.

Enfin, il faudrait mener davantage d'activités physicochimiques et biologiques sur les dérivés du mesquitol afin de cibler diverses applications dans les secteurs de l'agriculture, des détergents, des cosmétiques et des produits pharmaceutiques.

CONCLUSION AND RECOMMENDATIONS

1 Conclusion

This research study used two main mesquitol extraction techniques namely, Soxhlet and maceration method. Soxhlet method produced higher yield of mesquitol at 9.89 ± 0.442% compared to that of maceration method at 5.86 ± 0.264% for acetone extracts. Both extracts contained crude mesquitol that successfully underwent purification to yield large scale amount of mesquitol at 62.7 ± 0.47% for Soxhlet and 66.3 ± 1.53% for maceration extraction method. Mesquitol produced from these two methods acted as the main starting material for hemi-synthesis reactions that produced various mesquitol derivatives.

The chemical modification of mesquitol led to the introduction of different functional groups through formation of compound **[15]**, compound **[16]**, compound **[17],** compound **[18]** and compound **[19]** from esterification with carboxylic acids of different chain lengths, compound **[20]** from a reaction with amide formed from an amino acid (phenylalanine) and a carboxylic acid (caprylic acid), and compound **[21]** and compound **[22]** from a reaction with amide formed from succinic anhydride and amino acids (phenylalanine and alanine, respectively).

Physicochemical and biological tests indicated that mesquitol and its derivatives have very good antioxidant properties of approximately 5 μM. Functionalization of mesquitol preserved the antioxidant properties but improved the antibacterial properties for trimodular compounds namely compound **[20]**, compound **[21]** and compound **[22**]. Functionalization of mesquitol also improved the gelling properties for bimodular compounds namely compound **[17]**, compound **[18]** and compound **[19]** in water and antifungal properties for compound **[17]**. Combination of 0.1 ppm and 0.5 ppm tebuconazole, a synthetic fungicide, with different 1000 ppm and 5000 ppm extractives (mesquitol, catechin) showed that there exists synergy in their growth inhibition activities against TV and PP fungi. There was existence of synergy between a combination of mesquitol and catechin at 100 ppm, 500 ppm and 1000 ppm. These results implied that the combinations can be used for preparation of better wood preservatives. Wood durability test showed that most of the treatments are leachable with water, however lipophilicity of the treatment samples, such as in C12-1000, lowers leaching of the beech wood samples. The use of the mixtures with tebuconazole allowed very minimal weight loss differences. Lower solubility of tebuconazole affected the wood durability results thus less synergistic results in various combinations. In summary, the 15 treatments on beech wood were more effective for the PP fungi as compared to the TV fungi.

2 Recommendations

There is need to carry out more studies on mesquitol derivatives from the phenolic OH, instead of aliphatic OH. This will help in comparison of the physicochemical and biological properties of the compounds from the two sets of derivatives. Considering the fact that the antioxidant properties are derived from the catechol moieties of mesquitol, chemical modification of mesquitol at the phenolic OH positions might either improve or lower the antifungal properties of the derivatives.

Further studies on mesquitol derivatization by introduction of different functional groups, apart from carboxylic acid, amino acids and acid anhydrides, could also be conducted.

The study recommends carrying out wood durability tests on all the other bimodular and trimodular mesquitol compounds to assess their wood preservation capabilities. There should also be more studies on improving on the wood durability results by solving the solubility issue of tebuconazole.

Finally, there should be more physicochemical and biological activities carried out on the mesquitol derivatives to target various applications in agriculture, detergent, cosmetic and pharmaceutical industries.

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RÉSUMÉ

Mots-clés: *Prosopis juliflora*, bois, flavonoïde, mesquitol, antioxydant, hémisynthésis

Prosopis juliflora a été introduit dans diverses zones arides du Kenya pour être une solution à la désertification et aux pénuries de bois de feu, mais il est maintenant devenu un arbuste très envahissant car il a déplacé de nombreuses plantes indigènes, et il a un impact à la fois sur les moyens de subsistance et la biodiversité. C'est pourquoi il y a eu beaucoup d'intérêt sur cette plante pour trouver un moyen de faire bon usage de son excès de bois. La composition phytochimique des plantes de *Prosopis juliflora* montre que des flavonoïdes sont présents qui ont divers effets biologiques observés tels que des activités antifongiques et des activités antioxydantes. D'après la littérature, une grande quantité de flavonoïde appelé mesquitol a été extraite, isolée et caractérisée à partir de la partie bois de cœur de la plante. Par conséquent, compte tenu des rapports de littérature, les plantes *Prosopis juliflora* ont un effet positif sur l'alimentation humaine ainsi que sur la santé générale des êtres humains.

La recherche sur *Prosopis juliflora* n'est pas nouvelle au LERMAB. Un premier programme de recherche doctorale mené sur les propriétés du bois par Peter Sirmah a mis en évidence les grandes quantités de mesquitol dans le bois de cœur de cette espèce. Un second programme de recherche mené par Sarah Chepkwony sur la variabilité intraspécifique du contenu et de la composition des extractifs a démontré que *P. juliflora* est riche en différents flavonoïdes parmi lesquels le mesquitol représentait les principaux composés indépendamment de l'âge du bois et des comtés où les arbres ont été récoltés. Par rapport aux flavan-3-ols classiques, des métabolites secondaires connus pour fournir des rôles antioxydants chez les plantes, on pense que le mesquitol pourrait agir comme un agent antioxydant plus fort en raison de ses deux fractions catécholes agissant comme des agents de piégeage des radicaux mais aussi comme des chélateurs métalliques limitant la réaction d'oxydation supplémentaire. Les grandes quantités de mesquitol présentes dans le bois de cœur en tant que principal produit chimique ont constitué l'occasion d'envisager des dérivés modifiés du mesquitol aux propriétés améliorées, qui pourraient être utilisés pour différentes applications. Des molécules lipophiles ou amphiphiles aux propriétés antioxydantes, antibactériennes ou antifongiques peuvent donc être envisagées pour des applications en tant que tensioactifs ou gélifiants dans les cosmétiques, les produits pharmaceutiques ou la protection du bois.

Dans ce contexte, la présente recherche s'appuie sur le mesquitol extrait de *P. juliflora* et sa fonctionnalisation par acylation pour modifier l'équilibre hydrophobe/hydrophile de la molécule et améliorer son activité biologique pour différentes applications. Ainsi, les objectifs du projet sont:

- Extraire et purifier le mesquitol à grande échelle de *Prosopis juliflora* afin de démontrer la possibilité d'obtenir du flavan-3-ol de haute pureté à partir de la plante.
- Développer une méthode de synthèse de dérivés fonctionnalisés du mesquitol afin d'améliorer et/ou d'augmenter les propriétés du mesquitol.
- Étudier les propriétés physico-chimiques et biologiques des différents composés obtenus à partir du mesquitol hémisynthésis.

ABSTRACT

Keywords: *Prosopis juliflora,* wood, flavonoid, mesquitol, antioxidant, hemi-synthesized

Prosopis juliflora was formerly introduced to various dryland areas in Kenya to be a solution to desertification and shortages of fuelwoods, but now it has become a highly invasive shrub as it has displaced many native plants, and it is impacting both livelihoods and biodiversity. This explains why there has been a lot of interest on this plant to find a way of making good use of its excess wood. The phytochemical composition of *Prosopis juliflora* plants show that flavonoids are present that have various observed biological effects such as antifungal activities and antioxidant activities. From literature, a large amount of flavonoid called mesquitol has been extracted, isolated and characterized from the heartwood part of the plant. Therefore, considering the literature reports, *Prosopis juliflora* plants have positive effect on the human diet as well as general health of human beings.

Research on *Prosopis juliflora* is not new in LERMAB. A first PhD research program carried out on wood properties by Peter Sirmah highlighted the high amounts of mesquitol in heartwood of this species. A second research program carried out by Sarah Chepkwony on the intraspecific variability of extractives content and composition demonstrated that *P. juliflora* is rich in different flavonoids among which mesquitol represented the main compounds independently of the age of wood and of the counties, where the trees were harvested. Compared to classical flavan-3-ols, secondary metabolites known to provide antioxidant roles in plants, it is believed that mesquitol could act are stronger antioxidant agent due to its two catechol moieties acting as radical scavenging agents but also as metal chelators limiting further oxidation reaction. The high quantities of mesquitol present in heartwood as main chemical constituted an opportunity to envisage modified mesquitol derivatives with improved properties, which could be used for different applications. Lipophilic or amphiphilic molecules with antioxidant, antibacterial or antifungal properties can therefore be envisaged for applications as surfactants or gelling agents in cosmetics, pharmaceuticals or wood protection.

In this context, the present research is based on mesquitol extracted from *P. juliflora* and its functionalization through acylation to modify the hydrophobic/hydrophilic balance of the molecule and improve its biological activity for different applications. Thus, the objectives of the project are:

- To extract and purify mesquitol in large scale from *Prosopis juliflora* to demonstrate the possibility of obtention of high purity flavan-3-ol from the plant.
- To develop a method for synthesis of functionalized mesquitol derivatives in order to improve and/or increase the properties of mesquitol.
- To study physicochemical and biological properties of the different compounds obtained from hemi-synthesized mesquitol.