MOLECULAR PHYLOGENY, PHYTOCHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITIES OF SELECTED KENYAN POPULATIONS OF DODONAEA VISCOSA (SAPINDACEAE)

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DECLARATION

Declaration by the Candidate

This thesis is my original work and has never been presented for the award of an academic degree in any other university and should not be copied, or reproduced in any format without written authority from the author and/or University of Eldoret.

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DEDICATION

I dedicate this thesis to my wife, Mrs. Faith Langat, my children Adriene, Aiden, and Abigael, for their patience, love, support, and prayers throughout the entire study, as I was often away. I will always adore you more than words can express. May God bless you abundantly.

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ABSTRACT

The family Sapindaceae is diverse, with four subfamilies. The genus *Dodonaea* (Sapindaceae) is endemic to Australia and widespread in Kenya in various habitats. There are two varieties found in Kenya. The taxonomic relationship between them is quite confusing. The two have many ethnomedicinal uses. Microbial organisms cause several diseases in plants and animals and with time, have developed resistance to most conventional drugs. The phytochemical components of plants haave specific pharmacological effects on the human body and exert their therapeutic value. Secondary metabolites isolated from plants are also important in chemotaxonomy. This study provided a phylogenetic analysis that included nucleotide frequency and substitution rates, base composition disparity index and evolutionary divergence. Eleven Kenyan populations of *Dodonaea viscosa* had their ribulose-bisphosphate carboxylase (*rbcL*) and internally transcribed spacer 1 (*ITS1*) genes extracted and Sanger sequenced. They were aligned by MUSCLE (codons) and exported to MEGA 11 and PhyloSuite for analysis. Phylogeny was constructed using ML and ITOL used to edit the cladograms. Prior to phylogenetic analysis a nucleotide BLAST of the sequence genes was done to identify the closest ancestor of *Dodonaea viscosa*. The sequences were also subjected to analysis by PCA. An analysis of phytochemical constituents of DCM: CH_3OH leaf extract was conducted on Gazi 1C population. The agar well diffusion method was used to determine the antimicrobial activity, while antidiabetic activity was carried out on Swiss albino rats. Data on antidiabetic activity were organized in excel tables and analyzed using ANOVA, and paired sample t-test. Graphical method was used to analyze data on antimicrobial activity. From the phylogenetic analyses, a narrow genetic distance exists amongst the Kenyan populations of *Dodonaea viscosa*. The PCA showed more variations in the *Dodonaea* populations studied using the *rbcL* gene compared to using the *ITS1* gene. Phytochemical analysis identified 4 compounds from the leaves, which supplemented the bulk of data for chemotaxonomic analysis. This study revealed that the crude extract of *Dodonaea viscosa* was antidiabetic as it lowered blood sugar in STZ induced diabetic rats. Likewise, the crude extract showed antibacterial and antifungal activities, as evidenced by the inhibition zones, except in *Escherichia coli*. The antidiabetic activity of the roots and leaves of various populations did not differ significantly, at P-value of >0.05. Variance between the populations was also not statistically different, as the P-value was 0.742. More antidiabetic activity was observed in the reference drug, followed by the plant extract at a dose of 400 mg/kg bwt, with the lowest activity at 200 mg/kg bwt. Similarly, more antimicrobial activity was observed from the positive controls (Amoxicillin and Apron), followed by the plant extracts. More studies targeting other gene areas with different primers are recommended to establish a full relationship between the *Dodonaea viscosa* Kenyan populations. There is a need for more bioassays on the compounds isolated from this study to determine the biological potential of this widely distributed plant.

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LIST OF ABBREVIATIONS AND ACRONYMS

^{13}C NMR	^{13} Carbon Nuclear Magnetic Resonance
^{1}H NMR	^{1} Hydrogen Nuclear Magnetic Resonance
ANOVA	Analysis of Variance
atpB	ATP synthase Subunit Beta
BIC	Bayesian Information Criterion
BLAST	Basic Local Alignment Search Tool
CD_2Cl_2	Deuterated Dichloromethane
CH_3OH	Methanol
cpDNA	Chloroplast DNA
CTAB	Cetyltrimethylammonium Bromide
DCM	Dichloromethane
DM	*Diabetes Mellitus*
DNA	Deoxyribonucleic Acid
EtOAc	Ethyl Acetate
ETS	External Transcribed Spacer
GC	Gas Chromatography
GC-MS	Gas Chromatography–Mass Spectrometry
HKY	Hasegawa-Kishino-Yano
ITS1	Internal Transcribed Spacer 1
ITS2	Internal Transcribed Spacer 2
IUPAC	International Union of Pure and Applied Chemistry
JC+G	Jukes-Cantor + Gamma

MEGA	Molecular Evolutionary Genetics Analysis
ML	Maximum Likelihood
MRSA	Methicillin-resistant *Staphylococcus aureus*
NCBI	National Center for Biotechnology Information
NJ	Neighbor-Joining
NMR	Nuclear Magnetic Resonance
nrDNA	Nuclear Ribosomal DNA
nrRNA	Nuclear Ribosomal RNA
OGTT	Oral Glucose Tolerance Test
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
P^H	Potential Hydrogen Concentration
rbcL	Ribulose Bisphosphate Carboxylase
rDNA	Ribosomal DNA
RNA	Ribonucleic Acid
rpm	Revolutions Per Minute
STZ	Streptozotocin
T1D	Type 1 *Diabetes mellitus*
T2D	Type 2 *Diabetes mellitus*
TLC	Thin Layer Chromatography
TM	Traditional Medicine
UPMGA	Unweighted Pair Group Method with Arithmetic Mean

UV	Ultraviolet Radiation
ZOI	Zones of Inhibition

CHAPTER ONE

INTRODUCTION

1.1 Background information

The Order Sapindales comprises nine economically important families, encompassing about 479 genera and with approximately seven thousand species (Joyce *et al*., 2023). The Sapindaceae is a varied family of shrubs, trees, and lianas that belongs to the order Sapindales and is subdivided into four subfamilies, which include Dodonaeoideae, Hippocastanoideae, Sapindioideae, and Xanthoceroideae (Muftaudeen *et al*., 2020). This family has approximately 1900 species distributed in 142 genera (Buerki *et al*., 2010; Adeyemi *et al*., 2013). The genus *Dodonaea*, a member of the subfamily Dodonaeoideae (Hari & Vigi, 2024), comprises of over 70 species (Christmas *et al*., 2018). *Dodonaea viscosa* (L.) Jacq (Sapindaceae), commonly referred to as 'hopbush' or 'sanatha', originated from Australia from its most current common ancestor in the Late Pliocene to Early Pleistocene and then separated into two independent, geographically based intraspecific lineages (Harrington & Gadek, 2009; Priyadi, 2021; Malik *et al*., 2022). This plant was named *Dodonaea* in honour of Rembert Dodoens, a 16th-century Flemish physician-cum-botanist, while its specific name *viscosa* was derived from a Latin word viscosus, which means sticky (Lawal *et al*., 2014).

Dodonaea viscosa is widely dispersed over tropical, subtropical, and warm-temperate climates in Mexico, Arizona, Florida, India, Africa, South America, Northern Mariana Islands, and the U.S. Virginia Islands (Anilreddy, 2009). It is the only species of *Dodonaea* distributed outside Australia and is amongst the world's most widely dispersed transoceanic

plants (Harrington, 2008; Muqaddas e*t al*., 2018). Among the seven subspecies, the subspecies *mucronata, angustissima, cuneata,* and *spatulata* are restricted to Australia, while the other subspecies, i.e *burmanniana, viscosa,* and *angustifolia* have ranges beyond Australia (Christmas *et al*., 2015). The subspecies *viscosa* grows along the Kenyan Coast, while subspecies *angustifolia* grows in Kenyan Uplands (Kaigongi, 2020).

Dodonaea viscosa is a tree or shrub that thrives in windy, drought-prone, and rocky or saline soils (West, 1984; Rani *et al*., 2009; Zeki *et al*., 2019). The plant may also live successfully in secondary forests and open spaces and propagated using seeds (Rani *et al*., 2009; Afzal *et al*., 2017). It has simple, alternating leaves that are either stipule-free or have very short petioles reaching a maximum of 2.5 mm in length. The leaves measure 3–9 cm in length, 0.5-2 cm in width, subacute to apiculate, glabrous, oblanceolate to spathulate, and subsessile. The leaf blade is either broad to narrowly elliptical or oblanceolate, with 15–20 frequently overlapping pairs of lateral veins or a noticeable midrib on both sides. The margins are smooth, both surfaces glabrous but glandular, and the leaves are coated (especially when young) with viscid glandular exudates (Lawal & Yunusa, 2013). The inconspicuous inflorescent blooms create colourful capsules containing their fruits, which have different wings (2-4) (Khan & Ismail, 2019; Kuswanto *et al.*, 2022). The plant is considered dioecious because it produces either male or female blooms on separate plants (Orpin, *et al*., 2018).

Dodonaea viscosa grows to a maximum height of seven metres in most diverse habitats where it is found (Anilreddy, 2009). A viscous yellow resin covers it. The pedicle is 4–8

mm long, while its flower is greenish yellow. The terminal panicles are approximately 3 cm in length. The puberulous, persistent, oblong, 3 mm long sepals are connate at the base and measure 3-5 cm. The flowers are bisexual or unisexual. In female flowers, six to eight stamens are free and rudimentary. The ovary is superior and rectangular, flattened, made of 2-3 cells, and has a 2-3 lobed style. The anthers are oblong, having a maximum length of 3 mm and up to a length of 2 mm in bisexual flowers. The annulus of the disc is cushion-shaped. The male flower is simple: a 2.2 mm long triquetrous ovary and a 3 mm long, minutely papillose style. Capsule dimensions are 12 to 14 mm long by 15 to 19 mm wide. The valves are two to four and may be green or maroon in colour, with wings on the back. The subglobose, black seeds have a length of 4 mm (Rani *et al.*, 2009; Muqaddas *et al*., 2018). A defining feature of *Dodonaea viscosa* is the presence of sclerenchymatous tissue surrounding the vascular tissues as a bundle sheath (Venkatesh *et al*., 2008). Very slight morphological variations were observed in the field between the two Kenyan subspecies as represented in Figure 1.1.

Figure 1.1 a and b: *Dodonaea viscosa* plant displaying leaves, stems, and flowers. Source: Photographs (a) and (b) taken by Philemon Langat at Kabarnet (Baringo) and Gazi (Coast) respectively in May 2023

The plant has been used by indigenous peoples worldwide for traditional medication to cure a wide range of diseases, orally or as a poultice (Patel & Coogan, 2008; Al-Snafi, 2017; Shiaka *et al*., 2019). It is prescribed traditionally to cure many conditions, including rheumatism, dermatitis, hemorrhoids, stomach pains, uterine colic pains, splenic or liver pains, issues with smooth muscles, and skin rashes as an antipruritic. Infusions prepared from the leaves were used to cure fractures, gout, and snakebites (Rao & Pratap, 2022). Compounds isolated from its leaves, and flowers have shown significant biological activities (Shafek *et al*., 2015; Al Habsi & Hossain, 2018). *Dodonaea viscosa* is a common medicinal plant in East Africa (Tessema *et al.*, 2023). It is frequently used in traditional

medicine to treat a wide range of illnesses, including dysmenorrhea, irregular menstruation, fever, colds, malaria, rheumatism, pains, toothaches, and migraines (Lawal *et al.*, 2014).

Pharmacological investigations have revealed that subspecies *viscosa* had actions that were anti-diabetic, insecticidal, antimicrobial, cytotoxic, anti-fertility, wound-healing, anti-inflammatory, analgesic, anti-ulcer, antispasmodic, anti-diarrheal, and detoxifying (Al-Snafi, 2017). It has been documented that the plant has been used traditionally as an antispasmodic, an anti-inflammatory (roots), an antipyretic, and an antibacterial agent (Al-Snafi, 2017; Aremu & Pendota, 2021). Leaf extracts have shown antihyperlipidemic and hepatoprotective activities in alloxan induced diabetic rabbits (Ahmad *et al*., 2012). Its leaf juice or paste has been used in treating cancer (Kewessa *et al*., 2015; Malik *et al*., 2022). According to an ethnobotanical research on the uses of Pakistani plants *Dodonaea viscosa* was used to alleviate nausea and as fuel (Ibrar *et al*., 2007). Kigen *et al.*, (2017), notes that respiratory diseases such as sore throat, influenza, cough, pain, and running nose are treated with a concoction made from the barks, leaves, twigs, and roots of *Dodonaea viscosa*. In Kenya, this plant is also used in brushing teeth, according to an ethnobotanical study of the plants of Marakwet (Kipkore *et al*., 2014).

Dodonaea viscosa extracts have been reported to have a powerful antimalaria activity as shown by their ability to prevent mice from becoming infected by *Plasmodium berghei* (Mengiste *et al*., 2012; Amelo *et al*., 2014). Leaf infusions are used to treat cold, influenza, and back pain (Van Wyk *et al*., 2008). *Dodonaea viscosa* when in synergy with the leaves of *Rhamnus prinoides,* and *Brucea antidysentrica* it exhibits wound healing activity, as

evidenced by a notable rate of wound contraction and a shortened epithelization duration. (Tessema & Molla, 2021). In Kenya, *Dodonaea angustifolia* leaf exudates gathered from Taita Hills, Voi, Ngong Forest, and Nairobi have also demonstrated radical scavenging and antiplasmodial qualities (Omosa *et al*., 2016).

There are numerous additional non-medical applications for *Dodonaea viscosa*. *Dodonaea viscosa* has been utilized by the Malayali tribes of India for handicrafts, dry and live hedge construction, and roofing for temporary houses (Kannan *et al*., 2016). According to an ethnobotanical study conducted on the Banda tribe of Pakistan, as the plant dries, it is used as firewood and animal feed (Murad *et al*., 2013). In Kenya, the Pokot people also use this plant as fodder and for construction purposes (Wigrup, 2006). Pastoralists from Baringo County, where this plant mainly grows, use it in house construction and fencing as it is resistant to infestation by mites (Vehrs & Heller, 2017). Ecologically, it is a pioneer species colonizing barren habitats (Groenendijk *et al*., 2005), controlling soil erosion (Liu *et al*., 2022), and also having allelopathic ability (Al-Jobori & Ali, 2014; Barkatullah & Muhammad, 2010). Its allelopathic ability is depicted by a lower plant abundance in its canopy (Sühs *et al*., 2025).

Even with the numerous medicinal activities and non-medicinal uses, of the Sapindaceae family, its taxonomic classification has historically been fraught with more challenges, originating from Radlkofer's early work of 1931–1934 and onwards (Meyer, 1976). Despite offering preliminary insights, early morphological taxonomies lacked an evolutionary foundation. Since then, molecular phylogenetics has shown that traditional

subfamilial and tribe classifications contain substantial degrees of polyphyly and paraphyly (Harrington *et al*., 2005; Buerki *et al*., 2009). For example, taxonomic revisions were required since taxa such as *Arytera, Cupaniopsis,* and *Haplocoelum* were found to be non-monophyletic (Buerki *et al*., 2010). *Dodonaea viscosa,* the plant of interest, has been rated to be a difficult species arising from its polymorphic nature (Harrington & Gadek, 2009; Christmas *et al*., 2018; Khan & Ismail, 2019).

The taxonomic status of the two Kenyan taxa (i.e, *Dodonaea viscosa* and *Dodonaea angustifolia*) has been quite confusing, with the two being considered by most taxonomists to be a subspecies (Christmas *et al*., 2015; Hossain, 2019; Priyadi, 2021), though this does not agree with their evolutionary history (Christmas *et al*., 2018). Other taxonomists consider them as varieties (Rani *et al*., 2009; Kaigongi *et al*., 2020), as synonymous (Tessema *et al*., 2023), or as distinct species (Beentje, 1994). The present classification of *Dodonaea viscosa* has been based only on morphology (Kaigongi *et al*., 2020; West, 1984), with current studies proposing molecular data to unravel its relationships (Kaigongi *et al*., 2020). The taxonomic difficulties of *Dodonaea viscosa* could be due to the many significant taxonomic revisions resulting in more confusion (Christmas *et al*., 2018; Kaigongi *et al*., 2020).

1.2 Statement of the problem

Kenyan populations of *Dodonaea viscosa* do not have a proper taxonomic delimitation. Because of their polymorphism, the coastal and highland types often confused with one another. The current taxonomic ambiguity results from different taxonomists using their polymorphism as justification for drawing species boundaries. Prior classification systems for this species relied solely on morphological characteristics, with scanty molecular research (Christmas *et al*., 2018). Insufficient research has been done on the phytochemistry of *Dodonaea viscosa* leaves. Most people today struggle to access high-quality, reasonably priced healthcare, especially in underdeveloped nations. Because the current healthcare system is insufficient to meet the needs of the populace, it is imperative to investigate alternative approaches to healthcare delivery (Patwardhan & Patwardhan, 2005). Numerous diseases in plants and animals are caused by microorganisms. They have also grown resistant to the majority of the drugs over time. Their resistance has necessitated more research to look into the biological activities and possibly create new medications from various plants. (Hancock *et al*., 2012). Since there is currently no cure for *diabetes mellitus* and numerous detrimental effects on patients, managing the condition has taken a toll on many people worldwide. Additionally, not enough research has been done on the synergy of chemicals extracted from *Dodonaea viscosa*'s crude ethanolic extracts.

1.3 Justification of the study

There have been limited molecular studies targeting the *ITS1* and *rbcL* regions in *Dodonaea viscosa* populations, with no studies on the Kenyan populations. The delimitation of several genera in the family Sapindaceae remains uncertain due to insufficient molecular data, and some African genera have been poorly understood or improperly placed (Acevedo-Rodríguez *et al*., 2011). In several communities, *Dodonaea viscosa* has significant therapeutic value while additional research supports its ecological and non-medicinal functions. This economically important and extensively dispersed plant's taxonomic uncertainty necessitated a thorough taxonomic investigation. One important source for the creation of traditional medicines are plants which have been used previously to cure diseases caused by viruses, fungus, and bacteria. The majority of people are turning to traditional herbal therapies for the treatment and management of numerous ailments, including diabetes, because allopathic (conventional) medicines are expensive and can have negative side effects on the patients (DeRuiter, 2003). Because of the existence of bioactive molecules that give them their biological activity, some plant-based drugs have the potential to treat specific ailments and are more reasonably priced.

Antimicrobial studies show that the antibacterial activity of *Dodonaea viscosa* has not been sufficiently studied (Khurram *et al*., 2009). Similarly, the antidiabetic activity of *Dodonaea viscosa* has not been thoroughly investigated. Nandakumar *et al.,* (2018) also noted that *Dodonaea viscosa* is a plant with various pharmacological effects, with relatively few research studies being done hence, more research is needed to fully understand its pharmacological potential. This could also provide avenues for developing novel drugs for

managing microbes and human diabetes, which have been a problem for many decades. Some compounds show more biological activity in synergy as they complement one another, thus the necessity of researching the crude extract's activity of *Dodonaea viscosa*.

Therefore, the outcome from this study will provide more molecular data that will aid in resolving the taxonomic confusion in the two Kenyan subspecies of *Dodonaea viscosa* by taking into account the DNA sequence data from *rbcL* and *ITS1* genes. Phytochemical data from the leaves of the collected population will also provide supplemental data that will aid in resolving their taxonomic challenges. Antimicrobial and hypoglycemic activities of the *Dodonaea viscosa* populations will be determined to justify its ethnobotanical uses.

1.4 Objectives of the study

1.4.1 Broad objective

To determine the phylogenetic relationships amongst Kenyan populations of *Dodonaea viscosa* using DNA sequences, isolate and characterize compounds in the leaves, and determination of the biological activities of its crude extract.

1.4.2 Specific objectives

To accomplish the broader goal, this study laid out the following specific objectives:

1. To establish phylogenetic relationships of the Kenyan populations of *Dodonaea viscosa* using molecular sequence data from the *ITS1* and *rbcL* genes.
2. To extract, isolate, and characterize phytochemicals in the leaves *of Dodonaea viscosa* using silica gel column chromatography.

3. To determine the biological (antimicrobial and hypoglycemic) activities of crude ethanol extracts of *Dodonaea viscosa* populations against different microbes and male albino rats.

1.5 Hypotheses

To achieve the above stated objectives, the following guiding hypotheses were developed:

HO1: Internal transcribed spacer (*ITS1*) and ribulose-1,5-bisphosphate carboxylase (*rbcL*) genes sequences do not differ among the eleven populations of *Dodonaea viscosa.*

HO2: The leaves of *Dodonaea viscosa* Gazi 1C population do not contain detectable secondary metabolites.

HO3: Extracts of various populations *Dodonaea viscosa* do not have any significant biological (antimicrobial and hypoglycemic) activities.

1.6 Scope of the study

This investigation examined the phylogenetics of Kenyan populations of *Dodonaea viscosa* collected from Baringo, Uasin Gishu, Elgeyo Marakwet, and Kwale Counties. Phytochemical analysis was done on the leaves of Gazi 1C (Kwale County) population. The biological activities of the different populations were also determined. This research was conducted for 23 months, from May 2023 to April 2025.

CHAPTER TWO

LITERATURE REVIEW

2.1 Botanical profile of *Dodonaea viscosa* (Sapindaceae)

2.1.1 Taxonomic classification of *Dodonaea viscosa*

The genus *Dodonaea* has been classified mainly based on its morphological attributes. Two varieties of *Dodonaea* (i.e, Variety *viscosa* and Variety *angustifolia*) have been described in Kenya, though it seems to disagree with the metabolomics study done on the different populations of *Dodonaea* (Kaigongi *et al*., 2020). Taxonomically, *Dodonaea viscosa* has been classified into the following taxa. Kingdom: Plantae, Subkingdom: Viridiplantae, Infrakingdom: Streptophyta, Superdivision: Embryophyta, Division: Tracheophyta, Subdivision: Spermatophytina, Class: Magnoliopsida, Sub-Class: Magnoliales, Superorder: Rosanae, Order: Sapindales, Family: Sapindaceae, Sub-family Dodonaeoideae, Genus: *Dodonaea*, and Species: *Dodonaea viscosa* (Lawal *et al*., 2014; Medina *et al.*, 2021; Rao & Pratap, 2022).

2.1.2 Common names of *Dodonaea viscosa*

There are a variety of English names, including Broadleaf hopbush, Thin leaf hopbush, Sticky hopbush, Wedge leaf hopbush, Soapwood, Switch sorrel, and Native hopbush. Amongst the Afrikaans, it is named Gansies and Kankerbos while in Arabic, it is referred to as Daidon, Dodanaia, Shath, and Dodonia. On the same note, the names Candlewood, Switchsorrel, Native hopbush, Soapwood, Wedge leaf hopbush, Sticky hopbush, Giant hopbush, and Narrow leaf hopbush, Broad leaf hopbush have been commonly used in

Australia. Similarly, Brazilians call it Vassourovermelho, Vassoura-do-campo, Faxina-vermelha, and Vassouravermelha, while Pakistanis name it as Sanatha. On a global perspective, Broadleaf hopbush, Florida hopbush, Giant hopbush, Hopshrub, and Sticky hopbush are some of the names used for *Dodonaea viscosa* (Al-Snafi, 2017).

2.2 Steps in studying Molecular phylogeny of Sapindaceae and other organisms

There are five main steps (Figure 2.1) to be followed in studying the molecular phylogeny of organisms, as outlined by Kokubugata *et al*., (2019). The choice of the right molecular marker should also be considered (Patwardhan, 2014), with different models of evolution used in performing phylogenetic analysis (Choudhuri, 2014).

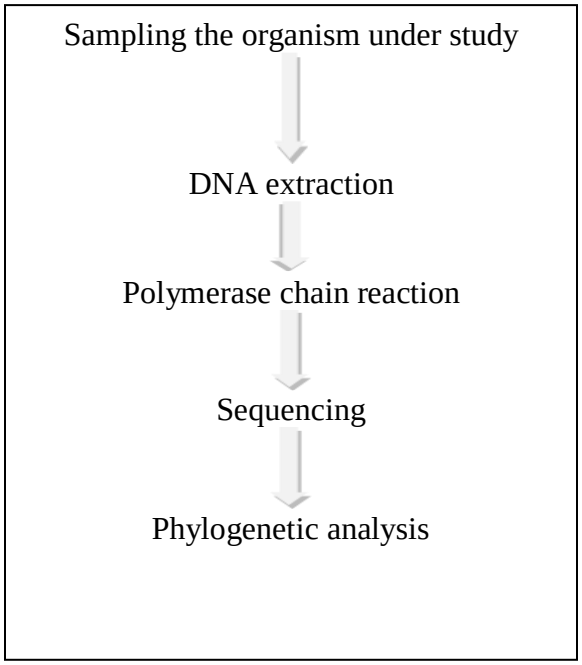

Figure 2.1: Steps in molecular phylogeny

2.3 Molecular markers and Plant genomes of taxonomic value

Due to their inherent stability, basic molecules of life such as DNA, RNA, and proteins have been employed as markers to identify organisms or evaluate the relationships amongst many organisms specifically (De & Bandyopadhyay, 2008). The most popular and suitable molecular markers for the majority of species are the DNA (Joshi *et al*., 1999). Both the nuclear and non-nuclear genomes of cell organelles, such as mitochondria and chloroplasts/plastids, contain plant genomes of taxonomic significance (Heslop-Harrison & Schwarzacher, 2011).

The nuclear genomes of plants exhibit structural differences in terms of the number of chromosomes, size, gene number and order, and number of genome copies per nucleus (Kellogg & Bennetzen, 2004). One of the most often used DNA markers in plant phylogenetic and DNA barcoding research is the internal transcribed spacer (*ITS*) of nuclear ribosomal DNA (nrDNA). It has been established that the *ITS* is a fundamental plant DNA barcode (Cheng *et al*., 2016). The ITS region of the 18S–26S nuclear ribosomal DNA has been found to be a valuable source of features for phylogenetic analysis in a number of angiosperm families. The two spacers in this region, *ITS1* and *ITS2*, are readily amplified by PCR and sequenced using well-known universal primers, even from the DNA of dried herbarium specimens. For phylogenetic relationships at a much lower level, such those between closely related species, the *ITS* sequencing data has also been helpful (Baldwin *et al.*, 1995). Figure 2.2 shows the different *ITS* sections of nuclear ribosomal RNA.

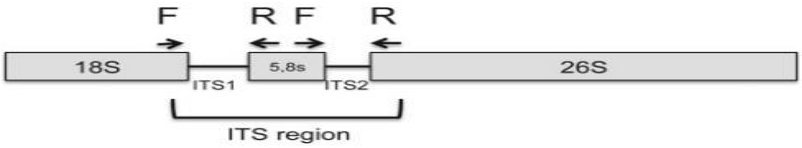

Figure 2.2: ITS region of nuclear ribosomal RNA and position of forward and reverse primers

The genomes of angiosperm chloroplasts (cp) are circular DNA molecules that range in size from 115 to 165 kb. A large inverted repeat (IR) region, which is separated into large-single-copy (LSC) and small-single-copy (SSC) portions, is present in two copies in these genomes (Wicke *et al*., 2011). The chloroplast genome regions *rbcL, matK, trnL,* and *trnH-psbA* are the most frequently used as plant DNA barcodes (Bell *et al*., 2016). Despite being small (~450 bp), the plastid intergenic spacer *trnH-psbA* is the most variable plastid region in angiosperms and may be easily amplified in a wide range of land plants (Kress *et al*., 2005). Data on chloroplast DNA sequences have been used for identification of plants, barcoding them, and determining the genetic relationships between different plant species (Nock *et al*., 2011).

Plant mitochondrial (mt) genomes are the largest; in angiosperms, they range between 200 and 700 kb, and in some species, they can grow to megabases (Gualberto *et al*., 2014). Their largely preserved genetic makeup and slower rate of genic evolution make them fascinating (Gualberto & Newton, 2017). They are species-specific due to the wide variation in traits including genome size, organization, and repeat sequences (Liao *et al*.,

2018). Even within the same species, the massive mitochondrial genomes of angiosperms are distinct due to recombination events involving repetitive regions, resulting in substantial genetic variation (Gualberto & Newton, 2017).

Molecular data using various genes has been used to determine the evolutionary relationships of numerous plants, including those with taxonomic challenges. Angiosperms have been reclassified as a result of the phylogeny of these organisms being deduced from three genes: *atpB*, *rbcL*, and *18S rDNA*. This is the most significant change to higher-level angiosperm taxonomy that has occurred in the past 200 years (Soltis & Soltis, 2000). With the use of various molecular data, several of the controversial issues about the phylogeny of the Lauraceae have been settled. For instance, Sassafras, Cinnamomum, Ocotea, and Nectandra were added to this genus to create the Cinnamoneae tribe (Tian *et al.*, 2021). A phylogenetic examination of the genera *Arabidopsis* and *Arabis* using nuclear ribosomal DNA sequences showed that they are paraphyletic, consisting of several distinct lineages (Koch *et al*., 1999).

Variations in the nucleotide sequence of the genes for chalcone synthase (*Chs*) and chloroplast (*matK*) encoded in the nucleus were used to determine evolutionary connections within the Brassicaceae family. It was discovered that the taxa of the tribes Sisymbrieae, Hesperideae, Brassicaceae, and Arabideae were separated into four separate lineages each, with the Brassiceae tribe being the only monophyletic group (Koch *et al*., 2001). The Cactaceae family's phylogenetic relationships were examined using Bayesian, maximum likelihood, and maximum parsimony techniques, which were constructed using

nucleotide sequences from *trnK-matK*. Although there was a lack of clarity regarding the relationships between subfamilies, three of the four now recognized subfamilies have enough evidence (Bárcenas *et al*., 2011). Using information from three sets of DNA sequences including the plastid *rpll6* intron, the nuclear 18S–26S *ITS* of rDNA, and an alcohol dehydrogenase gene, it was observed that the 12 species of Gossypium in the section Grandicalyx that are found in the Kimberley and Cobourg regions of North West Australia were all monophyletic (Seelanan *et al*., 1999).

2.4 Reasons for the use of DNA molecular markers in classification

The capacity to sequence DNA has helped to identify new species with precision and reveal the taxonomic position of a plant (Haider, 2018). The main advantage of molecular studies is that they can be done at any life stage, even those that have few morphological characteristics, such as seeds, seedlings, or fern gametophytes (Li *et al*., 2009). The DNA molecular markers are an objective measure of variation that reveals polymorphisms in DNA sequence that are not influenced by the environment (Van Deynze & Stoffel, 2006). Molecular markers have become instrumental in resolving phylogenetic relationships in plants due to their high variability and ease of amplification (Kress *et al*., 2005). The ease of quickly capturing large amounts of DNA sequence data, rapid analysis, and the difficulty of generating large morphological matrices have encouraged the use of molecular markers (Scotland *et al*., 2003; De & Bandyopadhyay, 2008).

2.5 Existing molecular taxonomy of Sapindaceae and related genera

The difficulties caused by the intricate infra-familial classification of the family Sapindaceae were addressed using data on nuclear DNA sequences produced from 123 taxa (86%). Twenty tribes were discovered in four subfamilies: two in the Dodonaeoideae, two in the Hippocastanoideae, and sixteen in the Sapindoideae. The monotypic subfamily Xanthoceratoideae has no known tribe (Buerki *et al.,* 2021). However, based on molecular evidence, Buerki *et al*., (2010) propose that *Xanthoceras sorbifolium* be removed from the Sapindaceae family, rendering the family monophyletic and moving it to the Xanthoceraceae family. The internal transcribed spacer (*ITS*) region of nuclear ribosomal DNA sequences was used to study the phylogeny and molecular differentiation of the Indian species of the genus Sapindus and other closely related taxa in the family Sapindaceae. The three Sapindus species were found to be separated into different clusters using the maximum likelihood technique (Mahar *et al*., 2017). The *ITS* (nrDNA) regions of *Gloeocarpus* (Sapindaceae) were sequenced and compared to previously published sequences of closely related species in order to assess its monophyly and its phylogenetic position. *Gloeocarpus* has a strong evolutionary position within the tribe Cupanieae, according to the strict consensus tree, which also provided good support for the genus' monophyletic classification (Arriola & Alejandro, 2011).

A phylogenetic analysis of *Dodonaea viscosa* was conducted using *ITS* and incomplete *ETS* sequences from plants collected in the Bonin Islands, Ryukyu Archipelago. This showed that the northern and central Ryukyus (Clade 1), the southern Ryukyus (Clade 2), and Chichi-jima of the Bonin Islands (Clade 3) constitute three different clades of

Dodonaea viscosa, suggesting that the species has migrated to Japan on three different occasions over time (Kokubugata *et al*., 2019). Although the complex has at least two evolutionary lineages, no specific morphological subunits correlate with the *ITS1*, *ITS2*, and partial *ETS* areas in *Dodonaea viscosa*, according to another molecular examination of these regions. To include *Dodonaea biloba* and *Dodonaea procumbens*, it was proposed that *Dodonaea viscosa* be categorized as an ochlospecies (Harrington & Gadek, 2009).

2.6 Phytochemicals and their role in plant classification

2.6.1 Classification of Phytochemicals

Phytochemicals are naturally occurring chemical compounds that have biological activity and are present in plants (Saxena *et al*., 2013). Plants contain a vast variety of phytochemicals (Pathania *et al*., 2015) which have been classified into two main types: primary and secondary metabolites (Wadood *et al*., 2013), while (Singh, 2016) identifies semantices as a third type of metabolite. Secondary metabolites are derived from primary metabolites, and their chemical structure and metabolic routes are limited to taxonomically related taxa, making them useful in classification (Vasu *et al*., 2009). The classification of plants based on secondary metabolites/chemical contents and their metabolic processes is commonly referred to as chemotaxonomy (Singh, 2016). Chemotaxonomic information is best used in conjunction with other sources of taxonomic evidence (morphology, cytology, anatomy, physiology, genetics, etc.) to establish a more robust classification system (Umoh, 2020).

2.6.2 Phytochemicals and the role of phytochemistry in resolving taxonomic problems

Phytochemicals are plant-produced constituents that prevent and treat various diseases in living organisms (Dillard & German, 2000; Prakash *et al*., 2012). They are essential in the survival of plants, protecting against herbivory and microorganisms, and regulating growth and development (Molyneux *et al*., 2007). The scientific method of detecting various classes of phytoconstituents in different parts of plants is referred to as phytochemical screening (Sharma *et al*., 2020). Phytochemical screening is crucial since the medicinal value of these plants lies in their phytochemical components, which have specific pharmacological effects on the human body (Akinmoladun *et al*., 2007) and, most importantly, create novel medications and therapeutic agents for the pharmaceutical industry (Wadood, 2013).

Taxonomically, phytochemicals and nucleic acids are valuable tools for delimiting taxa. They are classified as micro-molecules and macromolecules, with those of restricted occurrence being significantly important taxonomically (Sharma, 2013). Many taxonomically informative compounds have been identified in the Gentianaceae family, including iridoids, xanthones, mangiferin, and C-glucoflavones. Iridoids (mostly secoiridoid glucosides) appear present in every species studied, making them an excellent taxonomic identifier for this family (Jensen & Schripsema, 2001). A taxonomic study of the volatile oils of the genus *Zingiber* resulted in the separation of *Zingiber* into two groups. Cluster one contained a high phenyl propanoids and hydrocarbon chemicals found in *Zingiber nivieum, Zingiber pyoglossum, Zingiber junceum,* and *Zingiber* species 1. Cluster

two includes a high concentration of monoterpene hydrocarbons, oxygenated monoterpenes, and sesquiterpene hydrocarbons, including *Zingiber ottensii, Zingiber montanum, Zingiber officinale, Zingiber zurumbet,* and *Zingiber* species 2 (Theanphong *et al*., 2016).

In the sponge family, pyrroloiminoquinone alkaloids have contributed significant chemotaxonomic markers. Latrunculid sponges are the large repositories of pyrroloiminoquinone-type alkaloids, which have a wide range of biological activities, including cytotoxicity, fueling their research for anticancer medication development (Li *et al*., 2021). Gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) were used to identify essential oils from six *Salvia* species, resulting in three major clusters. *Salvia reuterana* and *S. macrosiphon* were clearly distinguished from the other species. Other species (*S. atropatana, S. sclarea, S. aethiopis,* and *S. oligophylla*) were separated into two subgroups. The first sub-cluster was *S. atropatana* and the second cluster contains three other species, including *S. sclarea, S. aethiopis* and *S. oligophylla* (Salimpour *et al.*, 2011). Chemotaxonomic indicators are more critical in bacterial identification. The presence/absence of biochemical markers such as amino acids and peptides (peptidoglycan), lipids (fatty acid, lipopolysaccharides, micolic acid, and polar lipids), polysaccharides and related polymers (teichoic acid, whole sugar), and other complex polymeric compounds are key to their classification and identification (Gokdemir & Aras, 2019).

2.6.3 Existing chemotaxonomic work on Sapindaceae and related genera

Fatty-acid profiles of sixteen Sapindaceae species from five tribes examined resulted in two primary clusters identified by multivariate analysis. The Paullinieae tribe belongs to Cluster One with elevated eicosenoic acid levels, and the second central cluster has lower eicosanoid acid levels (Coutinho *et al*., 2015). Through phytochemical analysis, pentamethoxylated flavonoids, which had not previously been documented in this family, were discovered in the aerial portions of *Cardiospermum corindum* L. (Sapindaceae). Pentamethoxylated flavonoids in Cardiospermum can serve as chemotaxonomic indicators (Silva *et al*., 2014). A chemometric study on the Kenyan populations of *Dodonaea viscosa* revealed closer relationships between Nanyuki, Machakos, and Coastal populations and between Narok and Nairobi populations (Kaigongi *et al*., 2020).

2.7 The practice of traditional medicine

2.7.1 The use of plants in traditional medicine

Plants form an integral part of human life, with Traditional medicine (TM) having existed since the beginning of the human species (Patwardhan *et al*., 2005). They are becoming increasingly popular as a source of medicine worldwide because of their natural origin, accessibility in local communities, affordability, simplicity of use and potential lack of complications (Abubakar & Haque, 2020). Among the well-known systems of TM are Siddha medicine, traditional Chinese medicine, Unani, and Ayurveda, whereas Australian, Central and South American, and African systems of medicine are less known systems that are lately becoming more prominent (Gurib-Fakim, 2006; Alves & Rosa, 2007). African Traditional Medicine has been used to treat a variety of illnesses, including diabetes,

hypertension, infertility, parasitic, bacterial, viral, and fungal infections, by using items primarily derived from medicinal plants (Oguntibeju, 2019).

It is estimated that 60% of the world's population lives in underdeveloped nations and relies on traditional medicine, primarily plant-based medications, for their essential medical requirements (Shrestha & Dhillion, 2003). Despite its obstacles in Africa, over 80% of people rely heavily on traditional medicine for primary healthcare (Oguntibeju, 2019). In Kenya, traditional medicines account for at least 70% of treatments for fundamental medical conditions (Kisangau *et al*., 2017). Most Kenyan populations, for example, the Samburu, Luo, and Maasai, primarily rely on medicinal plants as first-line treatments for various illnesses (Njoroge & Bussmann, 2006).

2.7.2 *Diabetes mellitus* (DM): Causes and its effects

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both (Padhi *et al.*, 2020; Wild *et al*., 2004). *Diabetes mellitus* has been identified as the most common metabolic disorder (Gispen & Biessels, 2000). A survey has established that globally, diabetes is affecting more males than females with several predisposing factors like obesity, differences in body mass index, differences in glucose homeostasis, genetic background, and lifestyle (Kautzky-Willer *et al*., 2016; Ciarambino *et al*., 2022). Today, it is one of the public health concerns as it affects more than 400 million people worldwide, with the unfortunate fact that it cannot be cured (Malviya *et al.*, 2010; Khursheed *et al.*, 2019).

Diabetes mellitus is classified into three types: type 1 *diabetes mellitus* (insulin-dependent *diabetes mellitus*) and type 2 *diabetes mellitus* (non-insulin dependent *diabetes mellitus*) (Singh, 2011) and gestational diabetes, which affects expectant women (Reece *et al*., 2009). Type 1 diabetes (T1D) affects youngsters and young adults and is sometimes referred to as "juvenile diabetes" or ketosis-prone polygenic diabetes (Singh, 2011). T1D is an autoimmune disorder that affects pancreatic cells, which reduces or impairs insulin production, while T2D is caused by the *beta* cells of the pancreas secreting less insulin and the insulin receptors not responding well to it (Wong *et al.*, 2017). It is believed that fructose overconsumption plays a significant role in the emergence of T2D (DiNicolantonio *et al*., 2015). Fructose is a component of sucrose, also known as table sugar, which can be made from sugar cane or beets and comprises one glucose molecule and one fructose molecule joined by an α1-4 glycoside bond. In addition, fructose and glucose are naturally occurring monosaccharides found in various vegetables, honey, and fruits (Malik & Hu, 2015).

DM is characterized by polyuria (excretion of large volumes of diluted urine), polydipsia (drinking excess amounts of water), and glycosuria (excretion of glucose in the urine) (Singh, 2011). DM is also associated with other long-term complications affecting body organs like the eyes, kidneys, heart, and nerve cells (Gispen & Biessels, 2000). The management of diabetes is a global problem until now, and successful treatment has not yet been discovered (Malviya *et al*., 2010). DM contributes significantly to substantial economic loss, which might obstruct a country's ability to progress (Mahabir & Gulliford, 1997). Its treatment using conventional medicine is faced with a myriad of challenges,

including high cost of the drugs, progression of the disease, and many undesirable side effects (DeRuiter, 2003). The unfortunate effects include serious social and financial challenges, as well as early disability and eventual death of the patient (Sukhikh *et al*., 2023).

2.7.3 Microbial organisms and resistance

Some of the most frequently utilized medications in human therapies include antibacterial and other antimicrobial drugs (Gürel & Aslan, 2019). Their ability to manage microbial infections was extraordinarily effective until they unconditionally did not submit to several of the initial effective medications (Hancock *et al*., 2012). This resistance is attributed to the increased usage of antimicrobial drugs (Khurram *et al*., 2009; Gürel & Aslan, 2019). Humans' widespread and frequently needless use of antimicrobials also heightens antimicrobial resistance (Michael *et al*., 2014). Increased resistance could also be attributed to genetic mutation of the microbes, which is a basis for their survival adaptability (Elena & Lenski, 2003). Studies have also supported the role of environmental mutagens in directing mutations of microscopic organisms (Miyahara *et al*., 2011).

Most importantly, microbes can genetically transmit and acquire resistance to drugs utilized as therapeutic agents (Cohen, 1992). Fortunately, plants as natural resources have been used for treating several illnesses brought on by bacteria, fungi, and viruses. This is attributed to secondary metabolites, for example, alkaloids, carbohydrates, flavonoids, glycosides, sterols, saponins, terpenoids, coumarins, quinones, and tannins in plants (Cowan, 1999; Khurram *et al*., 2009).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Collection of plant specimens

A pre-survey was done with the assistance, a taxonomist from the University of Eldoret, to ascertain the specific location of the *Dodonaea viscosa* under study. Collections were done in four Counties: three Counties from the North-rift (Uasin Gishu, Baringo, and Elgeiyo Marakwet) and one County (Kwale) from the Coast. From the field collections, it was evident that *Dodonaea viscosa* grows in association with other plants, including *Rhus natalensis, Plectranthus barbatus, Euphorbia candelabrum, Euclea divinorum, Erythrina abyssinica, Acacia abyssinica, Caesalpinia decapetala, Olea africana, Juniperus procera*, among others. The specific sites of the collection were as presented in Table 3.1.

Table 3.1: Place of collection, coordinates and altitude

COUNTY	PLACE	COORDINATES	ALTITUDE (Meters)
UASIN GISHU	Sergoit	N 0° 38’00.28’’, E 35° 23’ 11.89’’	2229
	Turbo	N 0° 38’00.28’’, E 35° 23’ 11.89’’	1875
BARINGO	Cheploch	N 0° 32’ 02.58’’, E 35° 27’ 31.15’’	1535
	Kabarnet	N 0° 32’ 03.13’’, E 35° 27’ 31.46’’	1725
	Koriema	N 0° 31’ 19.72’’, E 35° 27’ 13.09’’	1845
ELGEYO MARAKWET	Iten	N 0° 33’ 11.24’’, E 35° 26’ 38.11’’	2334
	Chepyogot	N 0° 32’ 03.13’’, E 35° 27’ 31.46’’	2324
	Kapchemutwo	N 0° 33’ 43.20’’, E 35° 26’ 55.13’’	2337
KWALE	Gazi 1C	S 4° 25’ 51.11’’, E 39° 32’ 25.15’’	14
	Gazi 3B	S 4° 25’ 86.23’’, E 39° 32’ 67.46’’	8
	Gazi M5	S 4° 26’ 56.23’’, E 39° 30’ 28.38’’	11

The leaves and roots of *Dodonaea viscosa* populations were collected from the locations as listed in Table 3.1 from May to June 2023. The identification was at the University of Eldoret and the University of Nairobi. They were transported to the University of Eldoret and processing and keeping in the herbarium. After hand washing a few samples under running water, they were allowed to air dry for 28 days before grinding to test them for biological activity and phytochemical analysis. Other samples were preserved in cooler boxes to await molecular analysis.

3.2 Standardized DNA Extraction Method

A modified CTAB protocol of Rathod *et al.*, (2018), described herein, was used to extract DNA at the University of Nairobi. A pre-chilled mortar and pestle were used to crush 0.5 g of young leaf tissue from various *Dodonaea viscosa* populations. They were crushed using 1 ml of lysis buffer, which contained 0.1 g of polyvinylpyrrolidone (PVP), 250 mM NaCl, 25 mM EDTA with a PH of 8.2, 0.5% SDS, and 200 mM Tris base for an extraction buffer of 1000 ml. The crushed extracts were moved to another autoclaved microcentrifuge tube. Every tube was handled in the same way, as detailed herein. Using a float, the microcentrifuge tubes containing the powdered homogenate were incubated at 65°C for approximately one hour. Using a cooling centrifuge (5415 R), the tubes were spun for two minutes at 4°C at 14000 rpm.

Using a micropipette, the supernatant was placed in newly labelled tubes, and the samples were mixed with an equal volume of chloroform: isoamyl alcohol (24:1). To guarantee

optimal pigment extraction in the chloroform layer, the mixture in the tubes were carefully mixed by slowly inverting them fifteen to twenty times. The samples were centrifuged at 14000 rpm for ten minutes at a temperature of 4°C. Using a micropipette, the supernatant was carefully transferred to new tubes. To each supernatant 12 µl of RNase (1 mg/ml stock) was added and gently mixed by inverting the tubes five to ten times. The mixtures were incubated at 37°C in a water bath for thirty minutes. The tubes were inverted four to six times to gently mix the samples with an equal volume of chloroform and isoamyl alcohol (24:1). After that, the samples were centrifuged for 10 minutes at 4°C at 14,000 rpm. Cold ethanol was added after transferring the aqueous layer to a new tube, and the mixture was incubated for 12 hours.

After being thawed on ice, the mixtures were centrifuged for 5 minutes at 14000 rpm and 4°C. Then 750 µl of cooled 70% ethanol was added to each tube's pellet after the supernatant was disposed of, and the tubes were gently inverted twice to clean the DNA pellet. After centrifuging the tubes for five minutes at 4°C and 14,000 rpm, the supernatant was removed using a micropipette. The DNA pellets were dried for 1 hour and suspended in nuclease-free water.

3.3 PCR amplification and electrophoresis

DNA amplification was done at the molecular laboratory at the University of Nairobi's Center for Biotechnology and Bioinformatics (CEBIB) in Chiromo Campus. The primers *ITS1* and *rbcL* were used for PCR amplification of the *ITS1* and *rbcL* sections of nrDNA and cpDNA, respectively (Korall & Kenrick, 2002; Patwardhan, 2014; Omelchenko *et al.*, 2022). The primers used in PCR are represented in Table 3.2.

Table 3.2: The primers used in PCR

Loci	Direction	Primer sequence	Reference(s)
ITS1	F	5'-GGAAGGAGAAGTCGTAACAAGG-3'	(Omelchenko *et al.*, 2022)
	R	5'-AGATATCCGTTGCCGAGAGT-3'	
rbcL	F	5'-ATGTCACCACAAACAGAAAC-3'	(Fouad *et al.*, 2019; Algarni, 2022)
	R	5'-TCGCATGTACCTGCAGTAGC-3'	

The primers used in this study were ordered from Macrogen, Europe. For each of the populations, a Master mix for PCR was prepared by the addition of 5µL Flexi DNA polymerase, 2µL Magnesium chloride ($MgCl_2$), 14.3µL Nuclease-free water, primers (0.5µL of forward sequences and 0.5µL of reverse sequences), 0.4µL dNTPs (dGTP, dATP, dCTP, dTTP), Taq DNA polymerase, and 2µL DNA. A final concentration of 25µL of master mix was constituted and placed in each PCR tube. A Thermal Cycler (PTC-100)

was used to conduct the amplification reactions. It was set up to include an initial denaturation at 94°C for three minutes, annealing at 58 °C for one minute, and an extension at 72 °C for seven minutes after 35 cycles. A gel electrophoresis was finally carried out to confirm whether amplification was done.

3.4 DNA sequencing, alignment, and Phylogenetic Analysis

The products were sent to Macrogen, Europe, for purification and subjected to a DNA Cycle sequencing Kit to sequence the DNA based on the Sanger method (Men *et al*., 2008; Kluesner *et al*., 2018;). The *ITS1* and *rbcL* gene sequences of *Dodonaea viscosa* obtained were later deposited in GenBank, and their accession numbers were obtained. The sequences were fed to MEGA 11 (Tamura *et al*., 2021) software, manually edited at the ends to remove incorrect bases that could compromise the quality of the DNA, and aligned by MUSCLE (codons). Alignment was necessary to identify the matches, mismatches, and gaps in the two gene sequences under study. The aligned sequences were finally exported as FASTA files to PhyloSuite. Through a bootstrap test of phylogeny, maximum likelihood (ML) trees were generated and transferred to ITOL (interactive tree of life), an online software for managing, annotating, and displaying phylogenetic trees (Yang *et al*., 2023; Letunic & Bork, 2024). Basic evolutionary analyses (nucleotide frequency and substitution rates, base composition disparity index & evolutionary divergence) were performed on the obtained DNA sequences as recommended (Kumar *et al*., 2008; Tamura *et al*., 2021). First, second, third, and noncoding codon positions were all included in the analysis. While the two genes were being analyzed phylogenetically, Hasegawa-Kishino-Yano substitution (HKY) (Takahashi & Nei, 2000) and Jukes–Cantor+gamma (JC+G) (Som, 2006) models

were used. The HKY model assumed that all four nucleotide sites under consideration had comparable or uniform substitution rates and patterns, while the JC+G model assumed that there were equal base frequencies and mutation rates with gamma-distributed variation. Studies show that the best model for explaining the pattern of nucleotide substitution during evolution has the lowest Bayesian Information Criterion (BIC) score, as suggested by Tamura *et al*. (2021). The bootstrap resampling test was used to test for phylogeny, and each ML tree built with 1000 iterations had 5000 bootstrap replications. A bootstrap score of more than 70 indicated strong support, about 50 indicated moderate support for the tree topology/monophyletic clade, and less than 50 indicated poor support (Chebet *et al*., 2022). The trees with the highest log probability were chosen to best illustrate the evolutionary relationships between the Coastal and highland *Dodonaea* populations under investigation.

Additionally, an ML trees were built using the concatenated alignments of the two genes. *Sinoradlkofera minor* was included as an outgroup in ML trees to represent its the closest ancestor of *Dodonaea viscosa*. *Sinoradlkofera* is a genus that belongs to the Sapindaceae family, just like the genus *Dodonaea* under study. The sequences for this outgroup were obtained from NCBI GenBank (KX527258.1 and OR199385.1 for *rbcL* and *ITS1* genes, respectively) and then aggregated with the *Dodonaea* sequences of the populations under study during the generation of the ML trees.

3.5 Principal component analysis of *Dodonaea viscosa* populations

Sequence data were subjected to PCA. Principal components are a few linear combinations of the original variables that maximally explain the variance of all the variables. PCA can

be used to show relationships between samples and variables (Bro & Smilde, 2014). In our case, PCA can show which genes are distantly related from the others, or the ones which have a lot of variations compared to the rest. PCA requires numerical data, hence the sequence data recorded by a set of five digits (A, T, G, C, and a gap (-) was replaced by boolean vector that is presented by 0 and 1 forming a sequence matrix that was later converted into numerical data. This transformation of DNA data into numerical data has merits that no information is lost and so it is completely reversible. This transformation was done in R statistical program following R script provided by (Konishi, 2019). Obtained numerical data were then subjected to PCA in R using FactoMineR and Factoextra R packages to identify principal components and quality of representation of the principal components.

3.6 Extraction, isolation, and characterization of pure compounds in the leaves of *Dodonaea viscosa* Gazi 1C population

The solvent extraction method described by Ingle *et al*., (2017), was used in this study. Then 973 grams of ground material was steeped in Dichloromethane (CH_2Cl_2): Methanol (CH_3OH) 1:1 (v/v) for 72 hours and thereafter filtered. Using a rotary evaporator, the extraction solvent was eliminated by evaporation. Extraction was done three times. The extract was air dried, and the total yield obtained as a percentage was determined using the formula below:

$$\textbf{Percentage yield} = \frac{\text{Total extract obtained in grams}}{\text{Total weight of the plant}} \times 100$$

The resultant semisolid was used to determine the phytoconstituents present. Adsorption was done by adding 25g of silica (60-120 MESH) and grinding it uniformly in a mortar and pestle. In preparation for the slurry, 250g of 60-120 MESH silica gel in Hexane was introduced down the column. The phytoconstituents were isolated using silica gel column chromatography as the stationary phase (Gunawan *et al*., 2008). Elution of the small fractions was done using 100% hexane, and with time, there was an increasing polarity of ethyl acetate EtOAc (1%, 2%, 3%, 4% …50%). Fractions collected were approximately 300 ml and combined based on their TLC profiles. Based on solubility, ligand interaction, molecular size, and polarity, the isolates were purified on Sephadex, column chromatography, and thin layer chromatography plates (Zhang & Xu, 2007; Bele & Khale, 2011; Abubakar & Haque, 2020; Jiang *et al*., 2021). Pure compounds formed single spots on the TLC plates. Different spectroscopic methods, including ultraviolet (UV) and nuclear magnetic resonance (NMR), were used to characterize the compounds isolated (Vicario *et al*., 2020).

3.7 Biological activities of *Dodonaea viscosa* populations

3.7.1 Crude extraction

The protocol developed by Arsene *et al*., (2021), with some adjustments, was the basis for the extraction. Populations of *Dodonaea viscosa* were extracted by maceration using ethanol solution (96% v/v). 100g of the ground plant material (leaves and root barks) from different populations were added to 500 ml of the solvent in conical flasks and shaken at 200 rpm for at least 24 hours at 25 ˚C. Filtration was done using a filter paper (Whatman No. 1), and thereafter the semisolid mass was dried at 80°C in a rotavap before being stored

in airtight containers. The final crude extract was stored at 4 °C and later assayed for biological activities. The volume and mass yields were calculated for each extract.

3.8 Antidiabetic activity of *Dodonaea viscosa* populations

Six populations of *Dodonaea viscosa* roots and leaves were studied for their antidiabetic properties using Swiss male albino rats. This was done within 24 hours to ascertain how the crude extract affected blood sugar levels in the rats under study.

3.8.1 Breeding of experimental animals

The Swiss albino rats were acclimatized for two weeks before the start of the breeding exercise. 10 male and 18 female Swiss albino rats were bought and subdivided into 2 cages of 5 males and 9 females to breed. After 7 days, the males were separated from the females. On giving birth, only adult male rats weighing 160-200g were used for the experiment, while the females were discarded humanely. The males were kept in standard environmental settings (24 ± 1 ºC) with 12-hour cycles of light and darkness and given a commercial meal from Unga Group Limited (UNGA.ke) and unlimited access to water.

3.8.2 Induction of diabetes

Streptozotocin (STZ) was administered intraperitoneally to male albino rats in groups of 4 after being starved for the previous night to make them diabetic. Each rat was given one dose concentration of 6.5 mg/100g of STZ freshly prepared in 1ml distilled water (Lanjhiyana *et al.*, 2011). Blood samples from the tail vein were taken on days 3 and 7 following injection to confirm stable hyperglycemia. Glucose levels were then calculated using a glucometer (On Call® Plus). Male albino rats with blood glucose levels greater than 11 mmol/L were deemed diabetic (Mukundi *et al*., 2017).

3.8.3 Experimental design

The rats under study were randomly placed in five cages on day 8 to receive different intraperitoneal treatments as shown in Table 3.3.

Table 3.3: Experimental design used

Animal group	Status	Treatment	No of rats
I	Normal control	Vehicle only	4
II	Diabetic control	Vehicle only	4
III	Diabetic	Reference drug (Insulin)	4
IV	Diabetic	200 mg/kg bw extract	4
V	Diabetic	400 mg/kg bw extract	4

3.8.4 Oral Glucose Tolerance Test (OGTT)

On day 9, the OGTT was conducted to assess the extract's immediate impact on glucose control. After fasting for the previous night (day 8), male albino rats in all groups received 2 g/kg of body weight glucose orally and thereafter receive plant extract (groups IV and V). A small amount of blood was drawn from the tail vein before and after administering the glucose solution (0, 3, 6, 12, and 24-hour intervals). This was done to determine the effects of the plant extract from *Dodonaea viscosa* and other treatments on blood sugar. The results were presented in mmol/L.

3.9 Antimicrobial activity of *Dodonaea viscosa* populations

3.9.1 Bacterial and fungal strains

In this study, a common strain of a fungus and gram-positive and gram-negative bacteria were used. They included *Bacillus cereus*, *Candida albicans*, methicillin-resistant *Staphylococcus aureus* (MRSA), and *Escherichia coli*. They were bought from the Department of Biological Sciences of the University of Nairobi.

3.9.2 Agar well diffusion method

With minor modifications, the protocol previously described by Keshebo *et al*., (2016) and Singh *et al*., (2020) was used to ascertain whether plant extracts had antimicrobial properties. A nutrient agar medium was used to culture bacteria, while potato dextrose agar (PDA) medium was used to culture fungi. They were prepared following the manufacturer's instructions. They were autoclaved at 121^0C for 15 minutes and cooled to 45-50 ^{0}C in a water bath. They were poured onto sterile 90 mm diameter petri dishes to a depth of 4 mm and inoculated with the chosen isolates by streaking the swab over the medium's surface three times to ensure even distribution. A sterile 6 mm cork borer was used to make agar wells, which were saturated by stock solutions of 50 µL of each extract at different concentrations. Amoxicillin (500 mg), an antibacterial drug, and Apron a fungicide were the positive controls used in the study. The plates were kept in the refrigerator for an hour to improve the extract's ability to diffuse before being incubated for 24 hours at 37°C. Following incubation, the inhibition zones formed around the dug wells were measured in millimetres (mm), and each experiment was done in triplicate. From the obtained measurements, the means of each were computed.

3.10 Data analysis and evaluations

The data obtained from molecular work was analyzed in Molecular Evolutionary Genetics Analysis (MEGA) version 11 software, aligned by MUSCLE (codons), and ancestral states inferred using the Maximum Likelihood (ML) method. A nucleotide BLAST of the sequence genes was done to locate regions/areas with local similarities between nucleotide sequences by comparing them with the GenBank sequences and to identify their closest ancestor. Fundamental evolutionary investigations that included base composition disparity index, evolutionary divergence, and nucleotide substitution rate measurements between *Dodonaea* populations were performed in MEGA 11. Data on antidiabetic activity were organized in Microsoft Excel and shown as mean ± standard deviation. Paired sample t-test was used to determine the activity of roots and leaves. Using the F-test, an analysis of variance (ANOVA) was computed to compare the activity of the various populations and determine the most effective population. Graphical methods were used to analyze data from microbial assays. Pure compounds isolated from *Dodonaea viscosa* Gazi 1C population were identified by comparing the spectral data of each sample component from proton NMR (^{1}H NMR) and carbon NMR (^{13}C NMR) with values reported in literature to elucidate the structure and name the isolated compounds (Inbaraj *et al*., 2010; Raksat *et al*., 2025). They were given names based on the International Union of Pure and Applied Chemistry (IUPAC), and where available, by their common or trivial names.

CHAPTER FOUR

RESULTS

4.1 Molecular Phylogeny of *Dodonaea viscosa* populations

4.1.1 Gel electrophoresis

Gel electrophoresis performed on eleven (11) populations of *Dodonaea viscosa* collected to showed the presence of genomic DNA. Figure 4.1 represents genomic DNA from the *ITS1* gene. The white bands in Figure 4.1 show the presence of DNA from the different populations.

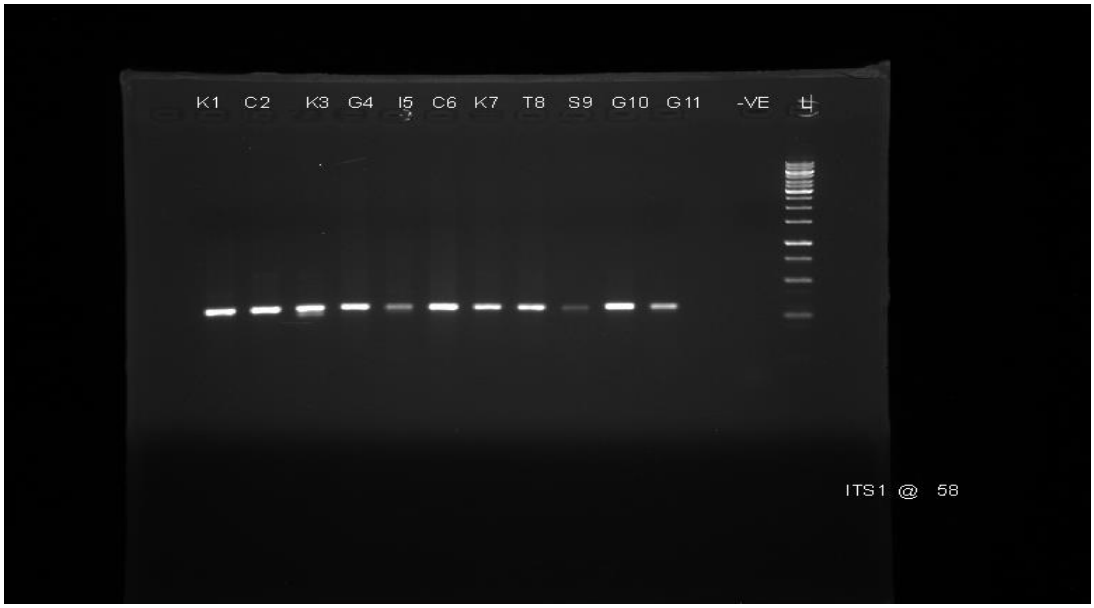

Figure 4.1: Gel electrophoresis image of the ITS1 gene from 11 populations with white bands showing genomic DNA. K1: Kabarnet, C2: Cheploch, K3: Koriema, G4: Gazi 3B, I5: Iten, C6: Chepyogot, K7: Kapchemutwo, T8: Turbo, S9: Sergoit, G10: Gazi M5 and G11: Gazi 1C.

Similarly, a gel electrophoresis was carried out on the *rbcL* gene sequences of the eleven (11) populations of *Dodonaea viscosa*. Figure 4.2 represents genomic DNA from the *rbcL* gene with white bands showing the presence of DNA from the different populations studied.

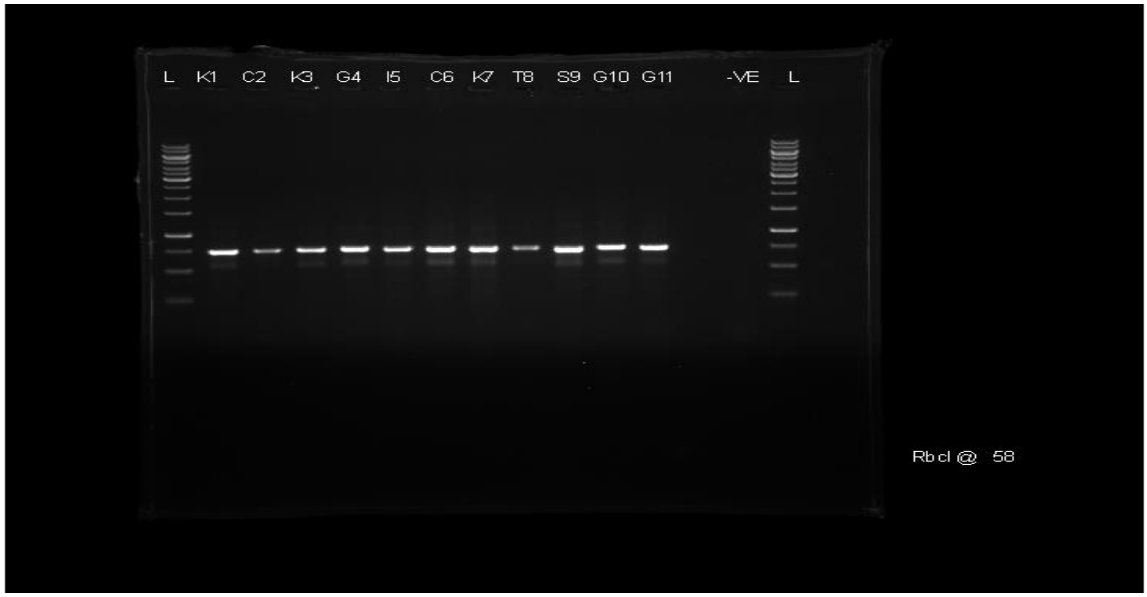

Figure 4.2: Gel electrophoresis image of the *rbcL* gene from 11 populations with white bands showing genomic DNA. K1: Kabarnet, C2: Cheploch, K3: Koriema, G4: Gazi 3B, I5: Iten, C6: Chepyogot, K7: Kapchemutwo, T8: Turbo, S9: Sergoit, G10: Gazi M5 and G11: Gazi 1C.

4.1.2 DNA sequencing and alignment of *ITS1* and *rbcL* genes

The results obtained were in three forms: AB1 File (.ab1), Adobe Acrobat Document (.pdf), and Text Document (.txt) file formats. The results included forward DNA sequences of the *rbcL* and *ITS1* genes. Sequencing assisted in identifying matching, mismatch regions, and gaps within the DNA. Figure 4.3 is a diagrammatic representation of a section of DNA

alignments from *rbcL* gene sequence of the different populations of *Dodonaea viscosa.* From the *rbcL* data set, there were 1729 nucleotide sequences, out of which 684 sites were conserved, 897 variable sites, 685 singleton sites, and 207 parsimony informative sites.

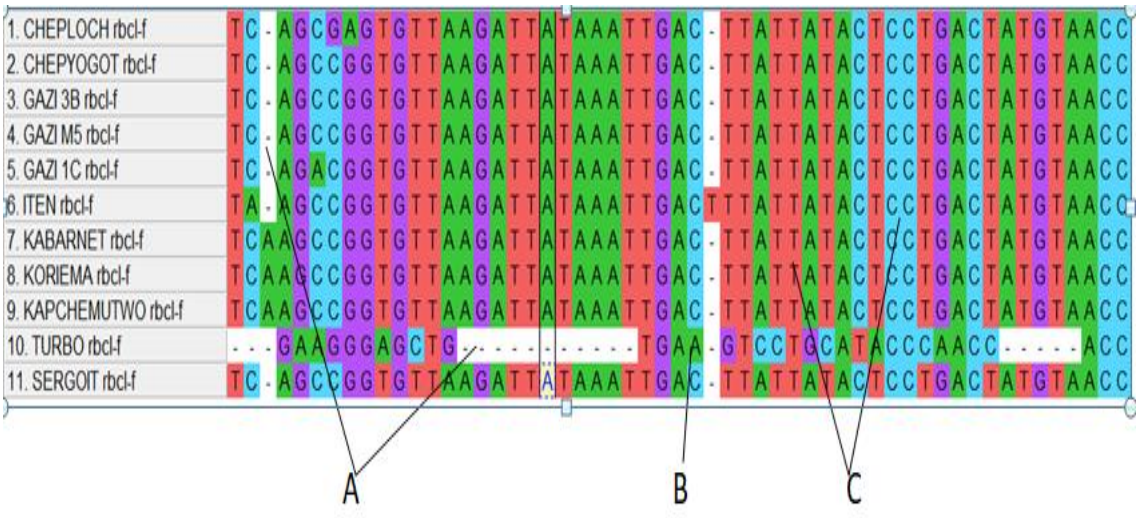

Figure 4.3: Multiple sequence alignments of *rbcL* forward genes. A: Gaps within rbcL genes; B: Mismatched Adenine; C: Matched Thymine and Cytosine sequences in the *rbcL* genes.

Similarly, DNA sequences obtained from the *ITS1* gene sequences of the various populations of *Dodonaea viscosa* gave the following pictogram (Figure 4.4) showing matches, mismatches, and gaps in the matrix obtained. From the *ITS1* data set, there were 3152 nucleotide sequences, out of which 381 sites were conserved, 2505 variable sites, 716 singleton sites, and 1672 parsimony informative sites.

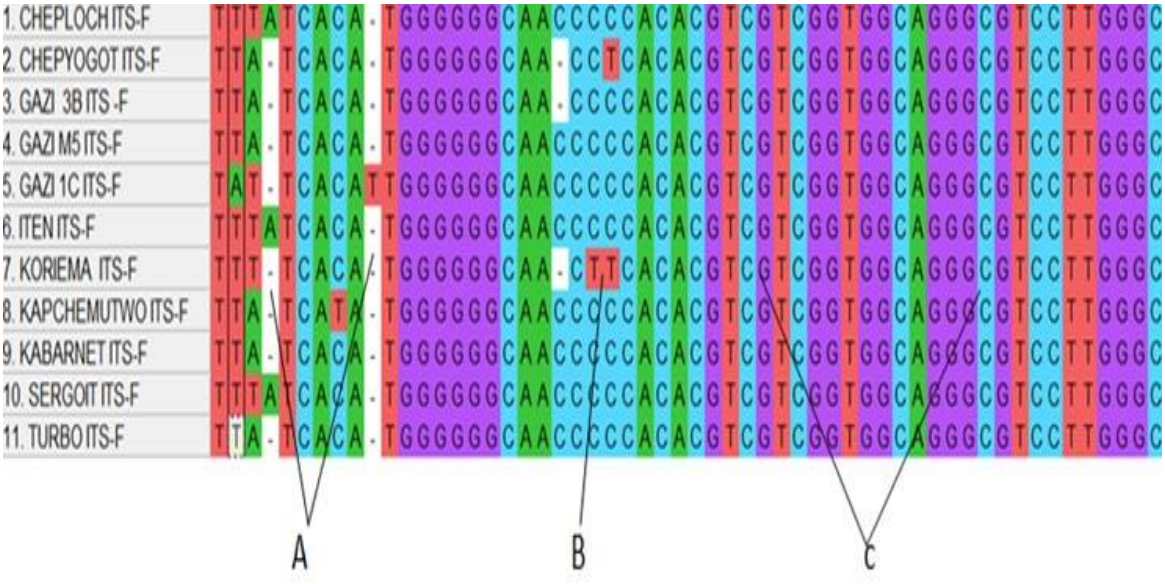

Figure 4.4: Multiple sequence alignments of *ITS1* forward genes. A: Gaps within *ITS1* genes; B: Mismatched Thymine; C: Matched Guanine and Cytosine sequences in the *ITS1* genes.

The obtained DNA sequences from the different populations were deposited in the GenBank public database with corresponding bibliographic and biological annotation for ease of accessibility by other taxonomists. Their accession numbers were also obtained as presented in Table 4.1.

Table 4.1: Kenyan *Dodonaea viscosa* populations accession numbers at Genbank

***Dodonaea viscosa* Populations**	**Gene**	**Accession Numbers at Genbank**	**Gene**	**Accession Numbers at Genbank**
Cheploch	*rbcL*	PQ901279	*ITS1*	PV056202
Chepyogot	*rbcL*	PQ901280	*ITS1*	PV056203
Gazi M5	*rbcL*	PQ901282	*ITS1*	PV056205
Gazi 1C	*rbcL*	PQ901283	*ITS1*	PV056206
Gazi 3B	*rbcL*	PQ901281	*ITS1*	PV056204
Iten	*rbcL*	PQ901284	*ITS1*	PV056212
Koriema	*rbcL*	PQ901286	*ITS1*	PV056207
Kapchemutwo	*rbcL*	PQ901287	*ITS1*	PV056208
Kabarnet	*rbcL*	PQ901285	*ITS1*	PV056209
Sergoit	*rbcL*	PQ901288	*ITS1*	PV056210
Turbo	*rbcL*	PQ901289	*ITS1*	PV056211

4.1.3 Evolutionary analyses

4.1.3.1 Nucleotide frequency and substitution rates of *ITS1* and *rbcL* sequences

From this analysis, the best nucleotide evolutionary model of both *rbcL* and *ITS1* sequences was the HKY + G model (Liu *et al*., 2017) with the lowest BIC value of 18721.288 and 12946.875 for *rbcL* and *ITS1* genes, respectively. According to the HKY + G model, Adenine (A), Thymine (T), Cytosine (C), and Guanine (G) nucleotide frequencies were 0.27, 0.25, 0.19, and 0.27, respectively, and the *rbcL* sequences' nucleotide substitution rates varied from the lowest value of 0.05 in AC and GC to the highest value of 0.11 in GA

and AG. Similarly, the frequency of nucleotides in the *ITS1* sequences for Adenine (A), Thymine (T), Cytosine (C), and Guanine (G) had respective sequences of 0.25, 0.19, 0.26, and 0.28. On the same note, the *ITS1* sequences' nucleotide substitution rate varied from the lowest value of 0.05 in CT to the highest value of 0.09 in TG and CG (Figure 4.5).

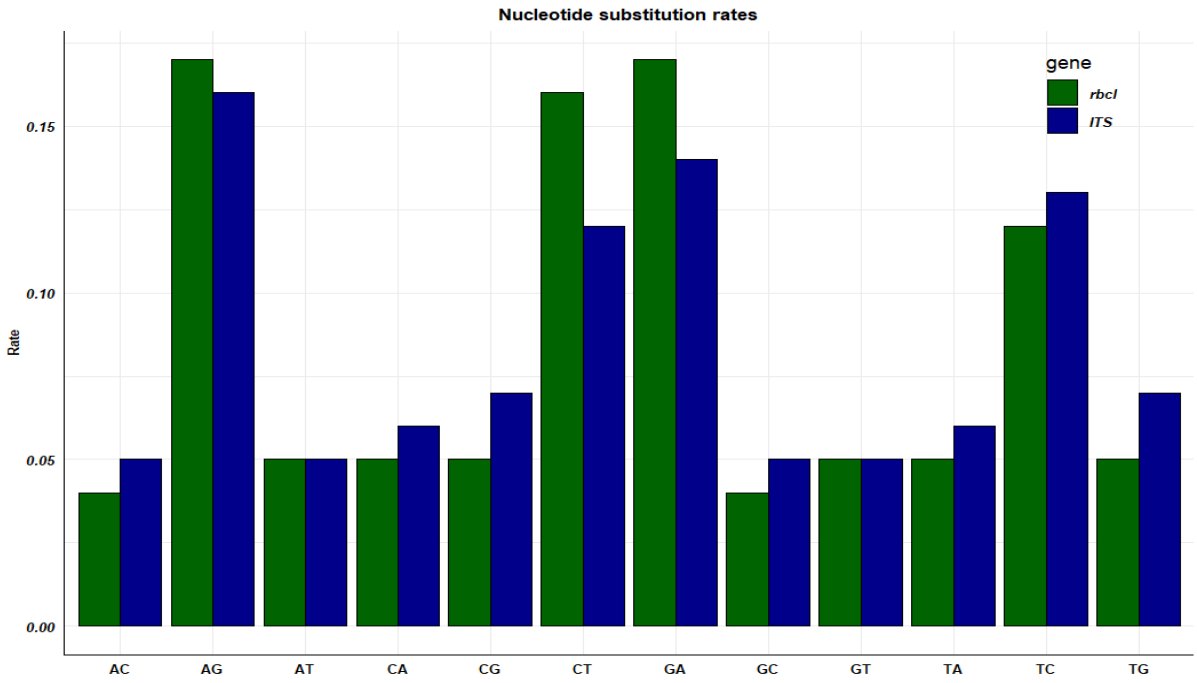

Figure 4.5: Nucleotide substitution rates in both *rbcL* and *ITS1* genes

4.1.3.2 Nucleotide Disparity Indices

According to the *rbcL* gene sequences, the highest value was found between the Turbo group and the rest of the groups, where the disparity index between the Turbo *rbcL* was >1 in all comparisons. Other higher disparity indices were found between Sergoit *rbcL* and Cheploch *rbcL,* and finally between Karbarnet *rbcL* and Cheploch *rbcL* genes. The other groups' comparisons gave low disparity indices of zero or near zero, implying close

relatedness. Hence, based on nucleotide disparity indices, distantly related groups included Kabarnet, Sergoit, and Turbo. Based on the *ITS1* genes, higher nucleotide disparity index of 3.55 was recorded in Gazi populations. In addition, a higher disparity index was also observed between Koriema and the other groups; indeed, a disparity index of 4.32 was observed between Koriema and the Cheploch groups.

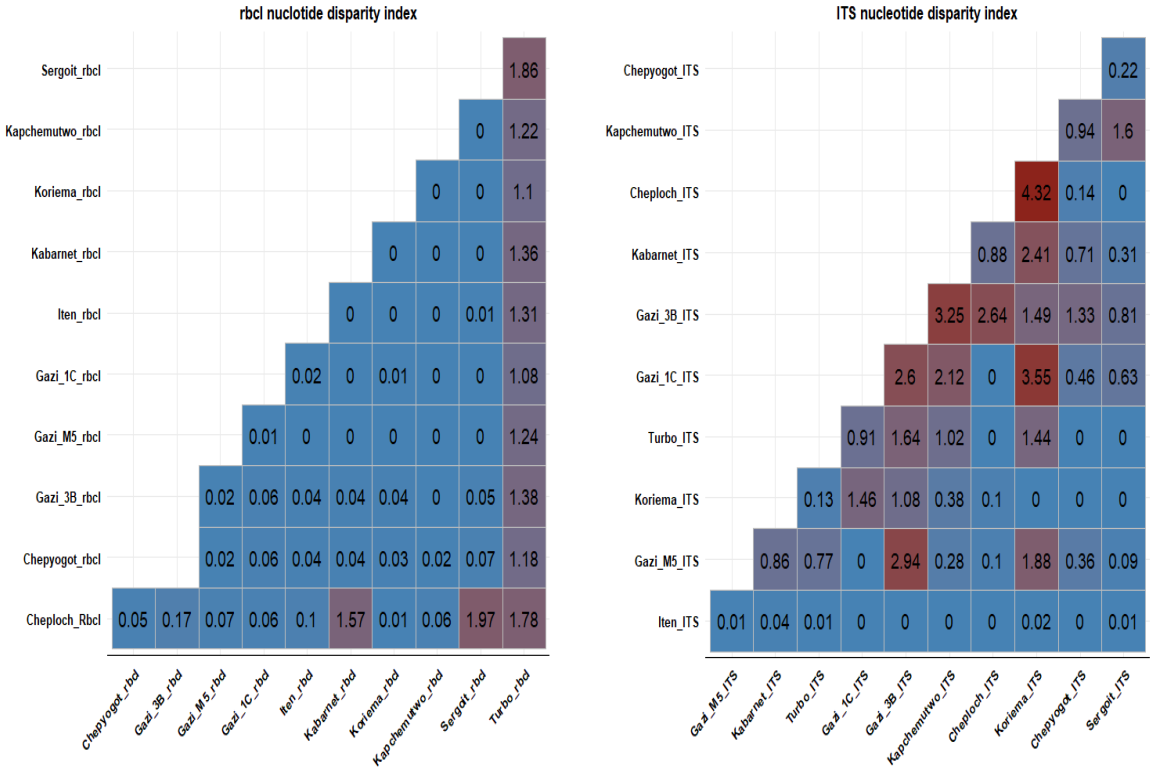

Figure 4.6: Nucleotide Disparity Indices amongst *Dodonaea viscosa* populations

4.1.3.3 Evolutionary divergence between *ITS1* and *rbcL* genes

ITS1 showed the lowest variation, ranging from 0.02 to 0.47, and was closely related to the populations under study. From the other *ITS1* genes, most comparisons gave distances of

0.03 to 1.23, implying that there were more substitutions/differences among the *ITS1* genes than the *rbcL* genes.

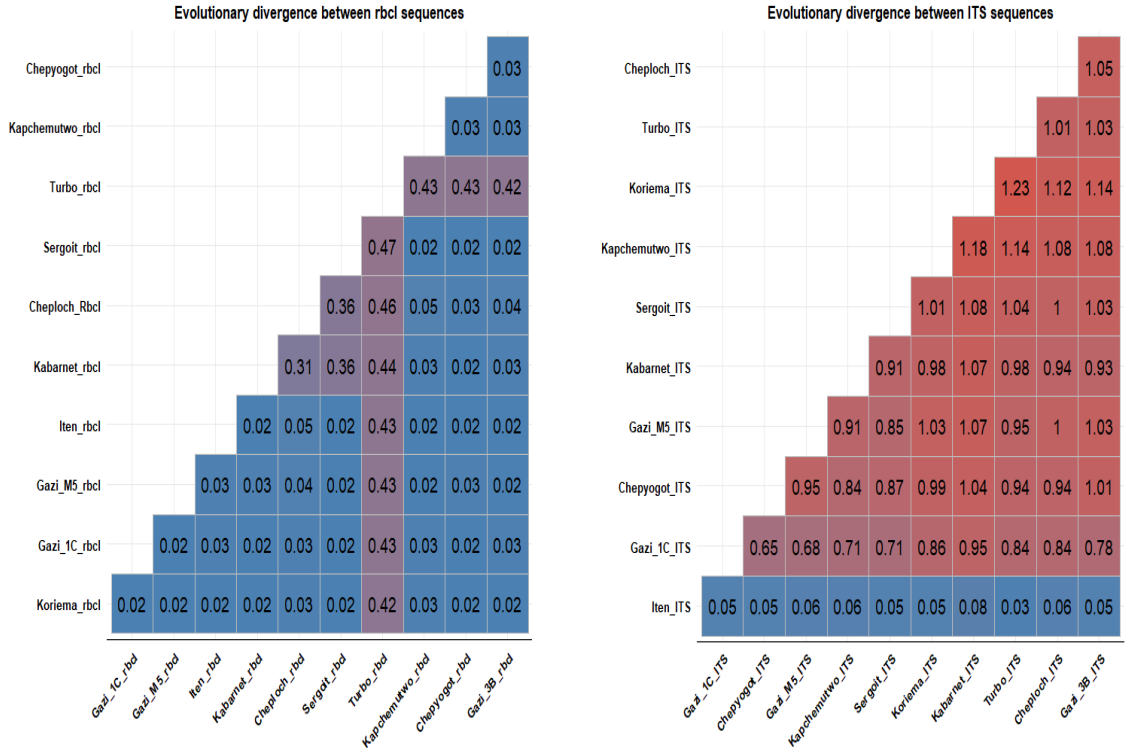

Figure 4.7: Evolutionary divergence of *ITS1* and *rbcL* genes

4.1.4 Phylogenetic trees developed from *ITS*1 and *rbcL* gene sequences

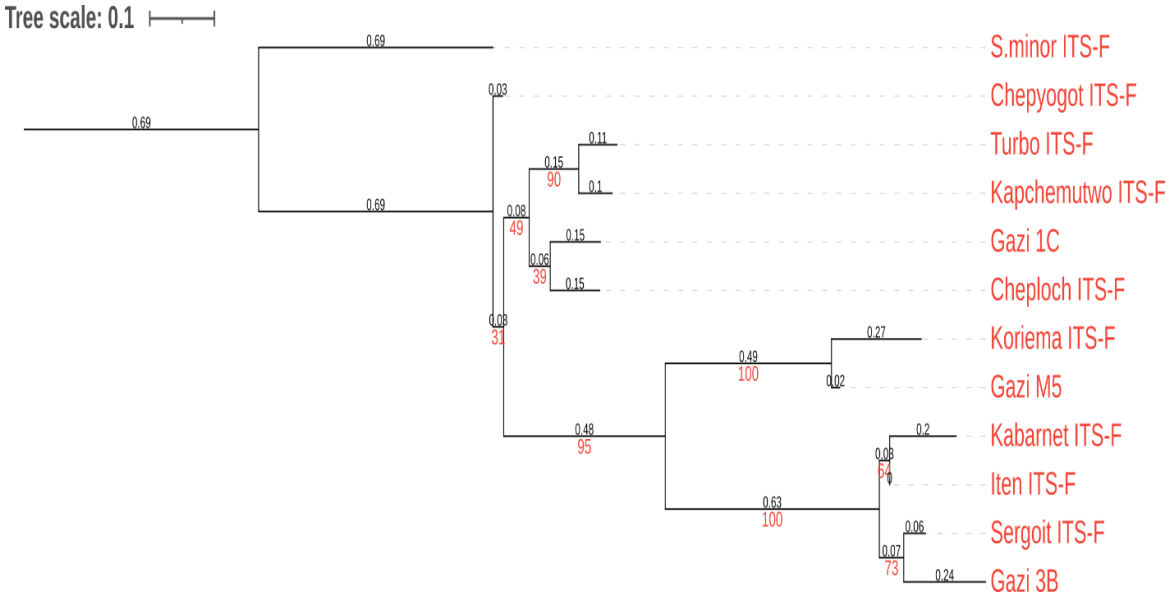

Figure 4.8: *ITS1* ML phylogenetic tree

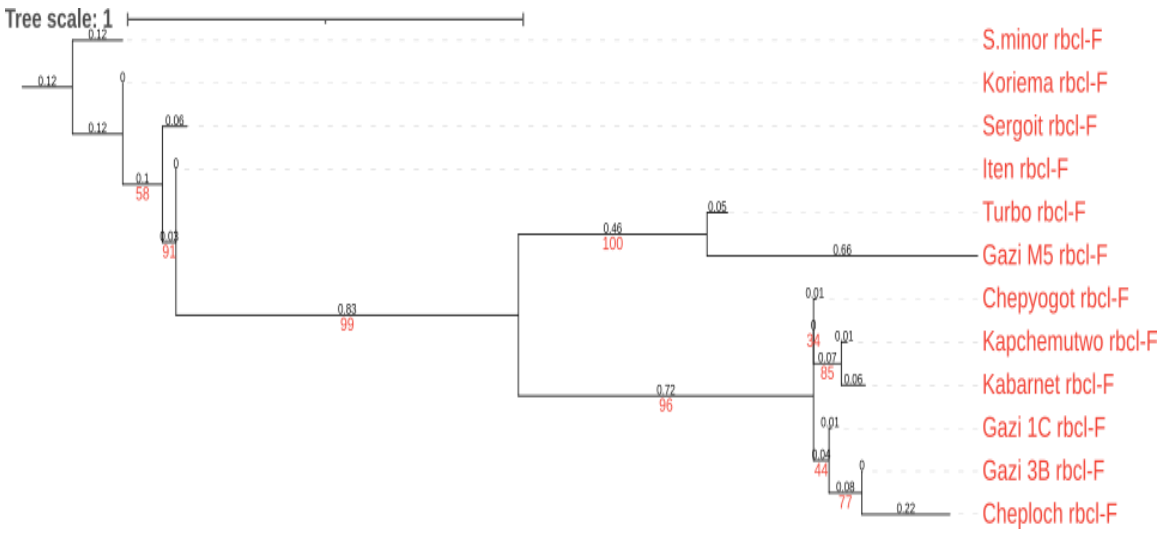

Figure 4.9: r*bcL* ML phylogenetic tree

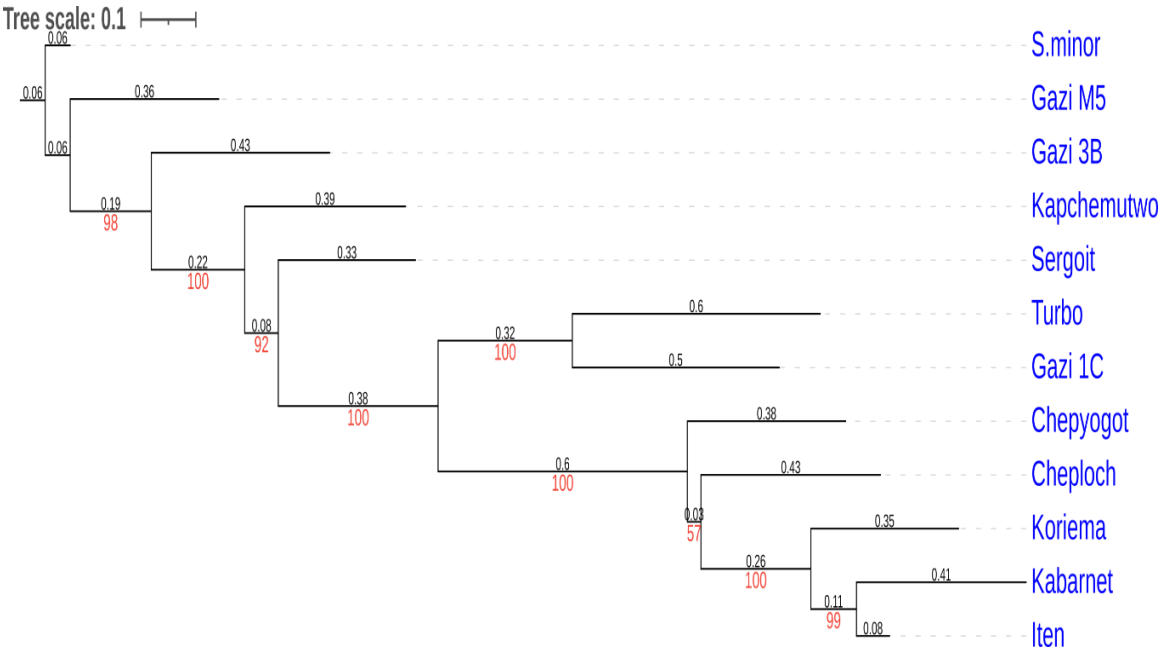

Figure 4.10*: rbcL*-F and *ITS1*-F concatenated ML tree

The *ITS1* phylogenetic tree shows one major and one minor clade. The major clade is made up of Turbo, Kapchemutwo, Gazi 1C, Cheploch, Koriema Gazi M5, Kabarnet, Iten, Sergoit and Gazi 3B populations whereas the minor clade is made up of only Chepyogot population. From the major clade, it is evident from one subclade that there is a closer relationship between the Coastal populations represented by Gazi 3B and Gazi M5 and the Upland populations represented by Koriema, Kabarnet, Iten, and Sergoit. This was evidenced by a high bootstrap value of 95 in the phylogenetic tree (Figure 4.8). However, the other subclade had a low bootstrap value of 49, represented by Gazi 1C, Turbo, Kapchemutwo, and Cheploch populations, which casts doubt on their ancestral relationship, as this clade was poorly supported. One Coastal population, Gazi M5, and Koriema, an Upland population, formed a clade that had a strong bootstrap value of 100, implying a strong support for that clade. The Chepyogot (Baringo County) population was

distantly related to all other populations studied. A closer phylogenetic relationship amongst the *Dodonaea viscosa* populations was also observed from the *rbcL* tree. All the Coastal populations, with most other Upland populations, were clustered under one clade, meaning they are monophyletic. This is backed up by a robust bootstrap value of 99. Koriema, an Upland population, was distantly related to the rest. Most clades of this phylogenetic tree were strongly supported with values of more than 70.

The branch lengths of the *ITS1* and *rbcL* cladograms were very short, indicating a narrow genetic distance among the various populations. The concatenated phylogenetic tree further supports that the Upland and Coastal *Dodonaea* populations are closely related. Most of the bootstrap values were more than 70, with a good number ranging from 90 to 100, implying strong support. However, only one clade, represented by Cheploch, Kabarnet, Koriema, and Iten populations, was moderately supported with a bootstrap value of 57.

4.1.5 Principal component analysis of *Dodonaea viscosa* populations

PCA identified two principal components (PC1 and PC2) among the *rbcL* and *ITS1* sequences, accounting for most of the variations seen in the *Dodonaea* populations. Moreover, it was also possible to identify which characters of *Dodonaea* contributed more to most of the variations (Quality of representation (Cos2) to the principal components (Figure 4.11). Cos2 shows that Gazi M5 *rbcL*, Gazi 3B *rbcL*, Gazi 1C *rbcL*, Kapchemutwa *rbcL*, Iten *rbcL*, Chepyogot *rbcL*, and Koriema *rbcL* contributed to most of the variation observed in the study populations. The PC1 accounted for 86.3% of the overall variation seen in *Dodonaea* populations. In factoring the *rbcL* genes, PCA grouped Sergoit *rbcL*, Cheploch *rbcL*, and Kabarnet *rbcL* populations in one cluster from the other *rbcL* groups.

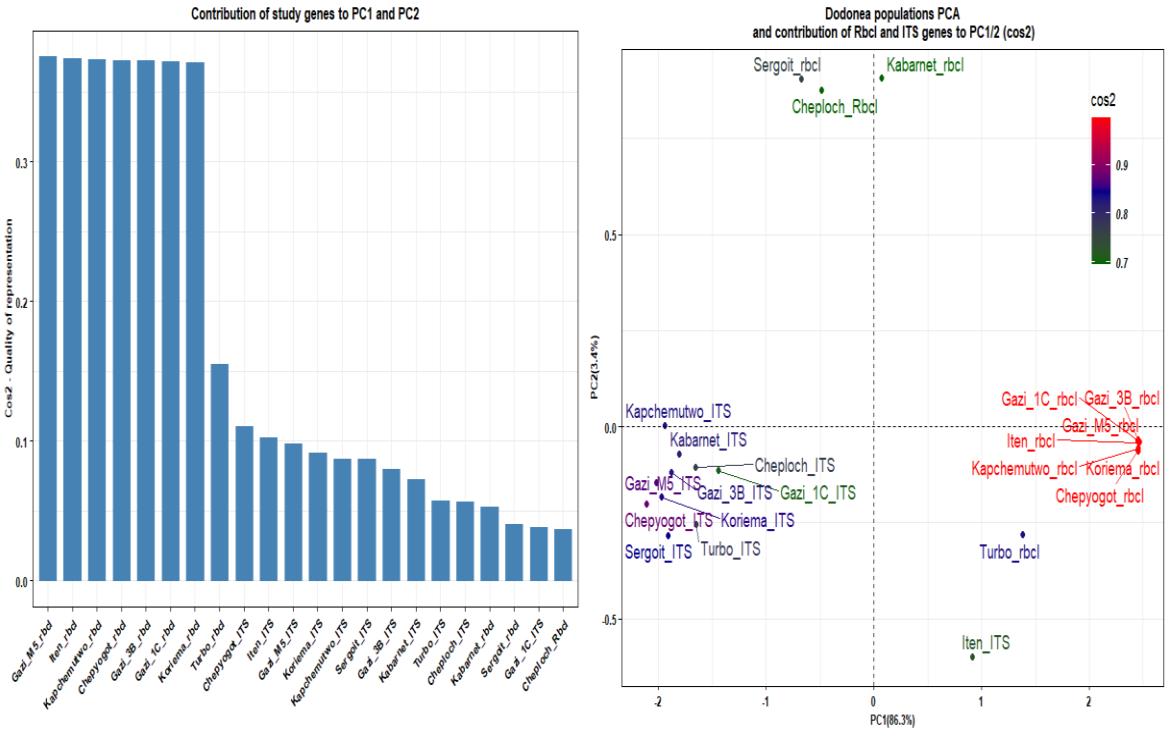

Figure 4.11*:* Quality of representation (Cos2) to PC1 and PC2

These groupings are represented by the dendrogram in Figure 4.12, which shows that *rbcL* genes separate *Dodonaea viscosa* populations into two clusters, where Sergoit, Cheploch, and Kabarnet groups have been separated from the rest of the groups based on *rbcL* genes. The *ITS1* genes have low representation on the overall variation, and PCA analysis of *ITS1* genes has grouped all *Dodonaea* populations in one cluster with Iten *ITS* population treated as an outlier.

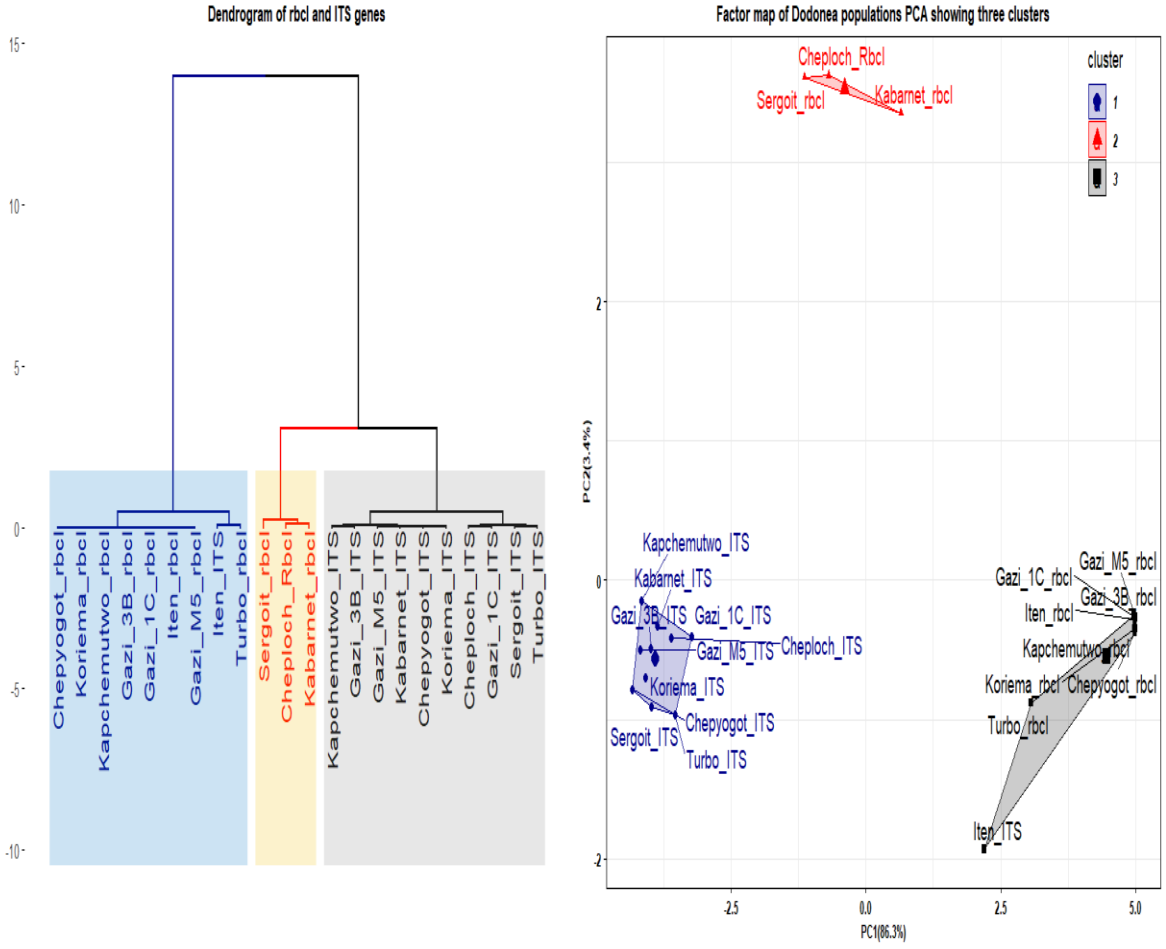

Figure 4.12: Dendrogram and Factor map of *Dodonaea* populations

4.2 Phytochemical isolation of pure compounds in *Dodonaea viscosa*

One population of *Dodonaea viscosa* was selected for phytochemical analysis. This were the leaves of the Gazi 1C population (Kwale County). Preliminary Thin Layer Chromatography (TLC) analysis showed that *Dodonaea viscosa* is a rich plant in phytochemicals. About 973g of *Dodonaea viscosa* leaf extract from the Gazi 1C population (Kwale County) gave 25g of a blue-black crude extract representing 25.6% of the total weight. A total of 417 fractions of the leaves of approximately 300 ml were collected in small vials. TLC was used to observe the separations and viewed under UV light and Iodine. Those fractions with similar profiles based on spot formation were combined. Pure compounds formed single spots on the TLC plates. NMR was used to identify the probable structure of the compound detected in pure compounds. Four compounds were isolated from the leaves of *Dodonaea viscosa* Gazi 1C population. The compounds were identified as: 2-(4,5-dihydroxy-2-methoxyphenyl)-5,7-dihydroxyl-3,6-dimethoxy-4H-chromen-4-one (Zhang *et al*., 2012) (1), Hautriwaic Acid (Salinas-Sánchez *et al*., 2012) (2), 5,7,4^{I}-trihydroxy-(3^{II}-methyl-2-buten-1yl)-3- methoxyflavanol (Zhang *et al*., 2012) (3), and Mkapwanin (4) (Omosa *et al*., 2010).

4.2.1 Characteristics of isolated compounds

i) **2-(4,5-dihydroxy-2-methoxyphenyl)-5,7-dihydroxyl-3,6-dimethoxy-4H-chromen-4-one**

Compound **1** was isolated as a pale yellow solid and assigned the molecular formula of $C_{18}H_{16}O_9$ which corresponds to a flavanol derivative. TLC showed a blue spot under UV light (254 nm). The ^{1}H NMR showed a downfield shifted singlet signal at δ_H 12.74 ppm,

indicating OH intramolecular hydrogen bonding. The spectrum showed the presence of the three sets of aromatic protons at δ_H 7.25 ppm, δ_H 6.66 ppm and δ_H 6.60 ppm. Peaks at δ_H 5.36 ppm indicated the presence of residual protons of the solvent CD_2Cl_2 while the peaks at 1.5 ppm indicated the D2O exchangable. Signals at δ_H 4.05 ppm, δ_H 3.98 ppm and δ_H 3.88 ppm indicated the presences of methoxy groups (Appendix 1 A). The ^{13}C NMR showed a signal at δ_C 178.24 ppm, indicating the presence of a flavonoid carbonyl. There was a presence of aromatic carbons from δc 90 -160 ppm. There was also presence of quaternary carbons. Signals at δc 62.38, δc 61.24 and δc 56.63 ppm indicating the presence of methoxy carbon atoms (Appendix 1 B). The compound was identified as 2-(4,5-dihydroxy-2-methoxyphenyl)-5,7-dihydroxyl-3,6-dimethoxy-4H-chromen-4-one (Zhang *et al*., 2012).

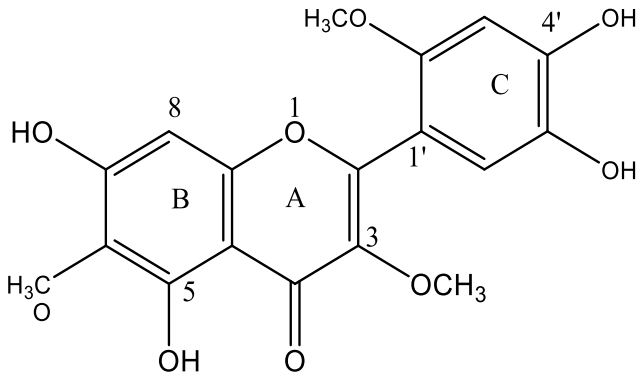

Figure 4.13: Structure of 2-(4,5-dihydroxy-2-methoxyphenyl)-5,7-dihydroxyl-3,6-dimethoxy-4H-chromen-4-one

Table 4.2: H (500 MHz) and ^{13}C (125 MHz) NMR spectral data for 2-(4,5-dihydroxy-2-methoxyphenyl)-5,7-dihydroxyl-3,6-dimethoxy-4H-chromen-4-one

Position	δ_H (*m*) Experimental	δ_H (*m*) (Zhang *et al*., 2012)	δ_C Experimental	δ_C (Zhang *et al*., 2012)
1				
2			153.32	157.4
3			138.27	138.7
4			178.24	179.4
5	12.74(1H, s)	13.04(1H, s)	152.29	153.6
6	3.98(3H, s)	3.88(1H, s)	136.92	131.8
7	6.60(1H, s)	9.12(1H, s)	155.86	157.7
8	7.25(1H, brd s)	6.57(1H, s)	93.79	94.4
9			151.67	153.0
10			102.64	106.3
1^I			110.53	122.3
2^I	6.64(2H, d)	7.78(1H, s)	113.77	130.6
3^I			150.81	127.0
4^I	7.88(1H, s)	9.74(1H, brs)	156.43	158.0
5^I			130.68	131.4
6^I	6.64(2H, d)	7.86(1H, s)	140.38	129.8
OCH_3-3	3.98(3H, s)	3.86(3H, s)	61.24	60.1
OCH_3-6	4.05(3H, s)	3.88(3H, s)	62.30	60.7
OCH_3-5^I	3.87(3H, s)		56.63	

ii) **Hautriwaic Acid**

Compound **2** was isolated as a colourless amorphous powder and assigned the molecular formula of $C_{20}H_{28}O_4$. It is a diterpene derivative compound consisting of carboxylic, methoxyl group and furan ring (Hamed Al Bimani & Hossain, 2020). The ^{1}H NMR spectra showed a downfield shifted singlet signal at δ_H 7.39 ppm, indicating hydroxyl. ^{1}H-NMR (500 MHz, CD_2Cl_2): δ_H 7.25 (1H, m, H-15/16), δ_H 3.79 (1H, dd, H-19), δ_H 0.91 (3H, d, H-17), δ_H 0.79 (3H, s, H-20) (Appendix 2 A). ^{13}Carbon-NMR showed a carboxylic carbonyl signal at 173.54 ppm. Morever, sp^2 carbon ranged from 111.41 ppm – 145.07 ppm, one methoxy carbon at 75.07 ppm while the rest of the signals indicated the presence of sp^3 carbons. There was also some isotopic impurity of the solvent used at a signal of δ_C 53.30 ppm. The ^{13}C-NMR (Appendix 2 B) data (125 MHz, CD_2Cl_2): δ_C 173.54 (C-18), δ_C 145.07 (C-15), δ_C 143.17 (C-4), δ_C 138.93 (C-16), δ_C 140.00 (C-3), δ_C 126.02 (C-13), δ_C 111.41 (C-14), δ_C 75.07 (C-19), δ_C 46.18 (C-10), δ_C 45.18 (C-9), δ_C 39.22 (C-5), δ_C 39.13 (C-11), δ_C 36.25 (C-8), δ_C 34.27 (C-6), δ_C 27.96 (C-2), δ_C 18.3 (C-7), δ_C 17.72 (C-20), δ_C 17.66 (C-12), δ_C 16.77 (C-1), δ_C 15.79 (C-17). Thus the compound was indicated as Hautriwaic acid (Salinas-Sánchez *et al*., 2012).

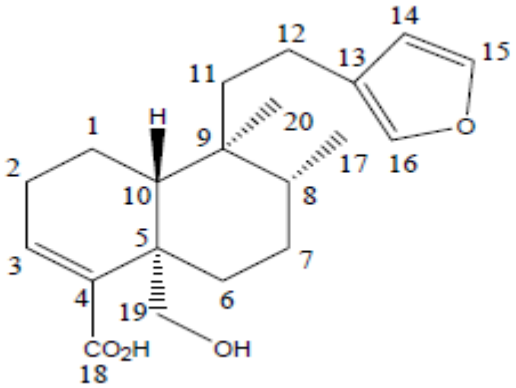

Figure 4.14: Structure of Hautriwaic acid

Table 4.3: H (500 MHz) and ^{13}C (125 MHz) NMR spectral data for Hautriwaic acid

Position	δ_H (*m*) Experimental	δ_H (*m*) (Salinas-Sánchez *et al*., 2012)	δ_C Experimental	δ_C (Salinas-Sánchez *et al*., 2012)
1			16.77	16.5
2			27.96	26.3
3		6.67(IH, t)	140.0	138.0
4			143.17	141.1
5			39.22	38.3
6			34.27	30.79
7			18.3	26.2
8			36.25	35.8
9			45.18	41.5
10			46.18	45.9
11			39.13	38.2
12			17.66	17.8
13			126.02	124.9
14		6.25(1H, m)	111.41	110.5
15	7.25(2H, d)	7.35(1H, m)	145.07	142.4
16	7.25(2H, d)	7.20(1H, m)	138.93	138.0
17	0.91(3H, d)	0.87(3H, d)	15.79	15.3
18			173.54	172.13
19	3.79(1H, dd)	4.141H,d)	75.07	64.5
20	0.79(3H, s)	0.76(3H, s)	17.72	18.1
19-0H	7.39(IH, s)			

iii) **5,7,4^{I}-trihydroxy-(3^{II}-methyl-2-buten-1yl)-3- methoxyflavone**

Compound **3** was isolated as a pale yellow powder and assigned the molecular formula $C_{22}H_{22}O7$. The ^{1}H and ^{13}C NMR spectroscopic data of the compound indicated this compound to be a flavonol derivative which comprised of one isoprenoid and one methoxy group (Zhang *et al*., 2012). From the ^{1}H NMR data, a sharp singlet on the downfield indicated the presence of a phenolic hydroxyl group. Aromatic sp^2 protons on ring A showed signals at δ_H 7.25 ppm to δ_H 6.25 ppm while for ring B the protons indicated symmetry at δ_H 7.62 ppm with unresonated broad signal. The ^{1}H NMR (Appendix 3 A) signals at δ_H 6.24 (1H, d, J = 1.2 Hz, H-6), δ_H 6.46 (1H, d, J = 1.2 Hz, H-8), δ_H 3.50 (2H, brd, J = 7.4 Hz, H2-1″), δ_H 5.68 (1H, brt, J = 7.4 Hz, H-2″), δ_H 1.77 (3H, brs, H3-4″), and δ_H 4.02 (2H, brs, H2-5″). From the ^{13}C NMR, (Appendix 3 B), a ketonic carbonyl showed a signal at δ_C 196.5 ppm. Two methoxy carbons attached to aromatic rings were assigned δ_C 58.40 ppm and δ_C 54.76 ppm while the sp^3 carbons showed signals at δ_C 24.14 ppm to δ_C 16.11 ppm. The sp^2 carbons were allocated from a range of δ_C 92.68 ppm to δ_C 163.5 ppm. The compound was indicated as 5,7,4^{I}-trihydroxy-(3^{II}-methyl-2-buten-1yl)-3-methoxyflavone.

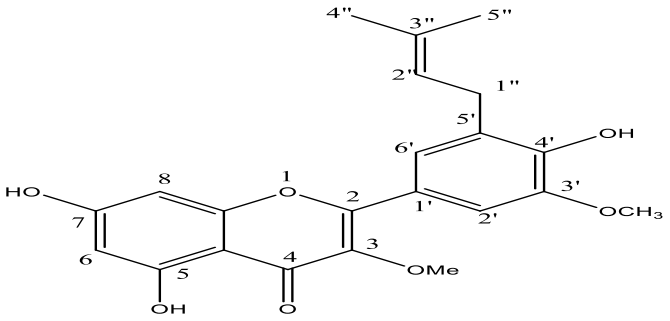

Figure 4.15: Structure of 5,7,4^{I}-trihydroxy-(3^{II}-methyl-2-buten-1yl)-3-methoxyflavone

Table 4.4: H (60 MHz) and ^{13}C (15 MHz) NMR spectral data for 5,7,4^{I}-trihydroxy-(3^{II}-methyl-2-buten-1yl)-3- methoxyflavone

Position	δ_H (*m*) Experimental	δ_H (*m*) (Zhang *et al*., 2012)	δ_C Experimental	δ_C (Zhang *et al*., 2012)
1			102.5	
2				157.0
3				139.1
4			196.5	179.9
5	12.74(1H, s)	12.82(1H, s)	146.1	153.6
6	6.25(1H, d)	6.24(1H, d)		131.7
7		---d	163.5	157.7
8	7.25(1H, brd s)		92.68	94.5
9				157.7
10			97.51	105.8
1^{I}			120.21	122.8
2^{I}	7.62(2H, brs)	7.82(2H. s)		128.5
3^{I}				128.9
4^{I}		---d	156.02	156.0
5^{I}				128.7
6^{I}	7.62(2H, brs)	7.82(2H, s)		128.5
1^{II}		3.50(2H, brd)	28.58	29.2
2^{II}	5.39(1H, t)	5.68(1H, brt)	108.21	122.7
3^{II}				133.9
4^{II}	1.73(3H, brs)	1.75(3H, brs)	16.11	17.5
5^{II}	1.77(3H, brs)	1.78(3H, brs)	24.24	25.9
OCH_3-3	3.94(3H, s)	3.86(3H, s)	58.40	60.1
OCH_3-3^{I}	3.87(3H, s)		54.76	60.67

iv) **Mkapwanin**

Compound **4** was isolated as a colourless oil and showed an oil patch on the TLC plate. It was then assigned a molecular formula of $C_{21}H_{26}O_4$ based on the ^1H NMR which showcased an integral value belonging to twenty six protons and in comparison to an existing reported literature (Omosa *et al*., 2010). The ^1H NMR (Appendix 4 A) showed a downfield shifted signals as δ_H 7.19(t) and δ_H 6.82(dd), indicating vinylic protons. The spectrum showed the presence methylene protons ranging from δ_H 2.5 ppm to δ_H 1.3 ppm and two methyl moieties at δ_H 0.88 ppm and δ_H 0.68 ppm. Peaks at δ_H 7.33 ppm indicated the presence of residual protons of the solvent while signals at δ_H 4.44(dd), δ_H 3.91(d) indicated the presences of protons in ortho position of an oxo group. The structure of the compound was elucidated as Neo-Clerodan-3,13-dien-16,15: 18,19-diolide which is also called Mkapwanin (Omosa *et al*., 2010). The ^{13}C NMR, of this compound was not detectable due to the minimal amount of the isolated compound.

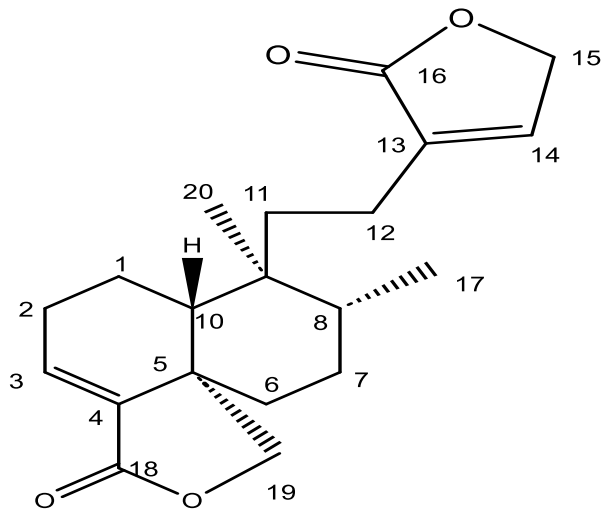

Figure 4.16: Structure of Mkapwanin

Table 4.5: H (60 MHz) and ^{13}C (125 MHz) NMR spectral data for Mkapwanin

Position	δ_H (*m*) Experimental	δ_H (*m*) (Omosa *et al*., 2010)
1	2.0-1.3(m)	1.17(1H, m), 1.07(1H, m)
2	2.42(1H, m), 2.32(1H, m)	2.40(1H, m), 2.23(1H, m)
3	6.82(1H, dd)	6.67(IH, t)
4		
5		
6	2.5-1.3(m)	1.93(1H, m), 1.25(1H, m)
7	2.5-1.3(m)	1.62(1H, m), 1.51(1H, m)
8		
9		
10	2.5-1.3(m)	1.75(1H, m)
11	2.5-1.3(m)	1.60H, m)
12	2.27(1H, m), 2.12(1H, m)	2.24(1H, m), 2.04(1H,m)
13		
14	7.19(1H, t)	7.14(1H, t)
15	4.86(1H, brd)	4.79(1H, d)
16		
17	0.88(3H, brd)	0.86(3H, d)
18		
19	4.44(1H, dd), 3.91(1H, dd)	4.30(1H,d), 3.92(1H, dd)
20	0.68(3H, s)	0.62(3H, s)

4.3 Biological activities of *Dodonaea viscosa* populations

4.3.1 Antidiabetic activity of populations of *Dodonaea viscosa*

The results of antidiabetic activity of *Dodonaea viscosa* were tabulated in Table 4.2. This also included results of their controls and the reference drug (insulin). The results were means of the blood sugar of 4 male rats presented in *mmol/L* including their standard deviations during the entire study period.

Table 4.6: Overall results (mmol/L) of antidiabetic activity of *Dodonaea viscosa* populations

Treatments	Duration	SERGOIT				GAZI 1C				CHEPYOGOT				KABARNET				TURBO				KORIEMA			
		ROOTS		LEAVES		ROOTS		LEAVES		ROOTS		LEAVES		ROOTS		LEAVES		ROOTS		LEAVES		ROOTS		LEAVES	
		Mean	STD	Mean	STD	Mean	STD	Mean	STD	Mean'	STD	Mean	STD	Mean	STD	Mean	STD	Mean	STD	Mean	STD	Mean	STD	Mean	STD
Group I	0Hr	7.7	1.2	7.7	1.3	7.4	1.1	7.7	0.2	4.9	0.6	7.0	0.8	7.6	0.7	8.0	0.7	5.4	0.4	7.9	0.6	7.6	0.5	7.3	0.3
	3Hr	7.9	0.5	8.0	0.4	7.9	0.5	8.1	0.8	5.3	0.5	6.6	0.2	7.6	0.4	7.9	0.6	5.4	0.5	8.0	0.9	8.5	0.3	7.6	0.7
	6Hr	8.0	0.1	8.0	0.1	8.3	0.4	7.8	0.6	5.3	0.2	7.5	0.9	7.3	0.5	7.4	0.9	5.6	0.4	8.3	1.1	8.3	0.6	7.2	0.5
	12Hr	7.9	0.4	7.8	0.6	7.5	0.5	7.8	0.8	5.4	0.4	7.3	0.8	7.5	0.8	7.6	0.9	5.4	0.7	8.5	0.5	8.5	0.5	7.1	0.7
	24Hr	8.0	0.4	7.9	0.6	7.8	0.3	7.7	0.7	5.3	0.2	7.0	0.6	7.9	1.3	7.9	1.1	5.5	0.3	8.6	0.3	8.9	0.7	7.0	0.4
Group II	0Hr	16.7	0.8	18.4	0.8	18.9	1.1	19.8	1.2	19.3	1.3	18.6	1.2	18.8	1.0	19.5	1.7	18.1	2.4	19.8	0.8	18.2	1.8	19.9	1.2
	3Hr	18.3	0.8	19.8	1.3	20.3	0.8	20.9	1.4	20.8	1.2	21.2	0.9	21.7	3.4	20.6	1.8	18.9	2.7	21.0	0.7	19.8	0.9	22.1	0.5
	6Hr	17.1	0.4	17.6	1.6	18.3	1.1	19.0	0.4	19.4	0.3	22.8	2.9	20.9	1.6	19.7	1.0	18.1	2.4	19.7	0.6	19.7	0.3	20.4	1.4
	12Hr	19.4	0.5	19.8	0.9	20.0	0.6	21.1	1.2	21.1	1.4	21.5	0.8	22.2	3.2	20.1	0.4	21.2	4.6	20.6	0.8	19.5	1.1	21.1	1.4
	24Hr	17.6	0.5	18.6	0.7	19.5	0.3	18.3	1.0	18.8	0.8	19.7	1.1	20.4	1.5	17.6	0.8	21.7	2.9	18.2	0.8	19.0	0.9	20.6	1.3
Group III	0Hr	16.5	0.7	19.0	0.8	15.1	2.6	20.5	2.2	15.2	1.0	19.9	1.3	12.6	1.7	17.4	0.8	18.9	1.0	18.9	1.3	17.9	5.1	17.4	0.9
	3Hr	7.0	0.4	6.3	0.3	7.8	1.5	6.8	0.5	9.0	0.3	6.8	0.5	9.0	1.4	6.6	0.5	9.3	0.5	7.3	0.1	8.5	0.9	7.1	1.1
	6Hr	6.5	0.4	5.7	0.2	6.9	0.5	6.2	0.5	8.5	0.1	6.2	0.6	7.8	0.9	6.2	0.3	8.3	0.4	6.9	0.1	7.3	0.9	6.6	0.6
	12Hr	5.9	0.5	5.1	0.2	5.5	0.5	5.6	0.4	8.2	0.1	5.7	0.3	6.2	0.7	5.8	0.5	8.2	0.5	5.8	0.3	6.1	0.8	5.4	0.5
	24Hr	5.4	0.5	4.4	0.4	5.0	0.3	4.9	0.4	7.9	0.1	5.3	0.3	5.7	0.7	5.5	0.4	7.8	0.4	5.4	0.4	5.3	0.1	5.2	0.2
Group IV	0Hr	18.2	0.6	16.8	0.7	16.3	1.3	18.8	0.6	16.2	0.9	17.3	0.8	19.5	1.8	19.4	1.3	19.0	1.5	18.2	0.8	17.4	0.7	18.7	0.9
	3Hr	10.8	0.6	7.0	0.2	11.3	1.7	9.2	1.5	22.8	1.3	9.0	1.1	14.4	3.1	13.7	3.9	20.1	1.0	11.5	0.6	12.7	2.5	11.6	2.3
	6Hr	8.4	0.7	6.6	0.2	9.8	0.7	8.5	1.0	13.1	0.5	7.7	1.1	11.5	1.3	10.1	2.6	13.0	1.4	9.2	0.4	10.5	0.5	9.9	1.0

Group V	12Hr	7.3	0.2	5.8	0.5	7.9	0.9	7.1	0.3	11.7	1.2	6.3	0.3	10.5	0.7	8.4	1.2	10.0	0.6	7.7	0.7	8.4	1.5	8.4	1.6
	24Hr	5.7	0.4	5.3	0.4	5.4	0.4	6.1	0.6	8.3	0.9	5.8	0.4	4.8	0.3	6.7	0.6	9.0	1.1	5.1	0.2	5.4	0.4	6.1	0.3
	0Hr	18.8	0.8	17.9	1.4	18.1	2.3	17.1	1.3	23.5	2.6	18.3	1.0	19.8	0.9	19.7	0.9	19.8	1.2	18.8	1.0	22.5	2.9	18.1	1.1
	3Hr	7.0	0.2	6.6	0.5	9.7	0.8	6.6	0.6	20.1	10.5	18.3	2.1	8.2	1.4	10.2	0.7	24.1	1.8	9.0	1.9	10.6	0.8	7.3	0.4
	6Hr	6.7	0.2	5.9	0.4	8.7	0.3	6.4	0.6	10.6	0.1	6.8	0.4	7.6	0.8	8.7	0.3	10.3	0.4	7.7	1.0	9.8	0.4	6.8	0.2
	12Hr	6.4	0.3	5.4	0.5	6.5	0.4	6.0	0.7	10.0	0.4	5.5	0.2	5.6	0.9	6.1	0.6	9.0	0.4	5.8	0.5	6.3	0.4	6.4	0.4
	24Hr	5.9	0.3	5.2	0.5	5.5	0.4	5.7	0.8	6.0	0.2	5.4	0.2	4.2	0.2	5.8	0.7	6.1	0.6	5.4	0.4	4.8	0.5	4.9	0.5

Table 4.6 shows that the rats in group I (normal control) had a low blood sugar level (below 11mmol/L) for the entire study period. Those in group II (Diabetic control) remained diabetic (above 11mmol/L) due to the effect of induced Streptozotocin (STZ). Diabetic rats treated with insulin hormone in group III reduced their sugar levels significantly to normal. The rats in groups IV and V, who were diabetic, were treated with *Dodonaea viscosa* crude extract of leaves and roots at corresponding doses of 200 mg/kg bw and 400 mg/kg bw respectively. The rats in these two groups also registered reduced blood sugar levels to normal from the 3rd to the 24th hour after treatment. The activity of roots and leaves of *Dodonaea viscosa* from different populations was compared using a paired sample t-test. Table 4.7 displays the following findings obtained:

Table 4.7: Comparison of the activity of roots and leaves

Population	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference		t	Df	Sig. (2-tailed)
				Lower	Upper			
SERGOIT ROOTS & LEAVES	.34200	1.24086	.24817	-.17020	.85420	1.378	24	.181
GAZI 1C ROOTS & LEAVES	.06700	1.63546	.32709	-.60808	.74208	.2050	24	.839
CHEPYOGOT ROOTS & LEAVES	1.32700	3.83749	.76750	-.25704	2.91104	1.729	24	.097
KABARNET ROOTS & LEAVES	.11600	1.64617	.32923	-.56351	.79551	.3520	24	.728
TURBO ROOTS & LEAVES	1.39100	4.01060	.80212	-.26449	3.04649	1.734	24	.096
KORIEMA ROOTS & LEAVES	.46600	1.61511	.32302	-.20069	1.13269	1.443	24	.162

From the comparison of the activity of roots and leaves, it was revealed that there was no significant difference between their activities. This was evident from the level of significance, which was >0.05. The activity of the different populations was also compared to find out the most effective population in reducing the blood sugar level. Table 4.8 shows the findings obtained.

Table 4.8: Comparison of the activity of different populations

Population	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
SERGOIT	50	10.4150	5.48297	.77541	8.8568	11.9732	4.40	19.77
GAZI 1C	50	10.9655	5.68194	.80355	9.3507	12.5803	4.88	21.10
CHEPYOGOT	50	11.9975	6.44692	.91173	10.1653	13.8297	4.88	23.50
KABARNET	50	11.5040	5.87524	.83088	9.8343	13.1737	4.22	22.20
TURBO	50	12.0185	6.10100	.86281	10.2846	13.7524	5.10	24.10
KORIEMA	50	11.4230	5.88139	.83175	9.7515	13.0945	4.83	22.45
Total	300	11.3873	5.89691	.34046	10.7173	12.0572	4.22	24.10

In this case, the F-statistic (F = 0.545) indicated that the variances between the groups are not statistically different. The high p-value (Sig. = 0.742) further confirmed this, because it exceeds the often-used significance limit of $\alpha < 0.05$.

The effects of *Dodonaea viscosa* extract at 200 mg/kg bw and 400 mg/kg bw on blood sugar decrease were also examined, as was the reference drug, insulin. The outcomes were displayed in Table 4.9:

Table 4.9: Comparison of the activities of Insulin, 200 mg and 400 mg/kg bw extracts

POPULATION		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean Lower Bound	Upper Bound	Minimum	Maximum
SERGOIT	Insulin	5	8.2150	4.65684	2.08260	2.4328	13.9972	5.35	16.48
ROOTS	200 mg/kg	5	10.0600	4.92064	2.20058	3.9502	16.1698	5.65	18.20
	400 mg/kg	5	8.9450	5.50963	2.46398	2.1039	15.7861	5.88	18.77
	Total	15	9.0733	4.73326	1.22212	6.4521	11.6945	5.35	18.77
SERGOIT	Insulin	5	8.0600	6.12642	2.73982	.4530	15.6670	4.40	18.95
LEAVES	200 mg/kg	5	8.3100	4.79298	2.14349	2.3587	14.2613	5.30	16.80
	400 mg/kg	5	8.1700	5.43767	2.43180	1.4182	14.9218	5.15	17.85
	Total	15	8.1800	5.07411	1.31013	5.3700	10.9900	4.40	18.95
GAZI 1C	Insulin	5	8.0450	4.11664	1.84102	2.9335	13.1565	4.95	15.13
ROOTS	200 mg/kg	5	10.1300	4.09197	1.82998	5.0492	15.2108	5.43	16.30
	400 mg/kg	5	9.6650	4.97027	2.22277	3.4936	15.8364	5.53	18.05
	Total	15	9.2800	4.18806	1.08135	6.9607	11.5993	4.95	18.05
GAZI 1C	Insulin	5	8.7800	6.59008	2.94717	.5973	16.9627	4.88	20.50
LEAVES	200 mg/kg	5	9.9200	5.08255	2.27299	3.6092	16.2308	6.05	18.75
	400 mg/kg	5	8.3400	4.92280	2.20154	2.2275	14.4525	5.65	17.13
	Total	15	9.0133	5.21421	1.34630	6.1258	11.9009	4.88	20.50
CHEPYOGOT	Insulin	5	9.7700	3.06275	1.36970	5.9671	13.5729	7.93	15.20
ROOTS	200 mg/kg	5	14.4000	5.48450	2.45274	7.5901	21.2099	8.25	22.78
	400 mg/kg	5	14.0350	7.40715	3.31258	4.8378	23.2322	6.03	23.50
	Total	15	12.7350	5.62882	1.45336	9.6179	15.8521	6.03	23.50
	Insulin	5	8.7650	6.26407	2.80138	.9871	16.5429	5.30	19.93

	200 mg/kg	5	9.2200	4.69735	2.10072	3.3875	15.0525	5.83	17.33
CHEPYOGOT LEAVES	400 mg/kg	5	10.8550	6.80880	3.04499	2.4008	19.3092	5.35	18.30
	Total	15	9.6133	5.62351	1.45199	6.4991	12.7275	5.30	19.93
KABARNET	Insulin	5	8.2500	2.75051	1.23007	4.8348	11.6652	5.70	12.58
ROOTS	200 mg/kg	5	12.1050	5.39555	2.41296	5.4055	18.8045	4.75	19.45
	400 mg/kg	5	9.0900	6.18025	2.76389	1.4162	16.7638	4.22	19.77
	Total	15	9.8150	4.93230	1.27351	7.0836	12.5464	4.22	19.77
KABARNET	Insulin	5	8.2750	5.10536	2.28319	1.9359	14.6141	5.45	17.38
LEAVES	200 mg/kg	5	11.6350	5.05030	2.25856	5.3642	17.9058	6.65	19.40
	400 mg/kg	5	10.0850	5.65289	2.52805	3.0660	17.1040	5.75	19.65
	Total	15	9.9983	5.08767	1.31363	7.1809	12.8158	5.45	19.65
TURBO	Insulin	5	10.4850	4.72218	2.11182	4.6216	16.3484	7.78	18.88
ROOTS	200 mg/kg	5	14.2150	5.06775	2.26637	7.9226	20.5074	9.03	20.08
	400 mg/kg	5	13.8600	7.69051	3.43930	4.3110	23.4090	6.13	24.10
	Total	15	12.8533	5.79952	1.49743	9.6417	16.0650	6.13	24.10
TURBO	Insulin	5	8.8500	5.66985	2.53563	1.8100	15.8900	5.40	18.90
LEAVES	200 mg/kg	5	10.3300	4.95339	2.21522	4.1796	16.4804	5.10	18.15
	400 mg/kg	5	9.3400	5.47459	2.44831	2.5424	16.1376	5.38	18.77
	Total	15	9.5067	5.01641	1.29523	6.7287	12.2847	5.10	18.90
KORIEMA	Insulin	5	9.0100	5.09836	2.28006	2.6795	15.3405	5.30	17.88
ROOTS	200 mg/kg	5	10.8900	4.55452	2.03684	5.2348	16.5452	5.35	17.43
	400 mg/kg	5	10.8000	6.93776	3.10266	2.1856	19.4144	4.83	22.45
	Total	15	10.2333	5.28287	1.36403	7.3078	13.1589	4.83	22.45
KORIEMA	Insulin	5	8.3200	5.13941	2.29842	1.9386	14.7014	5.15	17.40
LEAVES	200 mg/kg	5	10.9300	4.80193	2.14749	4.9676	16.8924	6.10	18.73
	400 mg/kg	5	8.6700	5.32134	2.37978	2.0627	15.2773	4.85	18.05
	Total	15	9.3067	4.86405	1.25589	6.6130	12.0003	4.85	18.73

The insulin hormone produced the greatest drop in blood sugar levels for every population, bringing them down to normal. Following insulin was 400 mg/kg bw and 200 mg/kg bw of plant extracts. Most of the populations of *Dodonaea viscosa* under study had significantly reduced blood sugar levels, except a few which had their blood sugar above

11 mmol/L. These included Turbo roots at 200 mg/kg bw, Kabarnet leaves at 200 mg/kg bw, Kabarnet roots at 200 mg/kg bw, and Chepyogot roots at both 200 mg/kg bw and 400 mg/kg bw. From this study, *Dodonaea viscosa* crude root and leaf extracts typically resulted in the reduction of rats' blood sugar levels under study. All the populations of *Dodonaea viscosa* studied showed this hypoglycemic activity.

4.3.2 Antimicrobial activity *Dodonaea viscosa* populations

The crude ethanolic extracts of *Dodonaea viscosa* populations were tested for their antimicrobial activities against three bacteria, *Bacillus cereus, Escherichia coli*, and methicillin-resistant *Staphylococcus aureus* (MRSA), and a fungus, *Candida albicans*. Among the three bacteria, there were two gram-positives (*S. aureus* and *B. cereus*) and one gram-negative, *E. coli*. Amoxicillin was used as a control for all bacteria, while Apron was used as a control for fungi. Each experiment was carried out three times, and the mean zone of inhibition.

The growth inhibition zones for *C. albicans* were 6 mm, 5 mm, and 3 mm at 100 mg/ml, 50 mg/ml, and 25 mg/ml, respectively, based on extracts obtained from the leaves of the Turbo population (Figure 4.17). At 12.5 mg/ml, the extract was unable to stop growth. The positive control prevented *C. albicans* growth by 17 mm. The growth of *B. cereus* was inhibited by 5 mm for 100 mg/ml. The same ZOI (5 mm) was observed at 50 mg/ml concentrations. This was followed by a diameter of 4 mm and 3 mm at 25 mg/ml and 12.5 mg/ml, respectively. The control prevented growth by 13 mm. The crude extract failed to

prevent the growth of *E.coli* at all concentrations. Finally, this extract also did not prevent MRSA growth, though inhibition was observed with the control at 20 mm.

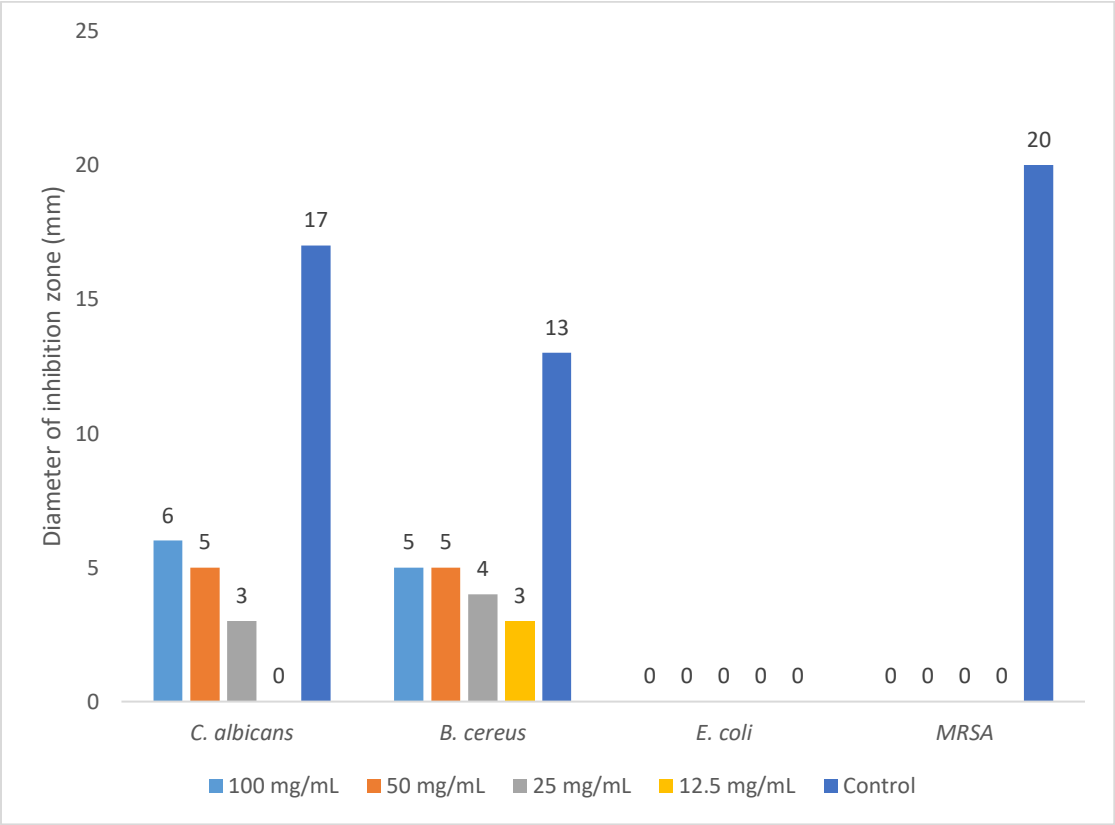

Figure 4.17: Growth inhibition zones of turbo leaves

The root extracts from *Dodonaea viscosa* Turbo population (Figure 4.18) also inhibited microbial growth in *C. albicans* and *B. cereus.* It prevented *C. albicans* from growing by 5 mm, 4 mm, and 2 mm at 100 mg/ml, 50 mg/ml, and 25 mg/ml, respectively. The lowest concentration (12.5 mg/ml) did not inhibit fungal growth, while its control showed 13 mm growth inhibition. Likewise, *B. cereus'*s growth was suppressed by the extract by 6 mm at 100 mg/ml and 5 mm for the other concentration, while its control inhibited growth by 13

mm. This extract did not prevent the growth of *E. coli* at all concentrations. Lastly, the growth of MRSA at all concentrations occurred except for its control at 21 mm.

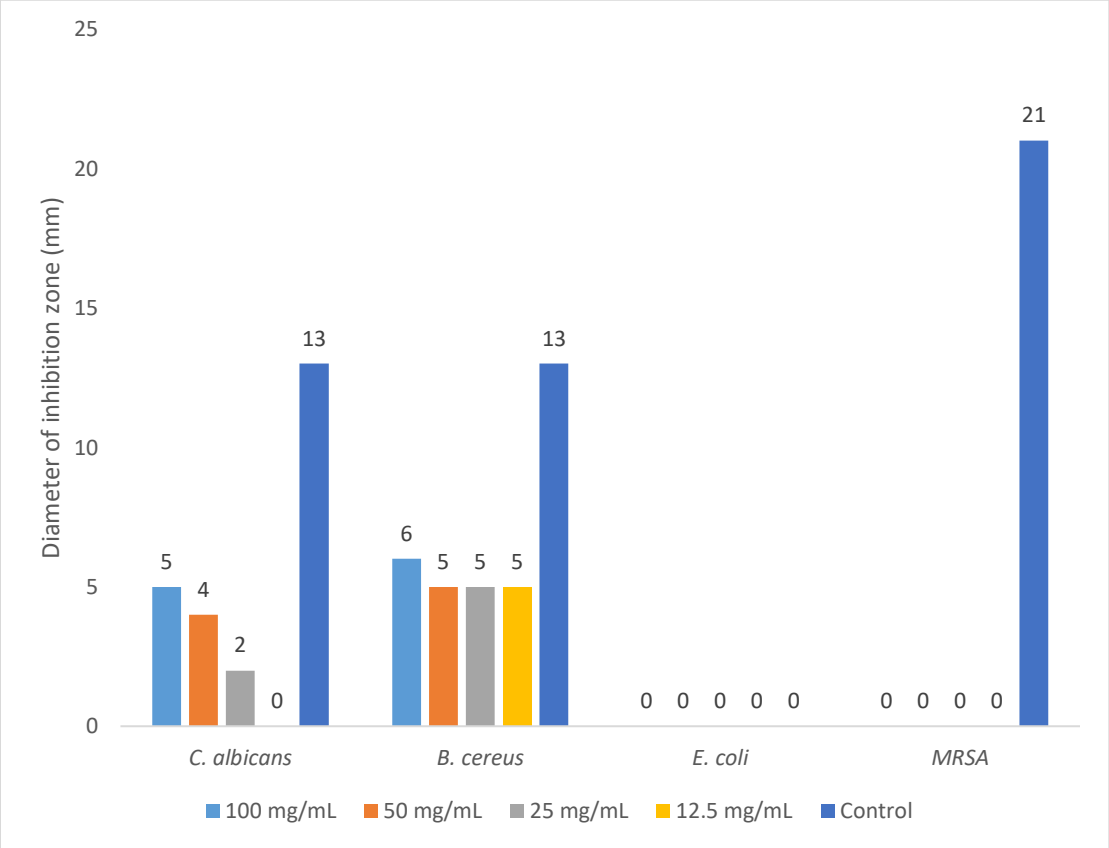

Figure 4.18: Growth inhibition zones of turbo roots

The leaves of the *Dodonaea viscosa* Gazi 3B population (Figure 4.19) also inhibited microbial growth. When the concentration of the extract was 100 mg/ml, 50 mg/ml, and 25 mg/ml, the growth of *Candida albicans* was inhibited by 5 mm, 3 mm, and 1 mm, respectively. Additionally, the extract prevented *B. cereus* from growing by 7 mm, 7 mm, 6 mm, and 5 mm at 100 mg/ml, 50 mg/ml, 25 mg/ml, and 12.5 mg/ml, respectively. Its control inhibited growth by 12 mm. This extract did not hinder the growth of *E. coli*.

Likewise, at 100 mg/ml, 50 mg/ml, 25 mg/ml, and 12.5 mg/ml, *Dodonaea viscosa* extract inhibited MRSA growth by 6 mm, 5 mm, 4 mm, and 4 mm, respectively. The highest inhibition zone by Gazi 3B leaves was observed in the control, which was 19 mm.

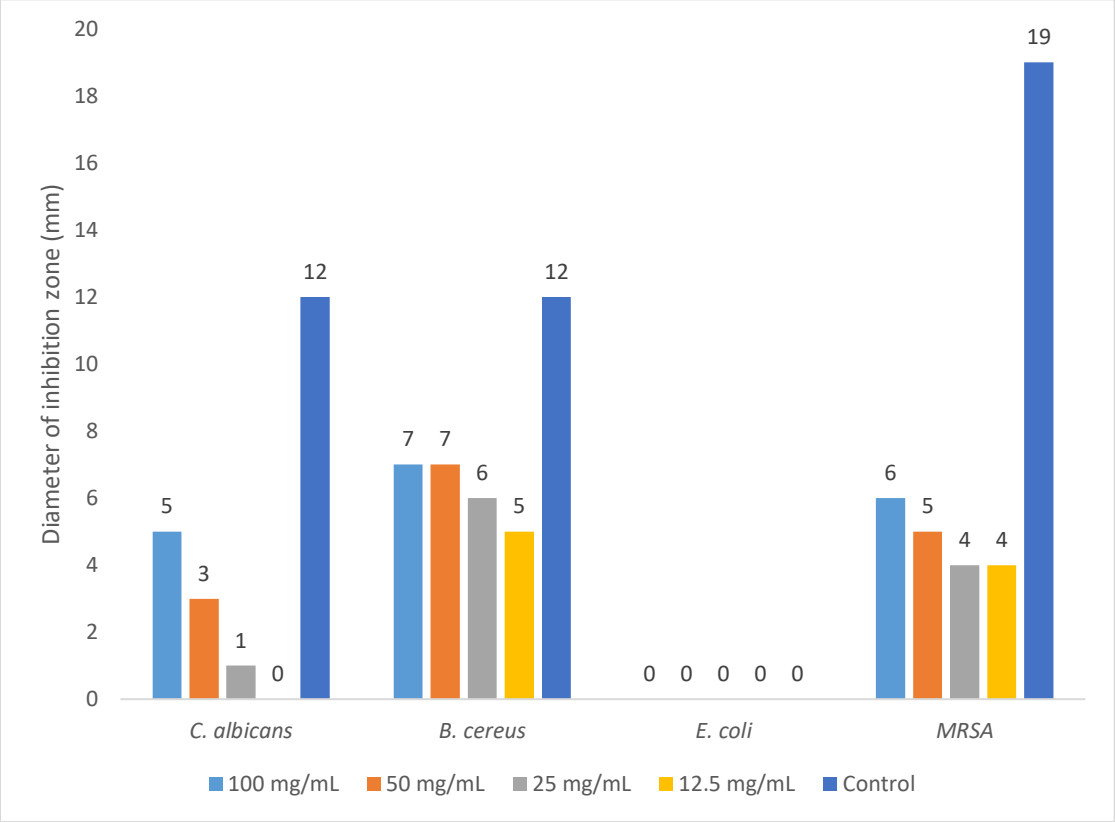

Figure 4.19: Growth inhibition zones of Gazi 3B leaves

The roots of the Gazi 3B population were also investigated for their antimicrobial activity (Figure 4.20). According to the findings, *Dodonaea viscosa* inhibited *C. albicans* growth at 100 mg/ml, 50 mg/ml, and 25 mg/ml by 7 mm, 4 mm, and 2 mm, respectively. A concentration of 12.5 mg/ml did not inhibit the growth of *C. albicans*, while its control inhibited growth at 13 mm. This plant extract also hindered *B.cereus* growth by 8 mm, 7

mm, 6 mm, and 5 mm at 100 mg/ml, 50 mg/ml, 25 mg/ml, and 12.5 mg/ml, respectively. Its control hindered growth by 12 mm. This extract also did not prevent the development of *E. coli* and MRSA on their media. The control for MRSA prevented the growth of this bacterium by 20 mm.

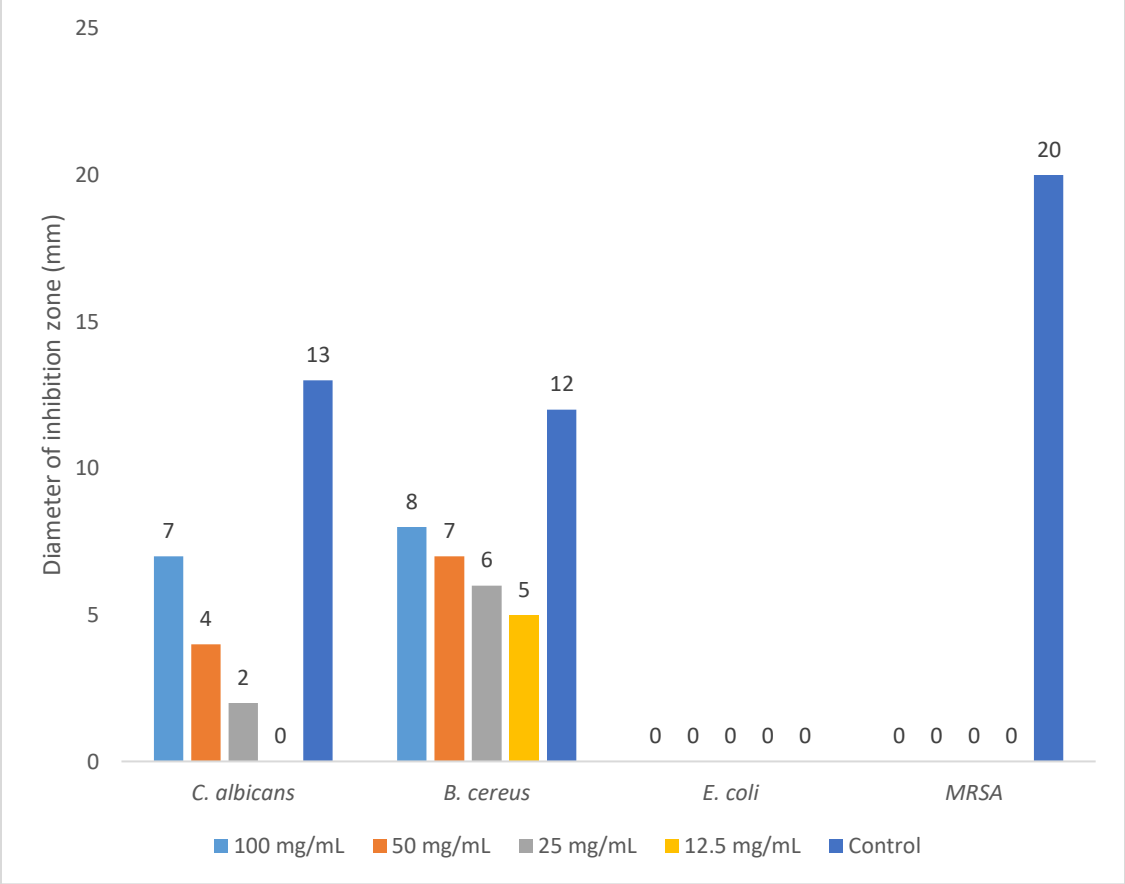

Figure 4.20: Growth inhibition zones of Gazi 3B roots

The leaves of another highland population, Sergoit, were also investigated. The results from this were closely similar to those of the Turbo and Gazi 3B roots (Figure 4.21). This root extract hampered the growth of *C. albicans* by 7 mm, 4 mm, and 2 mm at 100 mg/ml,

50 mg/ml, and 25 mg/ml. No fungal growth occurred at 12.5 mg/ml. Similarly, *Dodonaea viscosa* prevented the development of *B. cereus* by 8 mm, 7 mm, 6 mm, and 5 mm at 100 mg/ml, 50 mg/ml, 25 mg/ml, and 12.5 mg/ml, respectively while its control inhibited growth by 12 mm. The extract did not prevent growth of *E. coli* and MRSA growth at all concentrations. The control for MRSA prevented bacterial growth by a diameter of 20 mm.

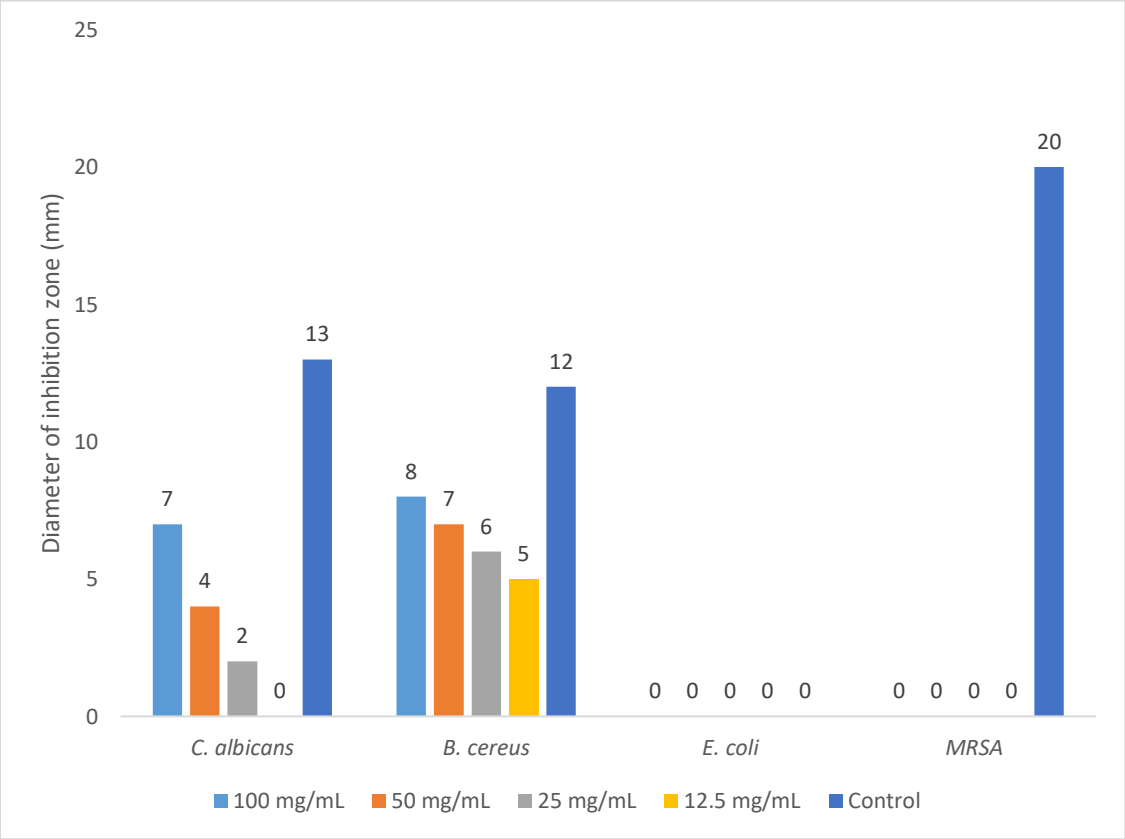

Figure 4.21: Growth inhibition zones of Sergoit leaves

Inhibition of *C. albicans* at 100 mg/ml, 50 mg/ml, and 25 mg/ml was 6 mm, 4 mm and 1 mm, respectively, in the Sergoit population for its roots (Figure 4.22). Like its leaves, a 12.5 mg/ml concentration was ineffective, while the control gave 11 mm inhibition.

Dodonaea viscosa root extract prevented the growth of *B. cereus* by 12 mm, 10 mm, 8 mm, and 3 mm from the highest to the lowest concentration. Its control was effective at 16 mm inhibition. The growth of *E. coli* was not prevented by this extract. *Dodonaea viscosa* extract prevented the growth of MRSA by 8 mm, 5 mm, and 1 mm at 100 mg/ml, 50 mg/ml, and 25 mg/ml, respectively, with no inhibition at 12.5 mg/ml, whereas its control also inhibited bacterial growth to a maximum of 11 mm.

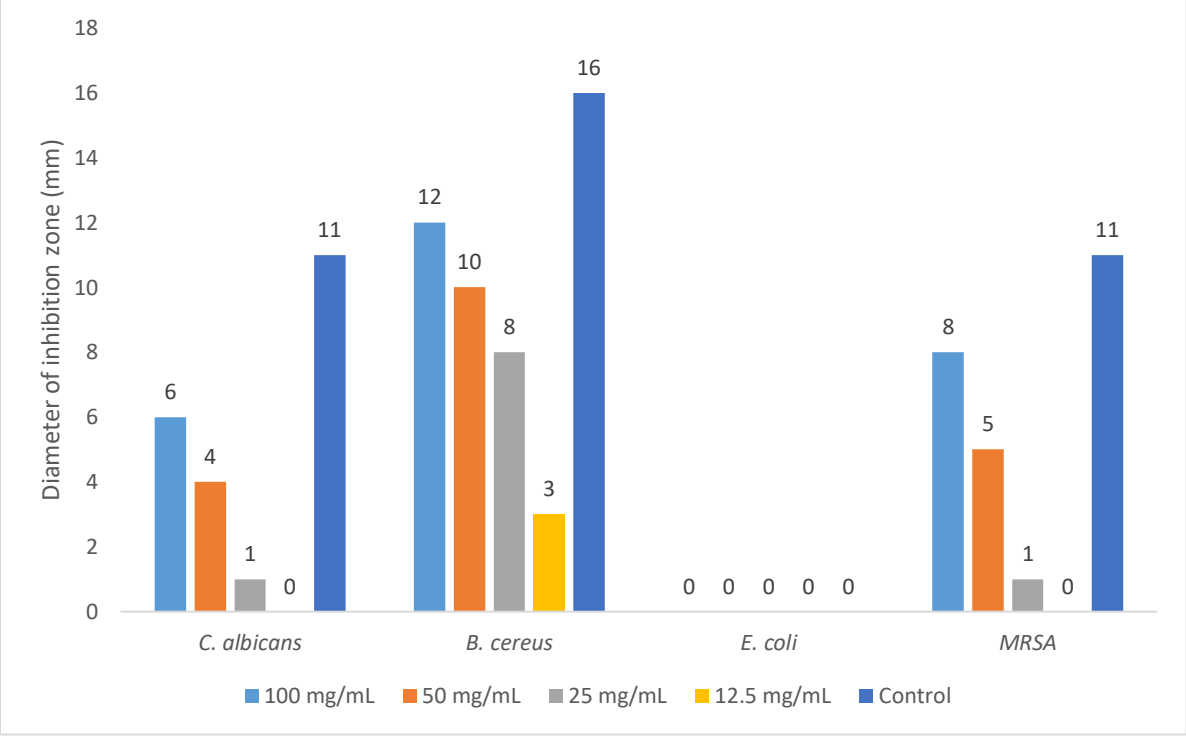

Figure 4.22: Growth inhibition zones of Sergoit roots

The Chepyogot population of *Dodonaea viscosa* inhibited the growth of *C. albicans* by 6 mm, 5 mm, and 2 mm with reducing concentration, with its control being effective at 15 mm (Figure 4.23). Similar low inhibition zones were observed in *B. cereus* of 5 mm, 4 mm, and 1mm at 100 mg/ml, 50 mg/ml, and 25 mg/ml, with no growth inhibition at 12.5

mg/ml. *Dodonaea viscosa* extract did not prevent growth of *E.coli* at all concentartions. Finally, *Dodonaea viscosa* effectively prevented growth of MRSA by 8 mm, 6 mm, and 2 mm, at 100 mg/ml, 50 mg/ml, and 25 mg/ml respectively with no inhibition at 12.5 mg/ml while its control also prevented by 12 mm.

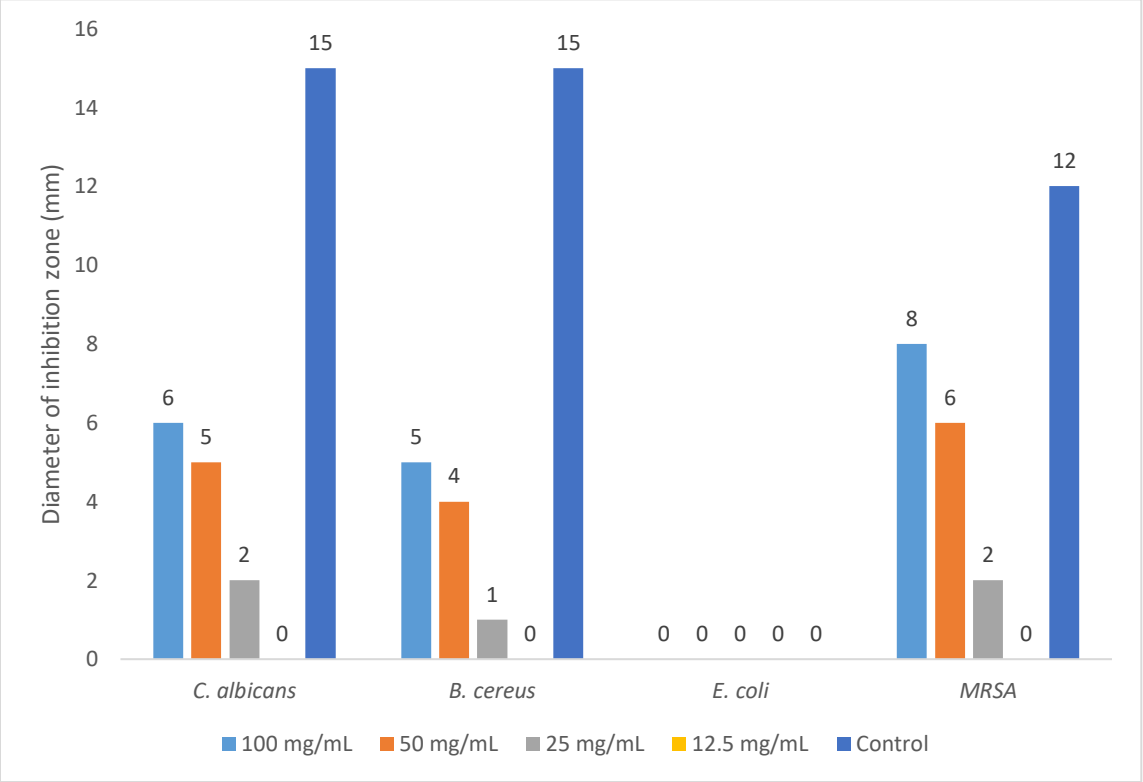

Figure 4.23: Growth inhibition zones of Chepyogot leaves

Like the leaves of the same population (Chepyogot), the roots prevented microbial growth except for *E. coli* (Figure 4.24). Starting with *C. albicans,* the inhibition zones obtained were 3 mm for the first three concentrations. An inhibition diameter of 2 mm was obtained at 12.5 mg/ml and 7 mm for its control. At the same time, *Dodonaea viscosa* extract inhibited the growth of *B. cereus*. Diameters of 6 mm, 5 mm, and 4 mm zones of inhibition were observable at 100 mg/ml, 50 mg/ml, and 25 mg/ml, respectively, with no inhibition

at 12.5mg/ml. This extract did not prevent the growth of *E. coli* at all concentrations, including its control. Lastly, this extract also inhibited MRSA by 7 mm at 100 mg/ml, 6 mm for both 50 mg/ml and 25 mg/ml, and 5 mm for 12.5 mg/ml. Its control prevented growth by 12 mm.

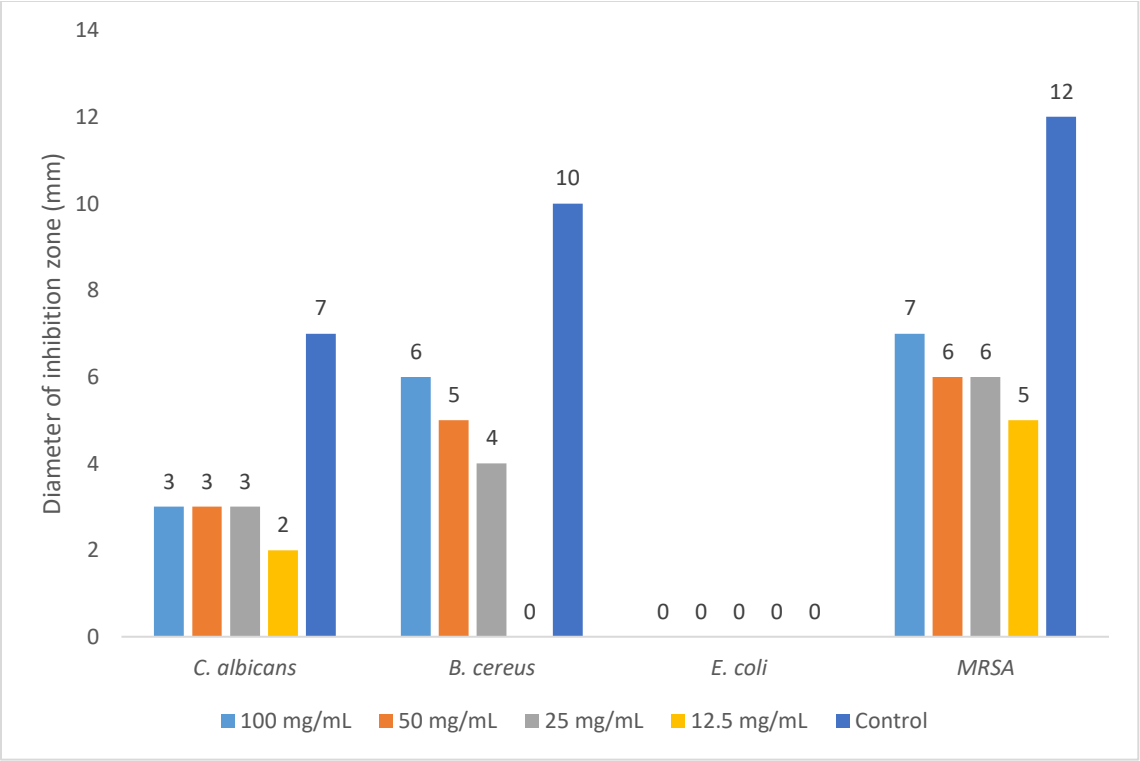

Figure 4.24: Growth inhibition zones of Chepyogot roots

The leaves of the Cheploch population were also significantly effective in preventing microbial growth, except against *E. coli* (Figure 4.25). The plant extract prevented the growth of *C. albicans* by 7 mm, 5 mm, and 4 mm at 100 mg/ml, 50 mg/ml, and 25 mg/ml, respectively. No inhibition was observed at 12.5 mg/ml, while its control was effective at 16 mm. *Dodonaea viscosa* extract also inhibited the growth of *B. cereus* by 9 mm, 8 mm,

and 5 mm at 100 mg/ml, 50 mg/ml, and 25 mg/ml, respectively, with no inhibition of growth at 12.5 mg/ml. The control of *B. cereus* showed growth inhibition of 14 mm. The extract was not effective in inhibiting the growth of *E. coli*. Finally, the extract inhibited MRSA growth by 8 mm, 6 mm, and 4 mm at 100 mg/ml, 50 mg/ml, and 25 mg/ml, respectively. A concentration of 12.5 mg/ml did not inhibit the growth of MRSA. A zone of 19 mm inhibition was observed for the control of MRSA.

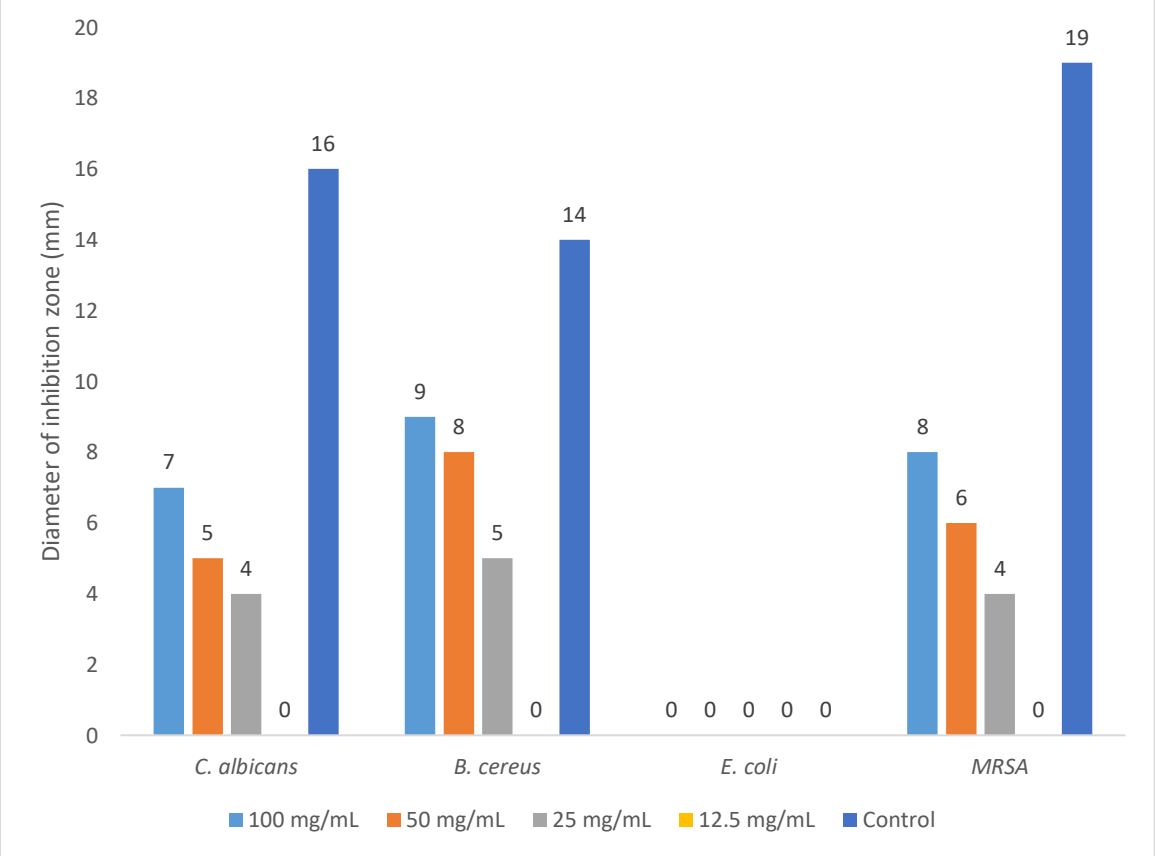

Figure 4.25: Growth inhibition zones of Cheploch leaves

The roots of the Cheploch population were the last population studied for antimicrobial activity (Figure 4.26). According to the findings, at 100 mg/ml, 50 mg/ml, and 25 mg/ml,

Dodonaea viscosa root extract prevented the growth of *C. albicans* by 7 mm, 6 mm, and 5 mm respectively. At 12.5 mg/ml, growth of *C. albicans* was not observed, with the control giving 16 mm inhibition. Similar inhibitory zones of 6 mm, 5 mm, and 2 mm were observed at 100 mg/ml, 50 mg/ml, and 25 mg/ml, respectively, whereas the control group gave 6 mm inhibition. *Dodonaea viscosa* did not prevent the growth of *E. coli* at all concentrations. Finally, a 6 mm inhibition was noted at 100 mg/ml, whereas MRSA showed a 5 mm diameter at 50 mg/ml, and 25 mg/ml. A final concentration of 12.5 mg/ml gave a 4 mm inhibition. Its control was 17 mm effective against MRSA.

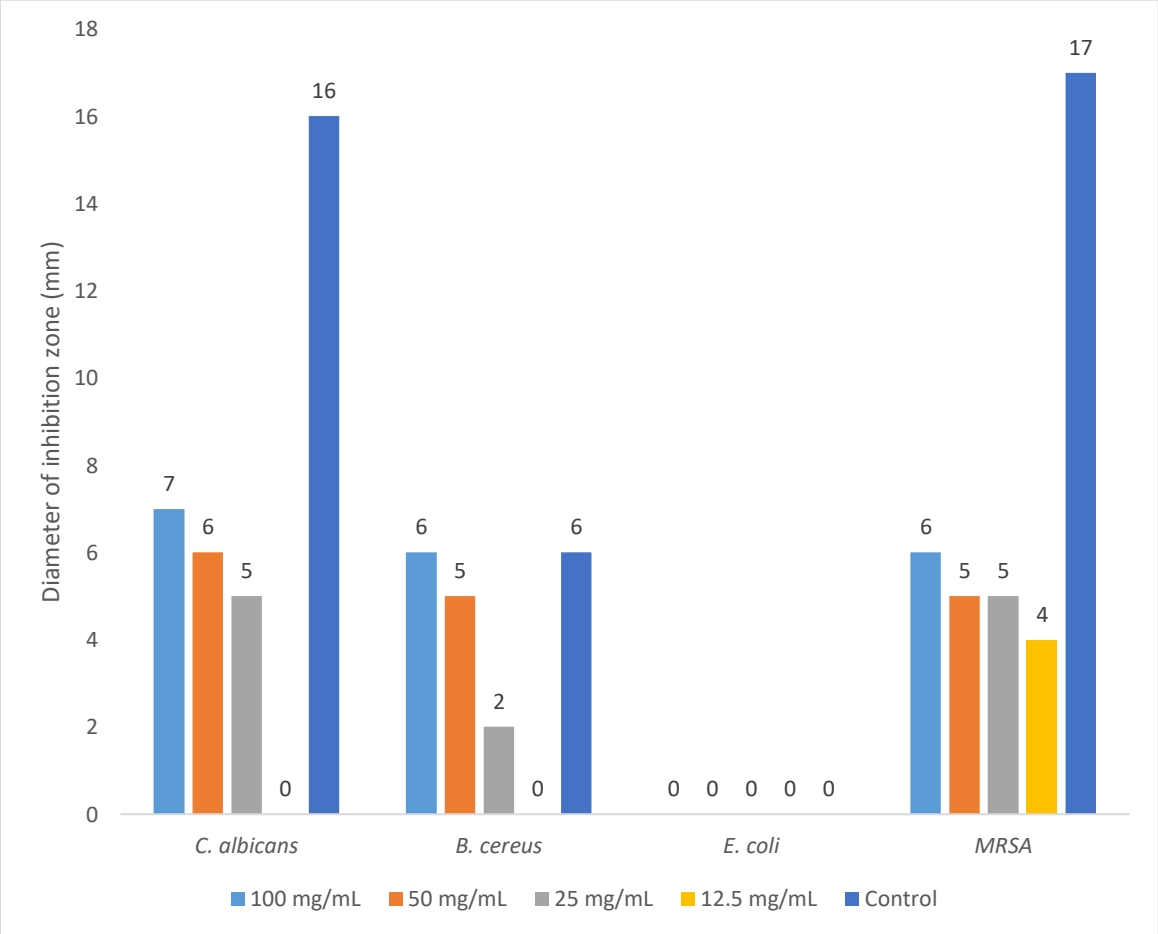

Figure 4.26: Growth inhibition zones of Cheploch roots

CHAPTER FIVE

DISCUSSION

5.1 Phylogenetic analysis of the Kenyan populations of *Dodonaea viscosa*

The diversity of living organisms can be determined using morphological, cytological, biochemical, and molecular markers (Bhandari *et al*., 2017), with this study focusing on molecular markers. Field observations showed that morphologically, the Coastal and Upland populations of *Dodonaea viscosa* showed very slight morphological variations, especially in leaf size, leaf colour, and plant size. Analyses of extracted DNA sequence data from the *rbcL* and *ITS1* genes were done to resolve their phylogenetic relationships. This study considered the relationships of Kenyan populations of *Dodonaea viscosa* collected from four counties (Table 3.1). These included one Coastal (Kwale) and three Upland counties (Baringo, Elgeiyo Marakwet, and Uasin Gishu). This was done to give a comparative study based on the molecular data in the Upland and Coastal populations of *Dodonaea viscosa* and finally infer their relationships.

Phylogenetic analyses (evolutionary divergence, nucleotide frequency & substitution rates, and base composition disparity index) and reconstructions were performed using MEGA 11 on the obtained DNA sequences. Results showed that the two genes' nucleotide substitution rates were generally low. On the same note, the nucleotide disparity index was also very low. The base disparity index gauges the homogeneity of substitution patterns among DNA sequences. In comparative analysis of the sequences, it is frequently assumed that the DNA sequences have undergone the same nucleotide substitutions over time. A violation of this important assumption significantly affects the accuracy of phylogenetic

inference and tests of evolutionary hypotheses. The base composition discrepancies were observed in Figure 4.6 with values for each site between sequence pairings amongst the studied populations. A lower nucleotide disparity index indicated a closer relationship. Disparity indices delineated the Coastal populations represented by the Gazi group from the highland population represented by the other groups. Although Koriema belongs to the highland group, the *ITS1* nucleotide substitution pattern differed from the other highland and the Coastal groups.

Determining evolutionary divergence is also crucial in phylogenetic reconstruction and molecular evolution studies. The number of nucleotide changes/substitutions between two sequences is the most accurate technique for calculating their distance from one another. Distance matrix methods such as UPGMA (Unweighted Pair Group Method with Arithmetic Mean) and NJ (Neighbour-Joining) are all based on sequence divergence. UPGMA presupposes identical rates of evolution to produce equal branch tips, while NJ is an algorithm that permits different rates of evolution such that the length of the branches corresponds to the amount of change. An evolutionary divergence between *Dodonaea viscosa* populations based on the two genes is displayed in Figure 4.7. Higher divergence suggests more substitutions between sequences and hence larger differences between sequences. In the *rbcL* gene sequences, most genetic distances were close to zero, implying that the *Dodonaea* populations were closely related. These results were indicative of fewer variations based on the *rbcL* genes than the *ITS1* genes.

This study agrees with previous taxonomic studies that suggested that the Upland and Coastal forms of *Dodonaea viscosa* be considered as varieties (Kaigongi *et al*., 2020; Rani *et al*., 2009). Phylogenetic reconstructions based on the two genes namely *ITS1* and *rbcL* sequences showed that Kenyan populations of *Dodonaea viscosa* could not be clearly distinguished, as they appear closely related. This is evident from the various cladograms obtained in this study, which have high bootstrap values of more than 70, indicating strong support (Chebet *et al*., 2022). From the *rbcL* gene sequences, Coastal (Gazi 3B and Gazi 1C) populations share one apomorphy with the Upland (Chepyogot, Kapchemutwo, Kabarnet, and Cheploch) populations, indicating a close relationship amongst them. Gazi M5, together with the Turbo populations, are also monophyletic. These relationships suggested a common ancestry amongst the 11 populations. From the cladogram of *ITS1* sequences, Koriema, Gazi M5, Kabarnet, Iten, Sergoit, and Gazi 3B populations were apomorphic with a bootstrap value of 95 as represented in Figure (4.8). Similarly, Gazi 1C, Turbo, Kapchemutwo, and Cheploch populations were also apomorphic with low bootstrap value of 49; hence, this clade was poorly supported. Chepyogot population seemed to be the distant relative of the other of the populations.

It is clear from these findings that the minimal nucleotide variations from phylogenetic analyses could be responsible for the observable observable variations (Buckler & Thornsberry, 2002) in *Dodonaea viscosa* populations studied. Larger variations in base composition disparity index shown by values greater than zero, explains the heterogeneity in their substitution rates, while low nucleotide substitution rates indicates a low rate of evolution in the different *Dodonaea viscosa* populations (Al-Atiyat & Aljumaah, 2014)

and a closer ancestry. The values obtained from phylogenetic analyses herein implies that there are minimal variations between the Kenyan populations of *Dodonaea.* A taxonomic study on populations of Kenyan *Dodonaea viscosa* sampled from Machakos, Nanyuki, and the Coast showed a closer relationship amongst the populations (Kaigongi *et al*., 2020). Hence, the hypothesis of this study, that the *ITS1* and *rbcL* gene sequences do not differ among populations of *Dodonaea viscosa*, holds and is strongly supported.

The minimal genetic diversity among *Dodonaea viscosa* could be explained by the differences in altitude, with more diversity in low altitude compared to high altitude (Adams & Hadly, 2013; Ohsawa & Ide, 2008). The populations studied were obtained along the Coast, whose altitude is low, and the Upland, whose altitude is higher. A investigation on chloroplast DNA sequences data from 15 plant species from the Longitudinal Range Gorge Region in southwest China showed that annual mean wet day and latitude had a weak but substantial positive correlation with genetic diversity (Wambulwa *et al*., 2022). A contrary study on anatomical structures of *Dodonaea viscosa* showed that there were no latitudinal or altitudinal trends, noting that variations were not controlled by ecological factors (Liu & Noshiro, 2003). Variability in the abiotic characteristics of the different environments under study may have led the genetic diversity of *Dodonaea viscosa* (Odat *et al.*, 2010). Similarly, environmental heterogeneity is known to lead to changes in the size of species populations and, consequently, genetic diversity, producing unimodal, positive, or negative relationships between species diversity and genetic diversity (Vellend, 2005).

Dodonaea viscosa populations could also react differently to global climate change, showing different microevolutionary processes that affected their genetic make-up due to changes in selective pressures like temperature (Pauls *et al*., 2013). The soil in the Uplands have a lower salinity compared to the Coast, whose salinity is comparatively higher. Hence, there could be a possible phenotypic adjustment of the two populations (Upland and Coastal) in response to differences in salinity (Song *et al*., 2024). Studies also show that the presence of microbes affect phenotypic responses or plasticity in plants in addition to the effects of the environment (Goh *et al*., 2013). On the same note, linked selection and mutations in *Dodonaea viscosa* could greatly affect the genetic polymorphism within this species, arising from the changes in biotic or abiotic environment (Ellegren & Galtier, 2016). Similarly, evolutionary processes, like genetic drift and migration, operate continually, changing the frequency of alleles in a population and affecting their genetic diversity (Bhandari *et al*., 2017). Climatic factors on *Dodonaea viscosa*, including, cold index, warm index, mean annual rainfall, hydrothermal synthesis index and yearly temperature range as studied on *Caragana microphylla* (Huang *et al*., 2016) could be responsible for the observed genetic diversity in *Dodonaea viscosa*. Anothe similar study has shown that alteration of rainfall regimes greatly affects the phenotype and genotype modification in different plants, as demonstrated by Avolio & Smith (2013).

In a narrow sense, a study on *Dodonaea viscosa* noted that the observable variations were due to environmental acclimation rather than genetic adaptation, as there was no clear genetic difference amongst populations sampled (Baruch *et al*., 2018). Other studies have shown that phenotypic plasticity is the source of phenotypic variations in plants, which

could be advantageous to the plants as it forms the basis of adaptability to different environments (Booy *et al*., 2000; Valladares *et al.*, 2007). Ecologically, plants respond to resource availability, affecting their phenotypic appearance (Coleman *et al*., 1994). Generally, plants display intraspecific or interspecific phenotypic plasticity. Plants exhibit flexible responses to a wide range of ecological factors, such as changes in the abiotic environment, disturbance, herbivory, parasitism, mutualistic connections, and neighbours' presence, absence, or identity (Callaway *et al*., 2003).

5.2 Biological activities of *Dodonaea viscosa* populations in relation to their phytochemical profiles

Results indicated that the biological activities of the root, and leaves of the different populations were not significantly different. This was supported by p-values, which are >0.05. This can be explained by the random sample employed in the collection of this plant and the existence of related chemicals that minimized variability. This therefore implies that the hypothesis holds, as there is not enough evidence based on the findings that there is no difference in the biological activities of *Dodonaea viscosa* populations in Kenya. The activities of the various populations were also not statistically different with a high P-value (Sig. = 0.742). Therefore, this study cannot rule out the null hypothesis based on this ANOVA test. This therefore indicates insufficient proof to conclude that the observed means of the groups are statistically different. In other words, at a significance level of α >0.05, the observed differences between the populations are likely due to random chance, rather than any true differences in the population means.

Antidiabetic activity was determined using only male rats. Studies have shown that females are affected by the estrous cycle, giving a possibility of inaccurate results, as their blood sugar levels are unstable. At the same time, female rats are also more resistant and sometimes milder to diabetes (Franconi *et al*., 2008). A comparison of the antidiabetic activity of insulin and *Dodonaea viscosa* extract at 200 mg/kg bw and 400 mg/kg bw showed that insulin led to the highest decrease in blood sugar level. This was followed by the plant extract at 400 mg/kg bw and 200 mg/kg bw, respectively. Insulin is a polypeptide that controls blood glucose levels and causes the liver, muscles, and adipose tissue to store glucose (Brunton *et al*., 2005; Rahman *et al*., 2021). Many plants studied from different families, including Sapindaceae, have phytochemicals responsible for their antidiabetic effects, with few showing precise mechanisms in *in vivo* conditions (Govindappa, 2015).

The antidiabetic activity studied showed that the Kenyan populations of *Dodonaea viscosa* crude extract have hypoglycemic activity. This was evident from the reduced blood sugar levels in streptozotocin (STZ) induced male diabetic rats. Blood glucose levels were significantly lowered by *Dodonaea viscosa* leaf and root extracts in the male rats under study at different concentrations. Results of an antidiabetic study of *Dodonaea viscosa* leaf extracts (Rao & Pratap, 2022) just like the present study resulted in reduced blood glucose in normal and alloxan-induced rabbits. In a similar study, the leaves of *Dodonaea viscosa* reduced the sugar levels in male alloxan-induced diabetic rats (Akhtar *et al*., 2011; Muthukumran *et al*., 2011). The same activity was shown by water and ethanolic extracts of *Dodonaea viscosa* against high-fat diet and low-dose streptozotocin (STZ) induced rats (Veerapur *et al*., 2010). An aqueous leaf extract of *Dodonaea viscosa* showed that the plant

has the potential to manage diabetes and associated symptoms (Luka *et al*., 2018). Similarly, *Dodonaea viscosa* showed a protective role against streptozotocin-induced hepatotoxicity, a condition associated with diabetes and nephrotoxicity in rats (Alanazi *et al.*, 2023). The leaves of *Dodonaea viscosa* in synergy with *Conocarpus lancifolius* and *Capparis spinose* showed a significant reduction in their blood sugar in alloxan-induced diabetic mice (Kadim *et al*., 2021).

Antimicrobial activity of the populations of *Dodonaea viscosa* was determined against different micropathogenic organisms. This study used the agar well diffusion method to identify the growth inhibition zones of four microorganisms (*C. albicans, B. cereus, E. coli, and* Methicillin-resistant *S. aureus (MRSA)*. From this study, *Dodonaea viscosa* has proven to have antimicrobial properties. This is visible from the zones of inhibition (ZOI), where each population displayed activity against the microbes used in this study. However, the ZOI were lower than in other studies involving the same plant with no synergistic activity of the numerous compounds in the crude extract reported. Plant crude extract contains numerous compounds, giving a possible synergistic effect of the various compounds against microbes and explaining their biological activity. This could be attributed to the differences in solvents used in the extraction process, differences in parts of the plant used, collection period, location of the plant, and the test organisms used (Esmaeel & Kamil, 2011; Mercedes *et al*., 2013). The extraction of active compounds using ethanol increases at low concentration (50%) compared to higher concentration (96%) (Hikmawanti *et al*., 2021), just like the present study, which was done with 96% ethanol. A similar survey (Hijazi *et al*., 2025) supports increased efficiency in the extraction of

bioactive compounds with a lower ethanol concentration. The activity of *Dodonaea viscosa* extract largely depends on the polarity of extraction solvents and the parts of the plant used (Malik *et al*., 2022). Notable synergistic effects of various compounds were identified in crude extracts of *Dodonaea viscosa* against Coxsackievirus B3 and rotavirus SA-11 (Shaheen *et al*., 2015), unlike the present study.

Zones of growth inhibition were observed with all the microbes studied except *E. coli* using *Dodonaea viscosa* extract, implying that *Dodonaea viscosa* has antimicrobial activity. A previous study involving crude methanol extract of *Dodonaea viscosa* also showed similar results in that it did not inhibit the growth of *E. coli* (Getie *et al*., 2003). Likewise, methanol, n-hexane, dichloromethane, and ethyl acetate extracts of *Dodonaea viscosa* were effective against various bacteria, but with minimal activity against *E. coli*, hence agreeing with the present study (Rao *et al*., 2015). Results of methanol extracts of *Dodonaea viscosa* inhibited the growth of MRSA and allowed growth in *E. coli* (Al-Snafi, 2017), unlike the current study. Another study by Khurram *et al*., (2009) showed that crude extracts of *Dodonaea viscosa* resulted in inhibitions against different microbes, including *E. coli*, unlike in the present study.

Many studies have shown that *E. coli* is a very resistant bacterium. It is known to cause infections that are quite challenging to cure in humans and veterinary animals (Poirel *et al*., 2018). The resistance of *E. coli* causes morbidity and mortality, which could be associated with the existence of specific virulence factors (VFs), including hemolysins, invasins, siderophores, adhesins, poisons, fimbriae, and capsules (Da Silva & Mendonça,

2012). A study on ethanolic and diethyl ether extracts of *Dodonaea viscosa* against *C. albicans* resulted in growth inhibition (Esmaeel & Kamil, 2011). A similar study involving 80% methanol crude extract of *Dodonaea viscosa* exhibited antibacterial activity against *Staphylococcus aureus,* just like the present ethanolic extract (Getie *et al*., 2003). From the present results, some populations of *Dodonaea viscosa* extract could not prevent the growth of MRSA. This could be due to multiple toxins produced by this microbe and the fact that it is a highly versatile microbe (Huseby *et al*., 2007; Bitrus *et al*., 2018). Another study involving cultivated *Dodonaea viscosa* in Iraq using agar well diffusion method resulted in inhibition of *B. cereus* and other microbes studied (AL-Azawi, 2017). This plant was effective against microbes in synergy with *Agathosma crenulata* and *Eucalyptus globulus* (Zonyane, 2013). Other studies involving the different plant parts of *Dodonaea viscosa* have also inhibited microbial growth (Shiaka *et al*., 2019; Malik *et al*., 2022). The present results, therefore, justify the application/use of the *Dodonaea viscosa* plant as a natural cure for managing microbial infections.

The biological (antidiabetic and antimicrobial) activities studied herein for *Dodonaea viscosa* could be explained by presence of several different compounds, including those isolated and characterized in this study and other studies involving the same plant. This study reports 2-(4,5-dihydroxy-2-methoxyphenyl)-5,7-dihydroxyl-3,6-dimethoxy-4H-chromen-4-one, Hautriwaic acid, 5,7,4^{I}-trihydroxy-(3^{II}-methyl-2-buten-1yl)-3-methoxyflavone, and Mkapwanin possibly responsible for its activity. Several secondary metabolites, such as alkaloids, terpenoids, triterpenoids, phenols, saponins, steroids, and flavonoids, have been isolated in *Dodonaea viscosa* and are responsible for its activity

(Lawal & Yunusa, 2013). The presence of kaemferol methyl ethers, lead molecules, and Zinc oxide Nanocomposites (Teffo, 2010; Alghamdi *et al.*, 2022; Priya *et al*., 2021) previously isolated and have antibacterial properties. Hautriwaic acid has been previously and currently isolated from *Dodonaea viscosa* and in other plants like *Pulicaria salviifolia* (Eshbakova & Saidkhodzhaev 2002). Similarly, Mkapwanin has also been isolated from *Dodonaea viscosa* leaf exudates (Omosa *et al*., 2010), just like the present study.

A study by Al Bimani & Hossain (2020), identified 3,5,7,3′,4′-pentahydroxyflavone and hautriwaic acid from the leaves of *Dodonaea viscosa* as having antimicrobial activities. A previous study by Ali *et al*., (2014) found that Hautriwaic acid isolated from *Dodonaea viscosa* similarly has hepatoprotective activity linked to *diabetes mellitus*. Similarly, Hautriwaic acid isolated from its leaves showed anti-inflammatory activity against female mice (Salinas-Sánchez *et al*., 2012). Notably, compounds previously isolated from the *Dodonaea viscosa* flowers have been proven to have antibacterial and anticancer activities (Raksat *et al*., 2025). Aerial parts of *Dodonaea viscosa* identified Pinocembrin with a fairly good antifungal activity and 5-hydroxy-3,6,7,40-tetramethoxy flavone together with 5,7-dihydroxy-30 -(4-hydroxy-3methylbutyl) 3,6,40-trimethoxyflavone with moderate antileishmanial activities (Mostafa *et al*., 2014). Research on ethanol extracts of its flowers has led to isolation of compounds with analgesic, antioxidant, and cytotoxic activities (Shafek *et al*., 2015). A study involving ethanol root extracts of *Dodonaea viscosa* found two triterpenoid saponins (dodonaeaside A and dodonaeaside B) with antiproliferative activities (Cao *et al*., 2009). Results of isolated clerodane diterpenoids and phenolics from

Dodonaea viscosa have also shown antioxidant and anticholinesterase activity (Muhammad *et al*., 2016).

Other compounds from *Dodonaea viscosa* include (6S, 9R)-vomifoliol-9-*β*-D-glucopyranosyle (1 -3)- *O*-*α*-L-rhamnopyranoside, viscosine, alkaloids, flavonoids, fixed oils and fats, steroids, phenolics, saponins, tannins, gums, mucillages, carbohydrates, reducing sugars, glycosides, catechin, diterpenoids, and trace elements (Al-Snafi, 2017; Muhammad *et al*., 2015; Muhammad *et al*., 2016; Sagara *et al*., 2021; Siddiqui *et al*., 2023). From the literature reviewed, more than eighty compounds have been isolated from the genus *Dodonaea*, mainly the aerial parts (stems and leaves), with most of them from *Dodonaea viscosa*, which are responsible for different biological activities (Beshah *et al.*, 2020).

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Despite the morphological variations of the Upland and Coastal populations of *Dodonaea viscosa,* the nucleotide substitution rates, nucleotide disparity index, and evolutionary divergence of both *rbcL* and *ITS1* genes in populations, were very low. The phylogenetic analyses, show that the genetic distance between Kenyan populations of *Dodonaea viscosa* is low. ML phylogenetic trees from the DNA sequences of both genes further supported the narrow genetic distance, as the Upland and the Coastal forms could not be separated. All the phylogenetic trees had strong bootstrap values. This study suggests that the Upland (*angustifolia*) and Coastal (*viscosa*) should be treated as varieties, and not as subspecies or distinct species, as proposed in other studies. PCA showed that *rbcL* gene contributed to more variation compared to *ITS1* gene. Phytochemical analyses have shown that *Dodonaea viscosa* is a rich plant in phytochemicals which is evident from the compounds isolated and described from the leaves of Gazi 1C population and those previously cited in literature.

The crude ethanol extracts from the roots and leaves of *Dodonaea viscosa* have the potential to exhibit slight antidiabetic and antimicrobial activities. These activities can be attributed to the numerous phytochemicals it possesses. From the findings, the activity of the roots and leaves did not significantly differ, as supported by a significant level of >0.05. Similarly, the variance between the different populations was not statistically different with a p=0.742. The crude extracts showed inhibition zones with all the microbes tested except *E. coli*. However, low zones of inhibition from the crude extracts were obtained, which can

be attributed to the toxic effects of high ethanol concentrations. More antidiabetic activity was observed from the reference drug (insulin), than from the 400 mg/kg bwt, and 200 mg/kg bwt of plant extract. Similarly, more antimicrobial activity was observed from the positive controls (Amoxicillin and Apron), followed by the plant extracts at different concentrations. This study justifies the use of *Dodonaea viscosa* in ethnomedicine by different communities worldwide. These findings also contributes phytochemical data that could potentially aid in resolving the existing taxonomic challenge on this plant.

6.2 Recommendations

Based on this research work, the following suggestions are therefore proposed;

i) Different primers targeting different gene areas shoud be used to provide more data that can aid in clearly distinguishing between the Coastal and highland populations of *Dodonaea viscosa*.

ii) Different extraction solvents and methods for the extraction of crude and isolation of pure compounds are to be used to target other compounds not extracted by ethanol and dichloromethane, respectively.

iii) Finally, more bioassays should be conducted on the pure compounds isolated from this study to determine their biological activities.

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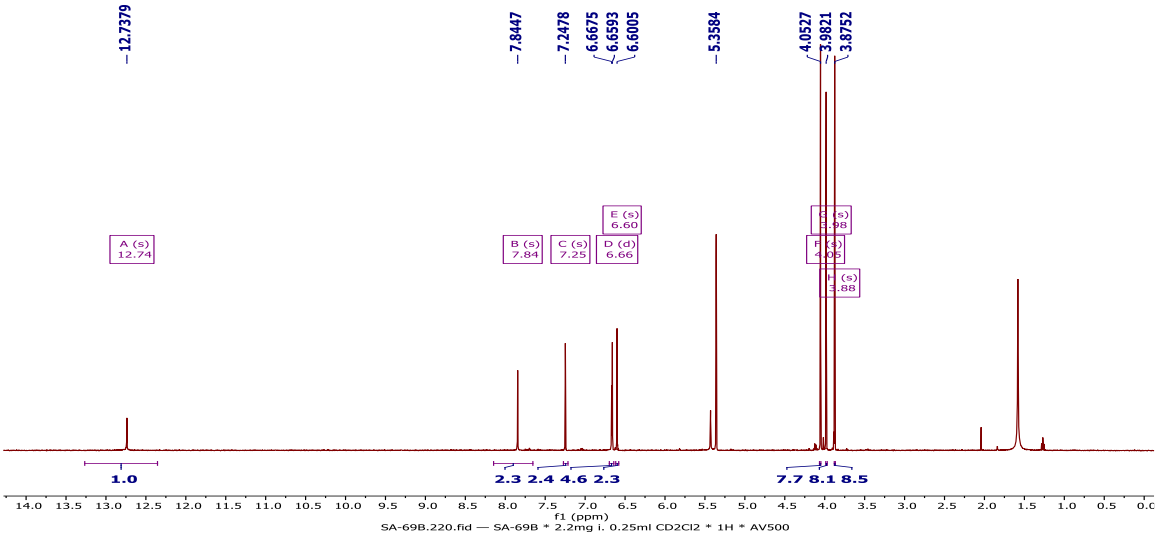
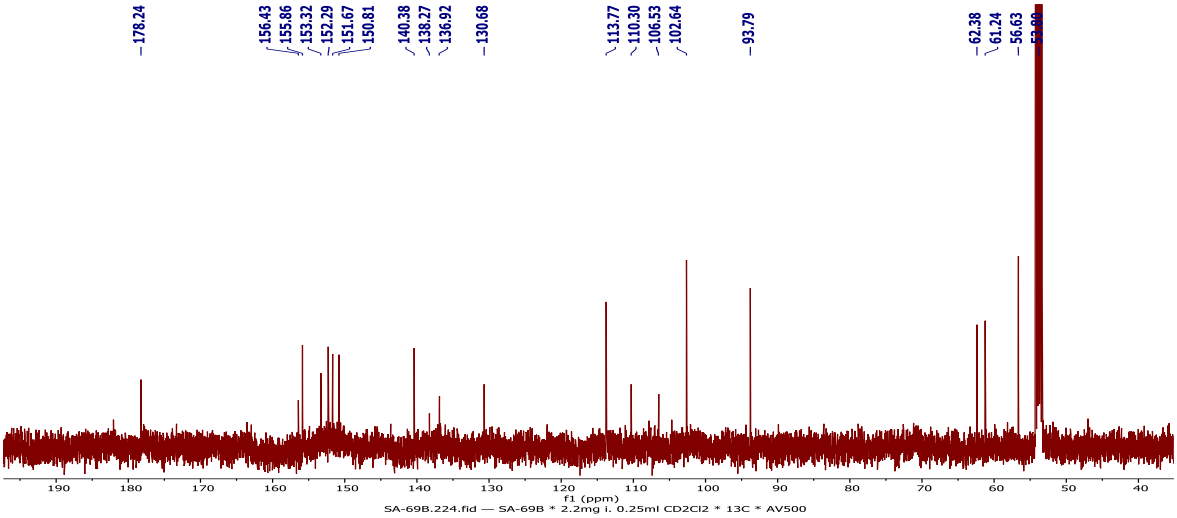
APPENDICES

Appendix I: NMR spectrum for 2-(4,5-dihydroxy-2-methoxyphenyl)-5,7-dihydroxy-3,6-dimethoxy-4H-chromen-4-one

Appendix 1A: ^{1}H NMR of 2-(4,5-dihydroxy-2-methoxyphenyl)-5,7-dihydroxy-3,6-dimethoxy-4H-chromen-4-one at 500 MHz in CD_2Cl_2 at 25 °C

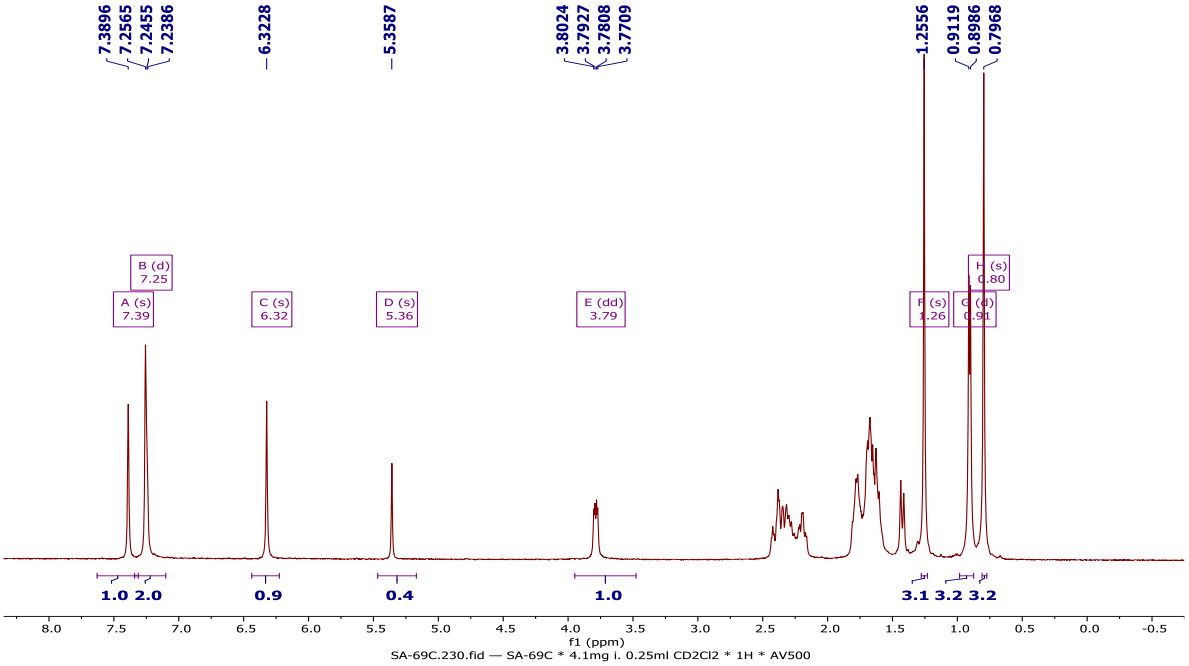


Appendix 1B: ^{13}C NMR of 2-(4,5-dihydroxy-2-methoxyphenyl)-5,7-dihydroxy-3,6-dimethoxy-4H-chromen-4-one at 125 MHz in CD_2Cl_2 at 25 °C

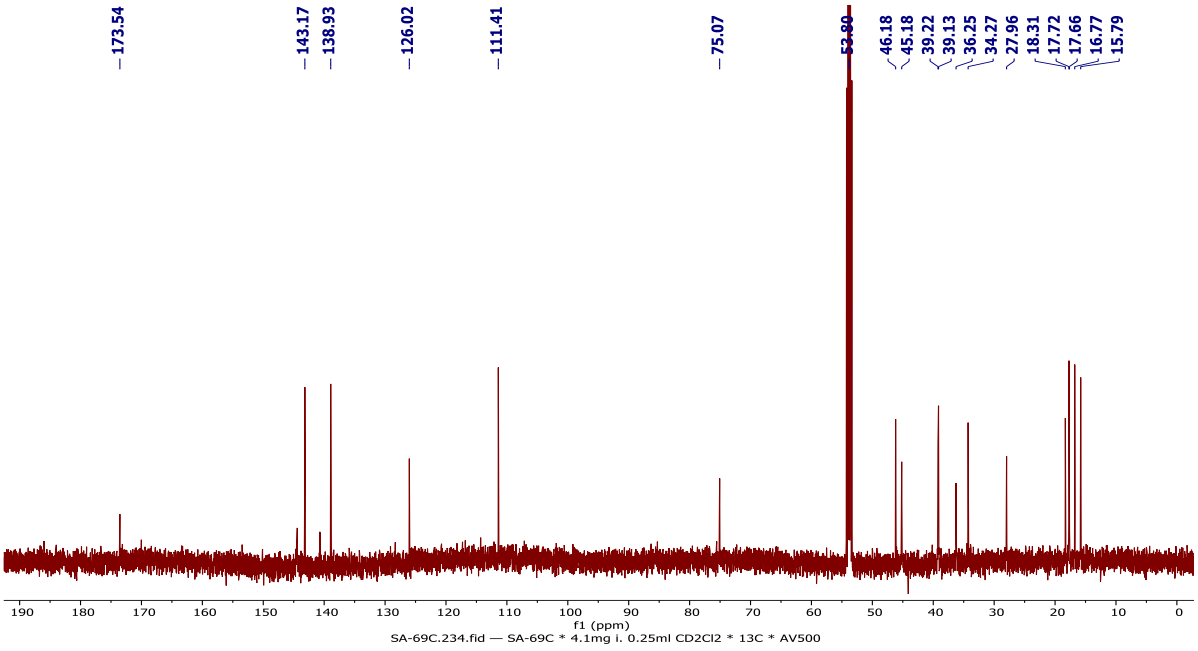


Appendix II: NMR spectrum for Hautriwaic acid

Appendix 2A: ^{1}H NMR of Hautriwaic acid at 500 MHz in CD_2Cl_2 at 25 °C

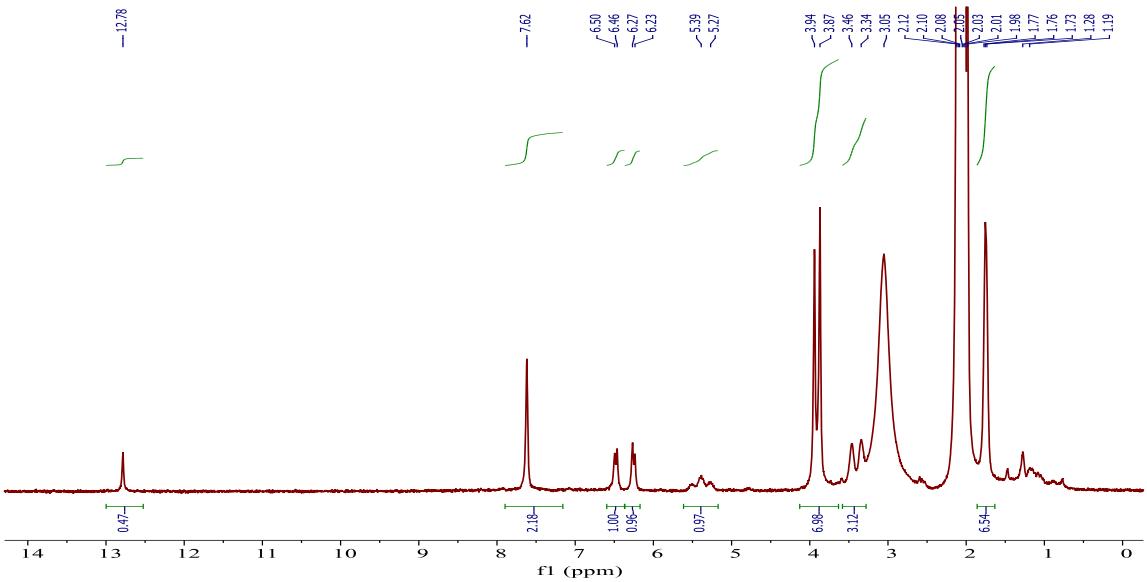


Appendix 2B: ^{13}C NMR of Hautriwaic acid at 125 MHz in CD_2Cl_2 at 25 °C

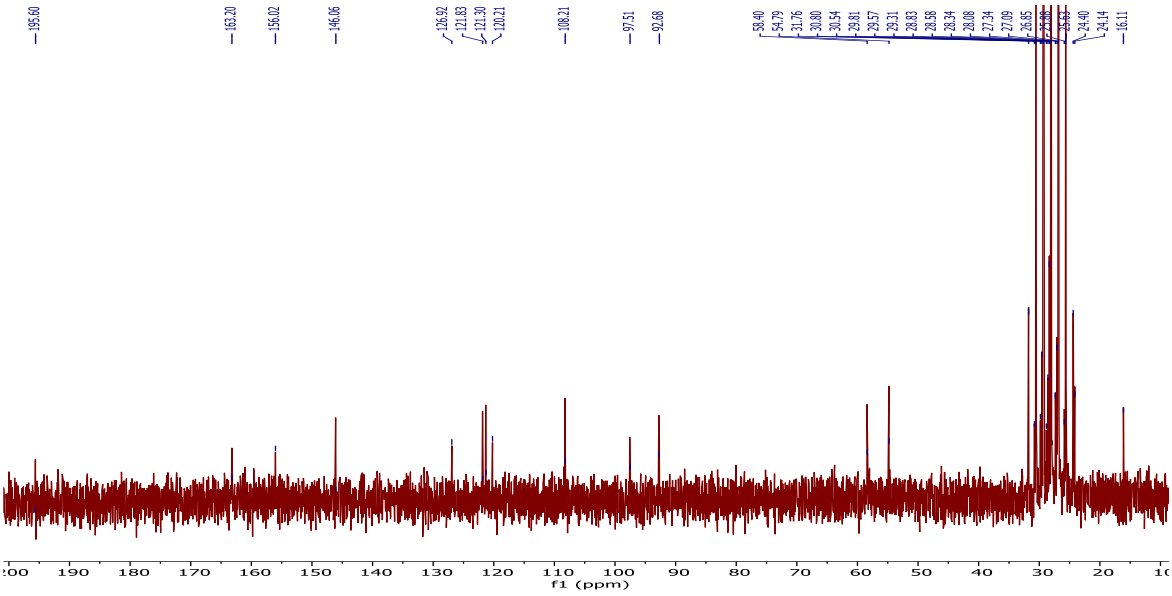


Appendix III: NMR spectrum for 5,7,4^{I}-trihydroxy-(3^{II}-methyl-2-buten-1yl)-3-methoxyflavone

Appendix 3A: ^{1}H NMR of 5,7,4^{I}-trihydroxy-(3^{II}-methyl-2-buten-1yl)-3-methoxyflavone at 60 MHz in acetone-d6

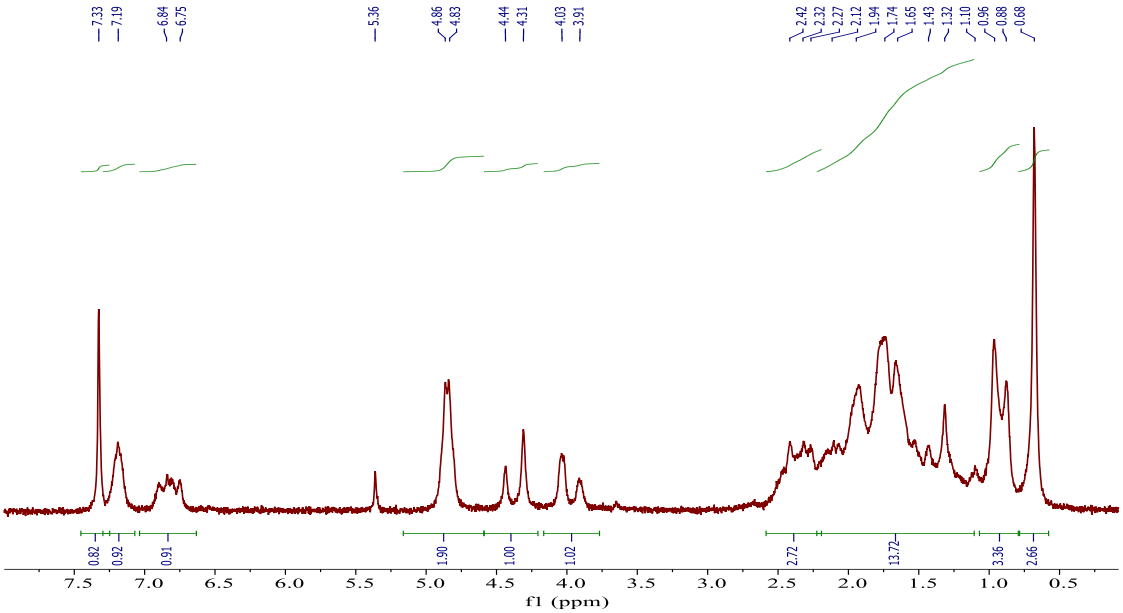


Appendix 3B: ^{13}C NMR of 5,7,4^{I}-trihydroxy-(3^{II}-methyl-2-buten-1yl)-3-methoxyflavone at 15 MHz in acetone-d6

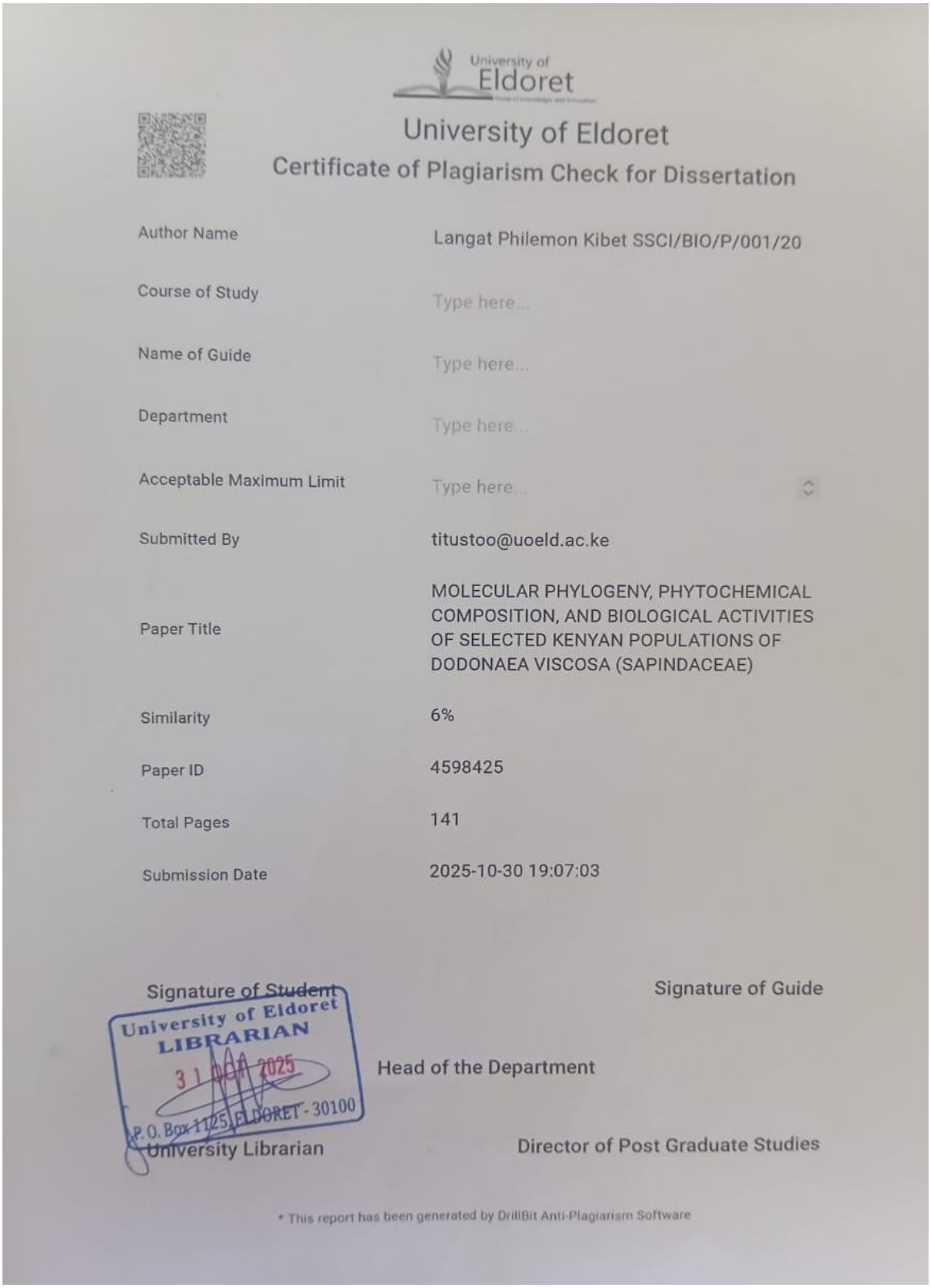


Appendix IV: NMR spectrum for Mkapwanin

Appendix 4A: ^{1}H NMR of Mkapwanin at 60 MHz in acetone-d6



Appendix VI: Similarity Page



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