PHYTOCHEMICAL COMPOSITION, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF *Albizia coriaria* LEAVES FROM THREE AGROECOLOGICAL ZONES OF UGANDA

TIMOTHY OMARA

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DECLARATION PAGE

Declaration by the Candidate

This thesis is my original work and has not been presented for a degree in any other University. No part of this thesis may be reproduced without the prior written permission of the author and/or Moi University.

Date: **15/11/2021**

Timothy Omara

(**MS/ACH/4316/20**)

Declaration by Supervisors

This thesis has been submitted for examination with our approval as University Supervisors.

Prof. Ambrose Kipchumba Kiprop  Date: **16/11/2021**

(Moi University, Eldoret, Kenya)

Dr. Viola Jepchumba Kosgei  Date: **16/11/2021**

(Moi University, Eldoret, Kenya)

DEDICATION

This thesis is dedicated to my wife Sarah Kagoya and my daughter Briella Macklin Anyango for their material, spiritual, emotional and financial support without which this work could have not been brought to fruition.

ABSTRACT

Oxidative stress-induced conditions and bacterial diseases constitute some of the major causes of mortality worldwide. Their treatment is becoming a challenge due to antimicrobial resistance, prohibitive costs, inaccessibility and side effects of the conventional drugs. Thus, traditional medicine is becoming popular in the treatment of these diseases in various parts of the world. The objectives of this study were to;(1) identify the secondary metabolites in extracts of *Albizia coriaria* leaves (EOACL), (2) determine the total phenolic and total flavonoid contents of EOACL, (3) establish the antioxidant activity of EOACL, (4) evaluate the antibacterial activity of EOACL, and (5) characterize the phytochemicals in the most active EOACL used in traditional treatment of oxidative stress-induced conditions and bacterial diseases in Uganda. The leaves were sampled from Jinja, Kole and Mbarara districts of Uganda, representing the South East, Mid Northern and Southern drylands agroecological zones, respectively. Shade-dried samples were ground into powder and successively extracted with ethyl acetate, ethanol and distilled water. The extracts were chemically profiled using classical phytochemical screening, ultraviolet-visible (UV-Vis) spectroscopy, Fourier transform infrared (FTIR) spectroscopy and gas chromatography-mass spectrometry (GC-MS). The total phenolic content, total flavonoid content, antioxidant and antibacterial activities were determined using; Folin-Ciocalteu method, aluminum chloride assay, 1,1-diphenyl-2-picrylhydrazyl assay and culture-based agar disc diffusion method, respectively. The results obtained varied for the three agroecological zones; Mbarara leaf extracts had many secondary metabolites and exhibited the highest bioactivities, followed by Kole and Jinja extracts. Phytochemical screening results indicated that phenols, alkaloids, saponins, flavonoids, cardiac glycosides and tannins were the major secondary metabolites in EOACL. These results were confirmed by UV-Vis spectra (with absorption maxima of 338 nm, 470 nm, 534 nm, 607 nm and 664 nm) and FTIR spectra which indicated the presence of O−H stretch (3370.27 cm^{-1}), C=O (1739.70 cm^{-1}), N−H (3261.46 cm^{-1}) and aromatic−C=C (1454.48 cm^{-1}). Total phenolic and flavonoid contents, and antioxidant activity were found to be highest for ethanolic extracts, with the highest contents (101.72 ± 0.22 mg GAE/ g DW and 13.23 ± 0.03 mg QE/ g DW) and antioxidant potential (IC_{50} = 18.65 ± 0.06 mg/mL) being for EOACL from Mbarara district. The high antioxidant potential of EOACL suggests their potential role in the prevention of oxidative stress-induced conditions. Antibacterial screening indicated that ethanolic extracts had the highest antibacterial activities with mean zones of inhibition of 6.00 ± 1.73 to 10.00 ± 1.73 mm, 5.00 ± 1.00 to 12.30 ± 1.53 mm, 17.00 ± 0.00 to 25.00 ± 2.65 mm and 9.00 ± 1.73 to 16.00 ± 1.73 mm for *Escherichia coli* (*E. coli*), *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella typhi*, respectively. Ethyl acetate EOACL from Kole and Mbarara were active against *E. coli* with inhibition zones of 3.00 ± 0.00 mm and 4.00 ± 2.00 mm respectively. Ethyl acetate EOACL from Jinja and all the aqueous extracts showed no antibacterial activity. Characterization of fractions of the most active (ethanolic) EOACL using GC-MS led to the identification of nine compounds: lupeol (**7**), lupenone (**8**), betulinic acid (**9**), benzyl alcohol (**12**), betulin (**13**), oleanolic acid (**14**), oleanolic acid acetate (**15**), undecanol (**16**) and pterin-6-carboxylic acid (**17**) of which compounds **13-17** are being reported for the first time in *Albizia coriaria*. In conclusion, EOACL were established to have compounds with antioxidant and antibacterial activities, giving credence to their use in traditional management of oxidative stress-induced conditions and bacterial diseases. Clinical trials using the active EOACL and the identified compounds should be done.

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LIST OF ABBREVIATIONS AND ACRONYMS

15-LOX	15-Lipoxygenase
A. coriaria	*Albizia coriaria* Welw. ex Oliver
ANOVA	Analysis of Variance
ATCC	American Type Culture Collection
CDC	Centers for Disease Control and Prevention
COX	Cyclooxygenase
DMSO	Dimethyl sulfoxide
DPPH	1,1-diphenyl-2-picrylhydrazyl
E. coli	*Escherichia coli*
EOACL	Extracts of *Albizia coriaria* leaves
FTIR	Fourier Transform Infrared
GC-MS	Gas chromatography-mass spectrometry
HR-ESI-MS	High-resolution electron spray ionization-mass spectrometry
IC_{50}	Half-maximal inhibitory concentration
MBC	Minimum Bactericidal Concentration
MIC	Minimum Inhibitory Concentration
mg GAE / g DW	Milligrams gallic acid equivalent per gram dry weight
mg QE / g DW	Milligrams quercetin equivalent per gram dry weight
NIST	National Institute of Standards and Technology
NMR	Nuclear Magnetic Resonance spectroscopy

OH	Hydroxide
P. aeruginosa	*Pseudomonas aeruginosa*
ROS	Reactive oxygen species
S. aureus	*Staphylococcus aureus*
S. typhi	*Salmonella typhi*
TLC	Thin Layer Chromatography
TFC	Total flavonoid content
TPC	Total phenolic content
UV-Vis	Ultraviolet-visible
ZOI	Zone of inhibition

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CHAPTER ONE: INTRODUCTION

1.1 Background of the study

Oxidative stress-induced conditions and diseases caused by pathogenic bacteria are some of the leading causes of preventable deaths in the world (Shrivastava *et al.*, 2018; Tincho *et al.*, 2020; WHO, 2020a, 2020c). Oxidative stress plays a fundamental role in the pathophysiology of various diseases and conditions including neurodegenerative diseases, atherosclerosis, cardiovascular dysfunction, drug toxicity, inflammation, carcinogenesis and ischemia-reperfusion injury (García-Sánchez *et al.*, 2020; Pleńkowska *et al.*, 2020). Some of the oxidative stress-induced conditions such as cancer, heart diseases and inflammatory disorders has been listed among the top killer diseases worldwide (Sung *et al*., 2021).

On the other hand, a significant percentage of annual global deaths are still attributed to bacterial infections which can be affordably prevented or cured (Holmes *et al.*, 2017; WHO, 2020b). Human immunodeficiency virus (HIV), bladder, stomach, cervical and liver cancers have been reported to be aggravated in patients with bacterial diseases (Abd-El-Raouf *et al*., 2020; de Martel *et al.*, 2020; WHO, 2020d). At least 55% of the 550,000 new stomach and bladder cancer cases worldwide are attributed to infections caused by bacteria such as *Helicobacter pylori* and *E. coli*. These bacteria play a part in the etiology of non-ulcer dyspepsia, duodenal and gastric ulceration, gastritis, bladder and gastric carcinoma by inducing inflammation, upregulating the generation of reactive oxygen species (inducing oxidative stress) and producing carcinogenic metabolites (Abd-El-Raouf *et al.*, 2020; Holmes Jr *et al.*, 2021; Parsonnet, 1995; WHO, 2020d).

Treatment of oxidative stress-induced conditions and bacterial diseases has become challenging in the face of antimicrobial resistance, prohibitive costs, limited access and side effects of the conventional drugs (Schultz *et al.,* 2020a). Because of the foregoing treatment challenges, some indigenous communities have folded back on the use of medicinal plants for managing such ailments. The World Health Organization (WHO) indicated that at least 50% of the world's population subsist on medicinal plants for their primary health care needs (WHO, 2019). Plants are preferred due to their availability, affordability, cultural acceptability and the belief that herbal medicines are more effective and safe than allopathic drugs (Schultz *et al.,* 2020b). It is therefore not surprising that oxidative stress-induced conditions and bacterial diseases are being treated using medicinal plants in various parts of the world, including Uganda (Schultz *et al.,* 2020a).

Uganda lies in the East African botanical plate and has more than 260 species of plants from over 160 genera being utilized in traditional medicine (Omara *et al.,* 2020a). *Albizia coriaria* Welw. ex Oliver (*A. coriaria*) is one of the treasured ethnomedicinal plants in Uganda. The plant, leaves, stem and root barks, roots, seeds and flowers of *A. coriaria* are used for treatment of diarrhoea, cough (tuberculosis), typhoid, cancers, fevers (malaria), coronary diseases, allergy, nausea, headaches, mental illness, anaemia, syphilis, constipation, post-partum haemorrhage, snakebites, sore throats, herpes zoster, menorrhagia, threatened abortion, skin diseases, jaundice and sore eyes (Omara *et al.*, 2020b; Schultz *et al*., 2020b).

Despite the widely reported therapeutic potential of *A. coriaria* by ethnobotanists, there are few reports on its phytochemicals (Obakiro *et al.,* 2020; Omara *et al.,* 2020b; Schultz *et al.,* 2021a). This study aimed at characterizing the phytochemicals in *A.*

coriaria leaves and evaluating its antioxidant and antibacterial activities to verify its claimed traditional use in the treatment of oxidative stress-induced conditions and bacterial diseases in Uganda.

1.2 Statement of the problem

Oxidative stress-induced conditions and bacterial diseases are some of the leading causes of mortality in the world (Shrivastava *et al.*, 2018; Tincho *et al.*, 2020; WHO, 2020a, 2020c). Treatment of these ailments using conventional drugs is expensive, carries several side effects due to lack of specificity and the drugs are usually inaccessible in rural settings. Further, pathogenic bacteria are developing resistance to the previously effective antibiotics (Schultz *et al.*, 2020a). Thus, there is need to search for novel, effective and cheaper drugs with new modes of action and less side effects (Domínguez *et al.*, 2020; Shrivastava *et al.*, 2018; WHO, 2017). Medicinal plants are being used as one of the readily available options for treatment of oxidative stress-induced conditions and bacterial diseases in various parts of the world (WHO, 2019). *Albizia coriaria* leaves, roots, root and stem barks are widely used for treatment of oxidative stress-induced conditions and bacterial diseases in Uganda (Asiimwe *et al.*, 2013; Musinguzi *et al.*, 2017; Nambejja *et al.*, 2019; Namukobe *et al*., 2011; Namukobe *et al.*, 2021; Schultz *et al*., 2020b). The compounds associated with the claimed bioactivities have only been partly characterized in the stem bark and roots (Byamukama *et al.*, 2015; Note *et al.*, 2010; Note *et al.*, 2009). This makes its use limited and has also led to over exploitation of the stem bark and roots (Tabuti and Mugula, 2007). Hence, there was a need for the current study to investigate the compounds in *A. coriaria* leaves.

1.3 Objectives of the study

1.3.1 General objective

The main objective of the study was to perform phytochemical screening, determine the total polyphenolic content, antioxidant and antibacterial activities and characterize the phytochemicals in the most active extracts of *A. coriaria* leaves from three agroecological zones of Uganda.

1.3.2 Specific objectives

The specific objectives of the study were to:

1. Phytochemically screen the secondary metabolites in ethyl acetate, ethanolic and aqueous extracts of *A. coriaria* leaves from Jinja, Kole and Mbarara districts of Uganda.
2. Determine the total phenolic and total flavonoid contents of extracts of *A. coriaria* leaves from Jinja, Kole and Mbarara districts of Uganda.
3. Establish the antioxidant activity of bioactive compounds in extracts of *A. coriaria* leaves from Jinja, Kole and Mbarara districts of Uganda.
4. Evaluate the antibacterial activity of extracts of *A. coriaria* leaves from Jinja, Kole and Mbarara districts of Uganda on *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella typhi*.
5. Perform bioassay-guided isolation and characterization of compounds in extracts of *A. coriaria* leaves from Jinja, Kole and Mbarara districts of Uganda.

1.4 Justification of the study

Bacterial infections and oxidative stress-mediated complications remain in the top list of the major causes of mortality reported globally (Shrivastava *et al*., 2018; Tincho *et al*., 2020; WHO, 2020a, 2020c). Further, they have been linked to cancer and increased HIV progression in immunocompromised patients (Anywar *et al.*, 2020a). *Albizia coriaria* leaves could be an alternative source of novel antioxidant and antibacterial drugs. However, these can only be developed and standardized if its phytochemical constituents, antioxidant and antibacterial activities are established. Therefore, there was need to investigate *A. coriaria* to determine the bioactive compounds in its leaves and their efficacy as antioxidant and antibacterial compounds. Further, establishing these efficacies could encourage the local communities to utilize the leaves instead of harvesting the stem bark and roots which causes the plant to dry (Tabuti and Magadula, 2007). Other than conserving the species, potential antibacterial compounds from *A. coriaria* leaves could be used in addressing the current antibiotic resistance crisis.

1.5 Research hypotheses (null hypotheses)

1. There are no significant differences in the secondary metabolites of *A. coriaria* leaves from Jinja, Kole and Mbarara districts of Uganda.
2. There are no significant differences in the total phenolic and total flavonoid contents of *A. coriaria* leaves from Jinja, Kole and Mbarara districts of Uganda.
3. The antioxidant potential of the *A. coriaria* leaves are the same.
4. *Albizia coriaria* leaves from Jinja, Kole and Mbarara districts of Uganda have no antibacterial activity.
5. The compounds in *A. coriaria* leaves from Jinja, Kole and Mbarara districts of Uganda are the same.

CHAPTER TWO: LITERATURE REVIEW

2.1 Taxonomy, morphology and geographical distribution of *Albizia coriaria*

2.1.1 Botanical classification

Albizia coriaria Welw. ex Oliver (*A. coriaria*) is a member of the genus *Albizia*, that is recognized worldwide for its ecological, economic and medicinal values (Kokwaro, 2009; Louppe *et al.*, 2008). The genus *Albizia* is traced to have first appeared in the literature by Durazzini in 1772. The Italian nobleman and naturalist, Filippo degli Albizzi was the first to introduce *A. julibrissin* Durazzin seeds from Constantinople to Florence (Italy) in 1749 (Krige, 2007; Nielsen, 1979).

The genus *Albizia* encompasses more than 185 fast-growing subtropical and tropical trees and shrubs in subfamily Mimosoideae of the family Fabaceae (Avoseh *et al.*, 2021; Lewis & Arce, 2005). *Albizia* is a pan tropical genus, with the species mostly indigenous to Africa, Madagascar, Asia, Australia and Southern North America (Abdel-Kader *et al*., 2001; Avoseh *et al.*, 2021; Janani *et al.*, 2014; Singab *et al.*, 2015).

The species *A. coriaria* bear resemblance to *Albizia ferruginea*, a close member of the *Albizia* genus which it is often confused with as they also share some medicinal uses. The commonly encountered synonyms of *A. coriaria* are *A. katangensis* De Wild. and *A. poissonii* A. Chev. (Tropical Plants Database, 2020). The taxonomic classification of *A. coriaria* is as follows.

Kingdom : Plantae

Phylum : Angiospermophyta

Class : Dicotyledonae

Order : Fabales

Family : Fabaceae

Subfamily: Mimosoideae

Genus : *Albizia* (Durazz. 1772)

Species : *Albizia coriaria* Welw. ex Oliver

2.1.2 Morphology, growth habit and geographical distribution

Albizia coriaria is a deciduous highly-branched slow growing tree, 6-36 m tall with an often twisted trunk (Agroforestry Database, 2009; Omeli, 2011; The Plant List, 2019). Young branchlets of *A. coriaria* usually appear hairy; the epithet ‘‘*coriaria*’’ describes the leathery texture of its upper leaf surfaces (Ganza, 2014). The leaves are bipinnate, oblong to elliptic 13-33 mm long, 5-17 mm wide and rounded. The flowers are characteristically white, sweet smelling with half-spherical heads and hanging red stamen filaments (**Figure 2.1.1**). The bark of *A. coriaria* is grey-black, rough and raggedly scaling. The fruit is a brown or purplish-brown pod with a tapered apex (Ganza, 2014). *Albizia coriaria* propagates vegetatively, using seedlings or wildings (World Agroforestry, 2019).

(a)

(b)

Figure 2.1.1. *Albizia coriaria* (a) tree, (b) fresh leaves and flowers (photos taken during sampling of *A. coriaria* leaves in Jinja district, Uganda).

Albizia coriaria thrives in various types of soils, typically at altitudes between 850 m and 1680 m above sea level. Thus, the tree is commonly known as ‘‘Giant albizia’’. It is found in Uganda, South Sudan, Kenya, Democratic Republic of Congo, Sudan, Zambia, Benin, Cote D’Ivoire, Ghana, Nigeria, Togo, Cameroon, Southern Angola and Tanzania where it is indigenous in transition zones between savannah and dry forests of tropical Africa (Hyde *et al.*, 2021; Kaba *et al.*, 2020; Katende *et al.*, 1995; USDA, 2021). In Uganda, it is a pioneer distinct tree usually found throughout the country on forest edges and wooded grasslands (Schultz *et al.*, 2021a).

2.2 Traditional medicinal uses of *A. coriaria* in Uganda

Albizia coriaria is one of the few plant species in Uganda that have been reported to cure both human and veterinary ailments. It is a treasured medicinal plant as evidenced by the existence of its name in various Ugandan local languages (Namukobe *et al.*, 2011; Schultz *et al.*, 2021a; Shehu *et al.*, 2018). It is called *Itek, Bata* in Lango (Opio *et al.*, 2017), *Musita* in Lusoga (Nampanzira *et al.*, 2015), *Mugavu* (Luganda), *Musiisa* in Lukiga (Lacroix *et al*., 2011; Mugisha *et al.*, 2014), *Etek, Etekwa* (Ateso), *Oyo* (Madi), *Latoligo, Ayekayek* in Acholi (Oryema *et al.*, 2010), *Chesovio, Kumoluko* or *Kiluku* in Lugishu (Olila *et al.*, 2007), *Musisa*, *Murongo* (Lunyankore), *Musisa*, *Musisye, Yenuberi* in Lunyoro (Kyazike, 2021), *Muyenzayenze* (Lukiga), *Musisiya* (Kwamba), *Mubere* (Lugwe) and *Ecailait* in Ngakarimojong (Gradé *et al.*, 2009; Katende *et al*., 1995; Omara *et al.*, 2020b; World Agroforestry, 2019). Different parts of *A. coriaria* are used for treatment of various diseases and disorders in Uganda (**Table 2.2.1**). The bark (stem bark) is the most used part of *A. coriaria*, followed by roots, leaves, flowers, whole plant and then seeds.

Table 2.2.1. Ethnomedicinal uses of different parts of *A. coriaria* in Uganda

Ailment(s) treated	Part(s) used	Preparation/administration	Reference(s)
Skin rash, wounds, syphilis, albino skin burns	Leaves, stem bark	Leaf powder mixed with jelly is applied for rashes. Decoction used for bathing to treat wounds and syphilis. For skin burns, mix bark powder with powder of *Albizia grandibracteata* leaves, add to jelly and smear	Asiimwe *et al.* (2021); Namukobe *et al.* (2021)
Cough, *Otitis media* (ear infection), poison	Flowers, leaves, stem bark	Bark chewed for cough; for ear infection, decoction of flowers and young leaves is dropped into the ear. Bark decoction with that of *Lannea schweinfurthii* (Engl.) is taken as an emetic for poisoning	Kyazike (2021)
Malaria, general infections, skin and soft tissue infections, toothache, inflammatory disorders (pain, redness, heat, swelling and wounds), stomachache, gastrointestinal tract infections, tuberculosis, blood, abdominal, bone marrow, cervical, intestinal, prostate, skin and throat cancers, erectile dysfunction (as an aphrodisiac), fibroids, heart diseases, hernia, HIV/AIDs, sexually transmitted infections, syphilis, typhoid fever, vaginal dryness and ulcers	Stem bark, stem, leaves, seeds	Decoction taken or used for herbal bath for infections. For toothache, the decoction is used to rinse the mouth without swallowing. For inflammatory disorders, powder is applied directly/topically or mixed with petroleum jelly and smeared on the body part	Asiimwe *et al.* (2021); Schultz *et al.* (2020b)
Stomach and skin lesions, cancer, fatigue, heart disease, allergy, nausea, headaches, mental illness, diarrhoea, cough, tuberculosis, anaemia and boosting immune system of people with HIV/AIDs	Bark	Decoction taken	Anywar *et al.* (2020a; 2020b)

Ailment(s) treated	Part(s) used	Preparation/administration	Reference(s)
Headache, fever, toothache. Applied as a wash to kill head lice	Leaves	Decoctions used externally. Used as a wash or steam inhalation against fever (including malaria)	Tropical Plants Database (2020); New Vision (2019)
Skin rashes and wounds	Leaves, bark	Leaves and bark pounded separately and compressed on the affected area	Nambejja *et al.* (2019)
Cough, diabetes, pleurisy, allergy, sore skin, chest congestion, worms, stomachache, colic pain, toothache, dysentery	Bark, roots	Decoction with rock salt taken or used for bathing in the case of skin rash. Pounded with rock salt and rubbed on the teeth for toothache. For hernia, the bark with that of *Erythrina abyssinica* are boiled while covered to retain steam and the cold decoction is taken. The root barks are chewed, and liquid swallowed for toothache, dysentery and stomachache	Gumisiriza *et al.* (2019)
Used as toothbrush	Unspecified	Not reported	TROFACO (2019)
Diarrhoea, chronic cough	Stem bark	Infusion taken	Shehu *et al.* (2018)
Sore eyes, strong cough/tuberculosis, skin diseases (e.g. ring worms), stomach ulcers, syphilis, constipation, stomachache	Leaves, roots, bark	Decoction taken	Asiimwe *et al.* (2021); Musinguzi *et al.* (2017)
Malaria	Flowers	Infusion drunk	Opio *et al.* (2017)
HIV/AIDS and its opportunistic ailments (gastrointestinal, bacterial, viral and fungal infections, diarrhoea, cough, tuberculosis, herpes zoster and respiratory infections)	Leaves, roots, stem bark	Decoction (100 mL) drunk twice daily or use for bathing. Boiled, honey is added and taken for respiratory ailments. Dry bark powder applied topically for viral infections	Asiimwe *et al.* (2013); Lamorde *et al.* (2010); Nyamukuru *et al.* (2017)
Influenza and its symptoms; diarrhoea, fatigue	Stem bark, roots	Decoction taken for influenza	Katuura *et al.* (2016); Kigenyi (2016)
Skin rash, cough in children, swollen rectum	Bark	Decoction used to bathe for rash and taken for cough. Boil and sit in the water for swollen rectum	Tugume *et al.* (2016)

Ailment(s) treated	Part(s) used	Preparation/administration	Reference(s)
Skin disorders, malaria	Bark	Decoction used to bathe for skin disorders or taken 3 spoons thrice daily for a week by children and adults, for malaria	Adia *et al.* (2014)
Bacterial and fungal diseases, gastrointestinal and viral infections	Leaves, stem bark, roots	Boil, bathe with or drink 100 ml daily. Bark powder applied on affected part for infections	Mugisha *et al.* (2014)
Tuberculosis, respiratory diseases	Stem bark, roots	Boil, add honey and drink 100 ml thrice daily	Bunalema *et al.* (2014); Orodho *et al.* (2011)
Cough, infertility in men, diarrhea	Stem bark	Decoction (500 ml thrice daily for adults and 250 ml once for children taken until recovery) for cough. For infertility, bark boiled with *Cymbopogon nardus* (L.) Rendle flowers in a local brew is drunk. Infusion taken for diarrhea	Namukobe *et al.* (2011); Lacroix *et al.* (2011)
Poultry diseases	Bark	Prophylactic decoction given	Nalubega (2010)
Cough, epilepsy, syphilis, stomachache	Roots	Not reported	Oryema *et al.* (2010)
Rinderpest/cattle plague (*Loleo*) and barrenness (*Atengina ekolupana*) in cows, East Africa coast fever	Bark, roots	Infusion/decoction given twice daily [a]	Grade *et al.* (2009); Tabuti *et al.* (2003)
Syphilis, anaemia, dermatological diseases, jaundice, eye diseases, cough, sore throat. Used as a general tonic and to concentrate human breast milk	Bark, roots	Half cup of decoction drunk twice daily for syphilis and anaemia	Asiimwe *et al.* (2021); Tabuti *et al.* (2007)
Cough in poultry	Leaves, stem bark	Infusion given	Olila *et al.* (2007)
Meat allergy	Bark	Decoction drunk	Ssegawa and Kasenene (2007)
Diarrhoea, cough, snakebites, amoebiasis, syphilis, lameness (*Butenge*)	Bark, leaves, roots	Bark infusion/decoction taken for diarrhoea/syphilis; bark/leaf infusion taken for snakebites; root/bark infusion used for bathing to treat	Tabuti *et al*. (2003)

Ailment(s) treated	Part(s) used	Preparation/administration	Reference(s)
		amoebiasis; root infusion with *tonto* [b] taken for pyomyositis. For lameness, *Steganotaenia araliacea* leaves are added to warm bark decoction & used to massage the limb	
Fever, constipation	Unspecified	Not reported	Nanyunja (2003)
Venereal diseases, sore eyes	Roots	Used as steam for sore eyes	Kokwaro (1993)
Menorrhagia, threatened abortion, post-partum haemorrhage	Bark	Not reported	Kokwaro (1993)

Note: [a] Decoction may also be prepared with milk, or an infusion is made with *Oncoba spinosa* roots, or roots of *Milicia excelsa* and *Securidaca longipedunculata* and a calf is given 500 mL daily. Infusion with *Clerodendrum myricoides* may also be prepared and given once daily in the morning.
[b] *tonto* is a traditional Basoga beer prepared from *Musa* × *paradisiaca* L. var. sapientum fruits.

In other countries, *A. coriaria* is used in the treatment of malaria, helminthiasis, tuberculosis, diarrhoea, breast, skin and uterine cancers, menorrhagia, hypertension, dermatological conditions, threatened abortion, venereal diseases, sore eyes, lungworms/ascaris worms (in cattle, sheep, goats), gastrointestinal infections, as toothbrush (*Miswak*) and mosquito repellent i.e. logs are burnt with cow dung (Araya, 2007; Bossard, 1993; Dharani *et al.*, 2015; Geissler *et al*., 2002; ICRAF, 1992; Jeruto *et al.*, 2010; Johns *et al.*, 1995; Johns *et al.*, 1990; Leiderer, 1982; Obakiro *et al.*, 2020; Ochwang'i *et al.*, 2014; Olala, 2014; Omara, 2020; Orwa *et al.*, 2007; Shiracko *et al.*, 2016; Sirama, 2014; Tsabang *et al.*, 2017).

2.3 Phytochemical studies done on *A. coriaria*

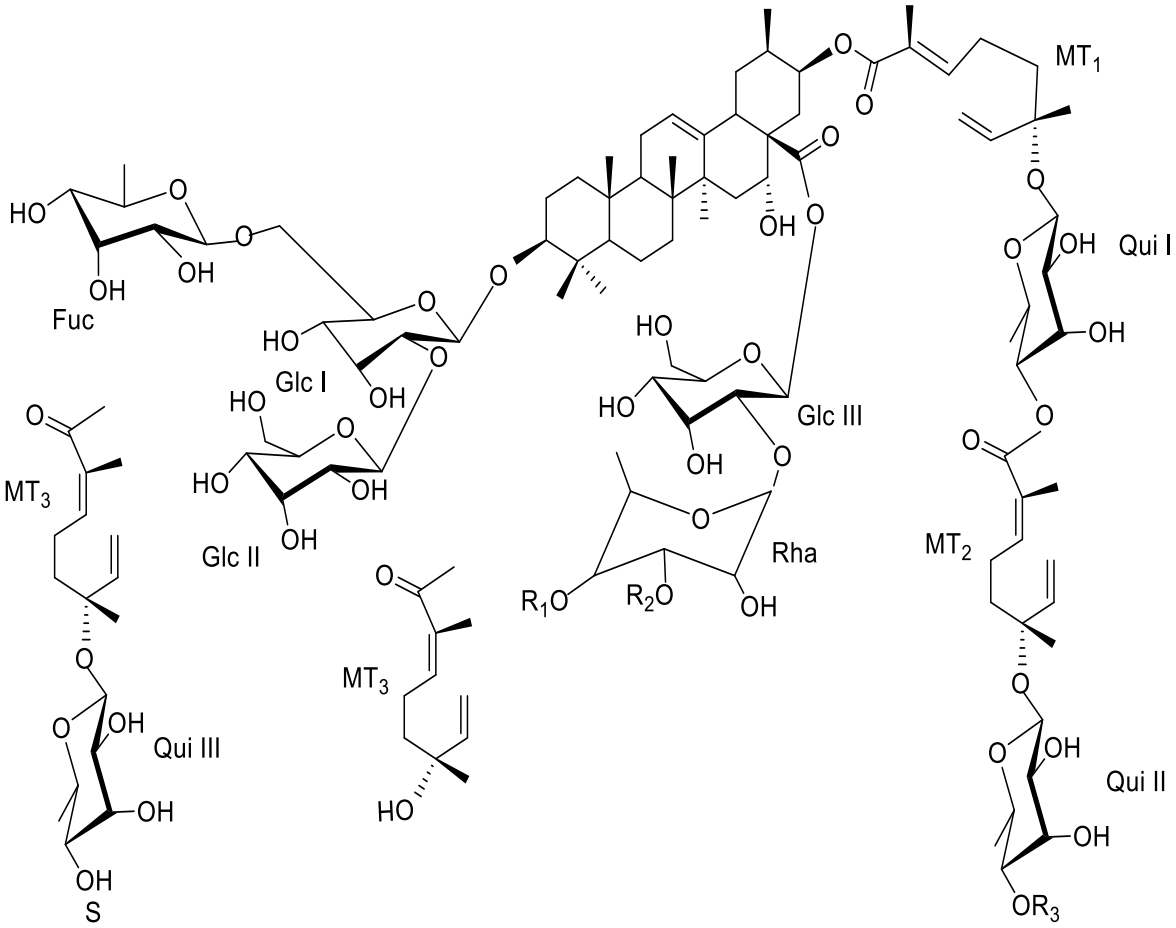
Phytochemical analyses of ether, ethanolic, methanolic and aqueous extracts of *A. coriaria* stem bark by Mengesha *et al.* (1997), Akanga (2008) and Agroforestry

Database team (2009) indicated that the active phytochemicals were tannins, saponins, alkaloids, flavonoids, steroids, triterpenoids, reducing sugars, flavone aglycones, volatile oils, polyuronides, glucides, sterols and coumarins.

Two new oleanane-type saponins: coriariosides A (**1**) and B (**2**), and a known saponin, gummiferaoside C (**3**) were isolated, purified and characterized using high-resolution electrospray ionization mass spectrometry (HR-ESI-MS) and extensive Nuclear Magnetic Resonance spectroscopy (NMR) from *n*-butanol fraction (obtained from methanolic extract) of *A. coriaria* roots by Note *et al.* (2009). **Coriaroside A** was identified as 3-*O*-{β-D-fucopyranosyl-(1→6)-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranosyl}-21-*O*-{(2E,6S)-6-*O*-{4-*O*-[(2E,6S)-2,6-dimethyl-6-*O*-(β-D-quinovopyranosyl)octa-2,7-dienoyl]-4-*O*-[(2E,6S)-2,6-dimethyl-6-*O*-(β-D-quinovopyranosyl)octa-2,7-dienoyl]-β-D-quinovopyranosyl}-2,6-dimethylocta-2,7-dienoyl}acacic acid 28-*O*-α-L-arabinofuranosyl-(1→4)-[β-D-glucopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl ester. **Coriarioside B** was deduced to be 3-*O*-{β-D-fucopyranosyl-(1→6)-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranosyl}-21-*O*-{(2E,6S)-6-*O*-{4-*O*-[(2E,6S)-2,6-dimethyl-6-*O*-(β-D-quinovopyranosyl)octa-2,7-dienoyl]-4-*O*-[(2E,6S)-2,6-dimethyl-6-*O*-(β-D-quinovopyranosyl)octa-2,7-dienoyl]}-2,6-dimethyl-6-hydroxyocta-2,7-dienoyl}acacic acid 28-*O*-α-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl ester. **Gummiferaoside C**, previously isolated from *Albizia gummifera* roots (Cao *et al.*, 2007) was identified as 3-*O*-{β-D-fucopyranosyl-(1→6)-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranosyl}-21-*O*-{(2E,6S)-6-*O*-{4-*O*-[(2E,6S)-2,6-dimethyl-6-*O*-(β-D-quinovopyranosyl)octa-2,7-dienoyl]-4-*O*-[(2E,6S)-2,6-dimethyl-6-*O*-(β-D-quinovopyranosyl)octa-2,7-dienoyl]-β-D-quinovopyranosyl}-2, 6-

dimethylocta-2,7-dienoyl} acacic acid 28-*O*-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl ester (**Figure 2.3.1**).

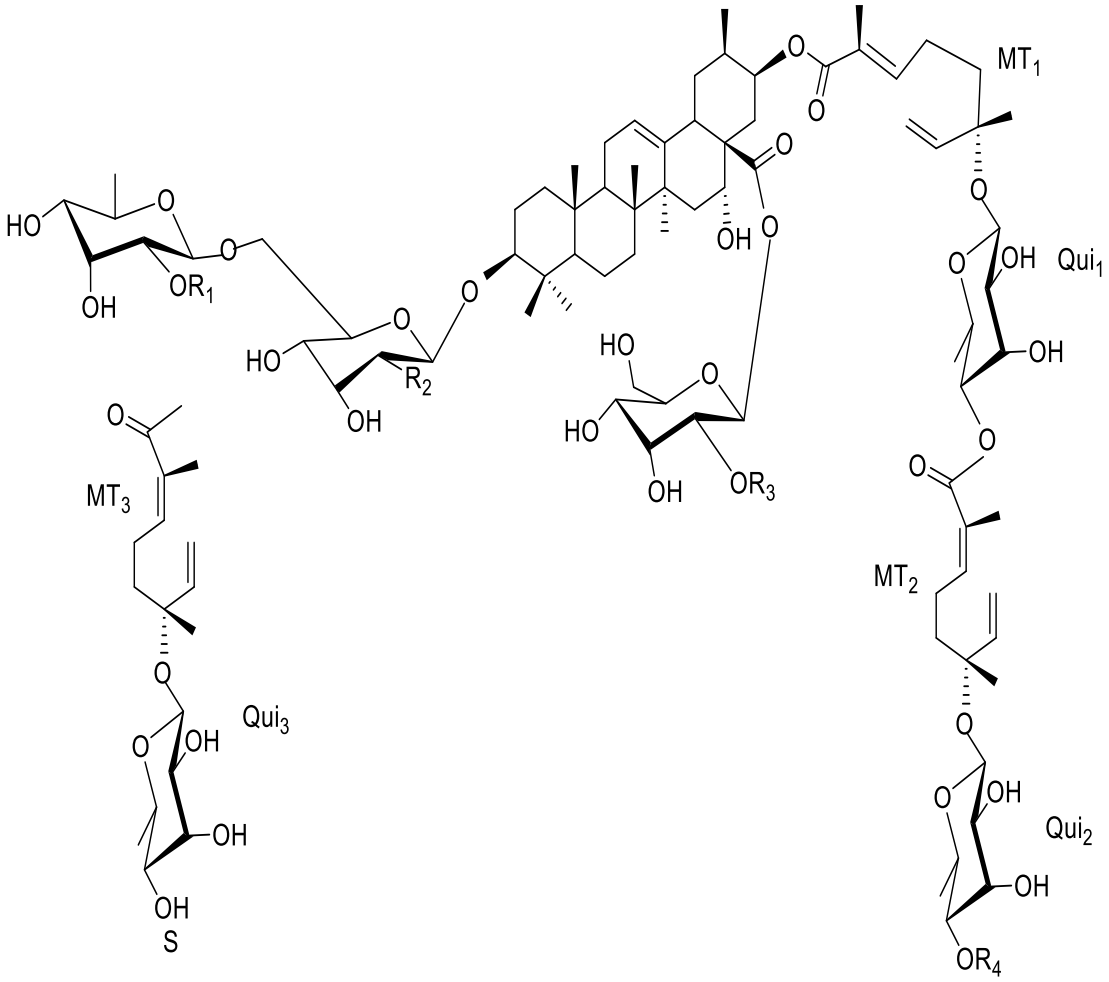
Further, HR-ESI-MS and NMR analysis of the chloroform-methanol-aqueous fractions of methanolic extract of *A. coriaria* roots revealed the presence of acacic acid glycosides which were previously reported in other *Albizia* species: *A. julibrissin*, *A. grandibracteata, A. procera, A. adianthifolia*, *A. gummifera* and *A. chinensis* (Note *et al.*, 2010). The coriariosides (triterpenoid saponins) were characterized as 3-*O*-[β-D-xylopyranosyl-(1-2)-β-D-fucopyranosy-l-(1-6)-2-(acetamido)-2-deoxy-β-D-glucopyranosyl]-21-*O*-{(2E,6S)-6-O-{4-*O*-[(2E,6S)-2,6-dimethyl-6-*O*-(β-D-quinovopyranosyl) octa-2,7-dienoyl}acacicacid-28-*O*-β-D-xylopyranosyl-(1-4)-α-rhamnopyranosyl-(1-2)-β-D-glucopyranosyl ester **(4)**, 3-*O*-{β-D-fucopyranosyl-(1-6)-[β-D-glucopyranosyl-(1-2)-β-D-dlucopyranosyl}-21-*O*-{(2E,6S)-6-*O*-{4-O-[(2E,6S)-2,6-dimethyl-6-*O*-(β-D-quinovopyranosyl) octa-2,7-dienoyl]-β-D-quinovopyranosyl-2,6-dimethylocta-2,7-dienoyl}acacic acid-28-*O*-α-L-rhamnopyranosyl-(1-2)-β-D-glucopyranosyl ester **(5)** and 3-*O*-[β-D-fucopyranosyl-(1-6)-β-D-glucopyranosyl]-21-*O*-{(2E,6S)-6-*O*-{4-*O*-[(2E,6S)-2,6-dimethyl-6-*O*-(β-D-quinovopyranosyl)octa-2,7-dienoyl)-β-D-quinovopyranosyl]octa-2,7-dienoyl}acacic acid-28-*O*-β-D-glucopyranosyl ester (**6**). These were named **Coriariosides C**, **D** and **E**, respectively (**Figure 2.3.2**).



Molecule	**R_1**	**R_2**	**R_3**
Coriarioside A (**1**)	Ara*f*	Glc	S
Coriarioside B (**2**)	Xyl	H	MT_3
Gummiferaoside C (**3**)	Xyl	H	S

Key: Ara*f* = α-arabinofuranosyl, Fuc = β-fucopyranosyl, Glc = β-glucopyranosyl, MT = monoterpenyl moiety (labelled 1 to 3), Rha = α-rhamnopyranosyl, Xyl = β-xylopyranosyl, Qui = Quinovose.

Figure 2.3.1. Structure of saponins isolated from *A. coriaria* roots.



Acacic acid type saponin	**R_1**	**R_2**	**R_3**	**R_4**
Coriarioside C (**4**)	Xyl	NHAc	Xyl (1→4) Rha	S
Coriarioside D (**5**)	H	O-Glc	Rha	S
Coriarioside E (**6**)	H	OH	H	H

Key: Glc = β-glucopyranosyl, Rha = α-rhamnopyranosyl, Xyl = β-xylopyranosyl, NHAc = 2-(acetamido)-

Figure 2.3.2. Structure of acacic acid saponins isolated from *A. coriaria* roots.

Nalubega (2010), Owuor *et al.* (2012) and Langat (2013) reported the presence of alkaloids, flavonoids, sesquiterpene lactones, cardiac glycosides, saponins and steroids in dichloromethane, aqueous and ethanolic extracts of *A. coriaria* stem bark. From petroleum ether extract of *A. coriaria* stem bark, Wanyama *et al.* (2011) detected the presence of coumarins, tannins, alkaloids, steroids, triterpenoids and reducing sugars. India (2015) reported similar results in Kenya in which methanol: dichloromethane (1:1, v/v) extract of *A. coriaria* bark extract contained tannins, alkaloids, flavonoids, saponins, cardiac glycosides and terpenoids.

Employing HR-ESI-MS and NMR, Byamukama *et al.* (2015) reported the presence of lupeol (**7**), lupenone (**8**), betulinic acid (**9**), acacic acid lactone (**10**), (+)-catechin (**11**) and benzyl alcohol (**12**) in ethyl acetate extract of *A. coriaria* stem bark (**Figure 2.3.3**).

To date, there are no reports on the phytochemicals in *A. coriaria* leaves, flowers and seeds. However, triterpenoidal saponins (usually containing aglycon parts as echinocystic acid, oleanolic acid, acacic acid, lactone or machaerinic acid γ-lactone) are commonly described in the *Albizia* genus (He *et al.*, 2020). The sugar residues are usually glucose, 2-acetamido-2-deoxy glucose, xylose, rhamnose, fucose or arabinose (Singab *et al*., 2015). In addition, some lignanoids, macrocyclic alkaloids, flavonoids and phenolic glycosides have been reported in this genus (He *et al.*, 2020).

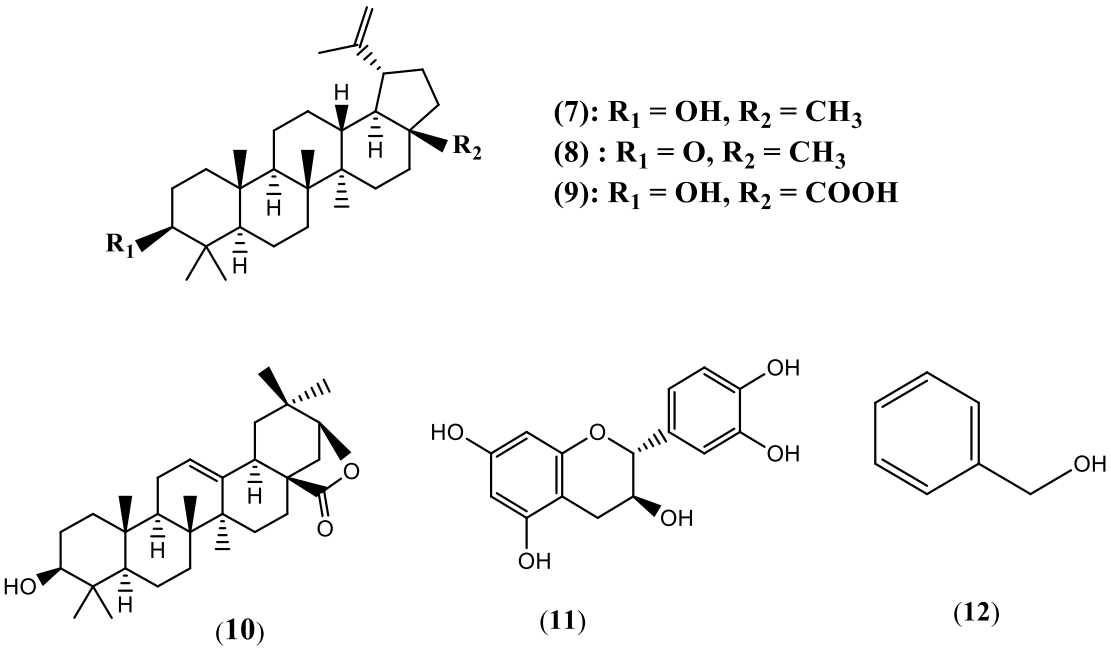


Figure 2.3.3. Structure of compounds isolated from *A. coriaria* stem bark.

2.4 Biological activities, toxicity and mutagenicity profile of *A. coriaria*

The genus *Albizia* is known for its various biological activities (He *et al.*, 2020; Singab *et al*., 2015). However, *A. coriaria* has not been fully investigated for its pharmacological activities to verify the claimed use of its various parts in traditional medicine (Byamukama *et al.*, 2015; Omara *et al.*, 2020b; Schultz *et al.*, 2021a). Some of the investigated bioactivities of its extracts and isolated compounds include antigiardial, molluscicidal, antiplasmodial, antimicrobial, antioxidant, anti-inflammatory and antiproliferative activities.

2.4.1 Antigiardial, molluscicidal and antiplasmodial activities

It has been reported that methanol extracts of *A. coriaria* roots and bark caused 100% death of *Giardia lamblia* trophozoites at 500 ppm and 1000 ppm (Johns *et al.*, 1995). A similar observation was made by Mengesha *et al.* (1997) in which methanol, ethanolic and aqueous extracts of *A. coriaria* bark resulted in 100% mortality of snails (*Biomphalaria pfeifferi*) at 50 ppm upon exposure to the extracts for six hours.

From another investigation, Owuor *et al.* (2012) reported that dichloromethane extract of *A. coriaria* stem bark was effective against D6 (chloroquine sensitive) and W2 (chloroquine resistant) strains of *Plasmodium falciparum* with half inhibitory concentration (IC_{50}) values of 6.7987 ± 3.04 μg/mL and 10.6797 ± 1.939 μg/mL, respectively. This was corroborated by another report in which methanolic extracts of *A. coriaria* stem bark had antiplasmodial activity with IC_{50} of 15.2 μg/mL and 16.8 μg/mL against D6 and W2 *P. falciparum* strains (Muthaura *et al.*, 2015). Aqueous extracts only elicited bioactivity against W2 strain with IC_{50} >100 μg/mL.

2.4.2 Antimicrobial, antioxidant and anti-inflammatory activities

Pseudomonas aeruginosa (inhibition diameter = 16 mm), *Bacillus subtilis* (inhibition diameter = 23 mm) and *Escherichia coli* (*E. coli*) with zone of inhibition diameter (ZOI) of 10 mm were reported to be susceptible to methanolic stem bark extract of *A. coriaria* indigenous to Uganda (Olila *et al.*, 2007). *Staphylococcus aureus* (*S. aureus*) was resistant to the methanol extract. All the bacteria screened were resistant to the petroleum ether extract in the study. Contrastingly, methanolic extract of *A. coriaria* stem bark harvested from Kenya elicited high bacteriostatic activity against *S. aureus* with ZOI of 18 mm (Luvonga, 2007). In the study, aqueous extracts recorded minimum

inhibitory concentration (MIC) of 12.5 mg/mL for *S. aureus*, 25 mg/mL for *Streptococcus pneumoniae* and 25 mg/mL for *Pseudomonas aeruginosa* (*P. aeruginosa*). The methanolic extract had MIC of 3.13 mg/mL for *S. aureus* and 25 mg/mL against *P. aeruginosa*. The aqueous extracts had minimum bactericidal concentration (MBC) of 12.5 mg/mL for *S. aureus*, 12.5 mg/mL for *S. pneumoniae* and 50 mg/mL for *P. aeruginosa*.

In another study conducted in Kenya, methanolic extract of *A. coriaria* stem bark had MIC of 12.5 mg/mL against *S. aureus* isolate, 25 mg/mL against *Shigella flexneri* and *Proteus mirabilis* and 50 mg/mL against *E. coli* isolate, clinical *S. aureus* and *E. coli* (Akanga, 2008). The hexane and acetone extracts of the stem bark however were not active on the tested microorganisms. Similarly, Nalubega *et al.* (2011) found that *A. coriaria* stem bark (aqueous and ether extracts) had bacteriostatic potential with ZOI of 1.6 mm and 1.9 mm, and 1.5 mm and 1.7 mm against *Streptococcus faecalis* and *S. aureus* but no activity was recorded against *E. coli* and *Salmonella typhi* (*S. typhi*). Ethanolic extracts used comparatively in the study had ZOI of 1.7 mm, 2.0 mm and 1.6 mm against *Streptococcus faecalis*, *S. aureus* and *E. coli* but did not inhibit *S. typhi*. The MIC was 0.5 g/mL for both *S. faecalis* and *S. aureus*.

Byamukama *et al.* (2015) indicated that the ethyl acetate extract of *A. coriaria* stem bark had the highest ZOI of 18 mm and 17 mm against *E. coli* and *P. aeruginosa*. The methanolic extract had a ZOI of 8 mm against *P. aeruginosa* but did not inhibit the growth of *E. coli*. The aqueous extract had no bioactivity against all the bacterial strains tested. The authors argued that the bacterial species tested were only susceptible to ethyl acetate extract as the inhibition diameters were within the range for standard antibiotics such as ampicillin (ZOI = 16-22 mm), doxycycline (ZOI = 18-24 mm) and tetracycline

(ZOI = 18-25 mm). The ethyl acetate extract had a MIC of 125 mg/mL on *E. coli* and 250 mg/mL on *P. aeruginosa* while the MBC was 125 mg/mL for *E. coli.* India (2015) found that methanol: dichloromethane (1:1, v/v) stem bark extract of *A. coriaria* had moderate antibacterial activity against *Bacillus subtilis*, *S. aureus* and Methicillin resistant *S. aureus* with ZOI of 12 mm, 10 mm and 13 mm, respectively. The MIC and MBC were in the range of 1.875 to 3.75 mg/mL. It however, showed very low antibacterial activity (ZOI of 6 mm each) against *E. coli* and *S. typhi*.

Ethanolic, methanolic and dichloromethane: methanol (50:50, v/v) extracts of *A. coriaria* stem bark exhibited anti-mycoplasmal activity against *Mycoplasma mycoides* subspecies *mycoides* (Afadé, B 237, Gladysdale, PG1 and V5) with IC_{50} of 0.227 ± 0.114 mg/mL, 0.137 ± 0.092 mg/ml and 0.327 ± 0.110 mg/mL (Kama-Kama *et al.*, 2016). *Mycoplasma mycoides* subspecies *capri* (Y-Goat, 95010, G1313.94, M-18 and G1255/94) had IC_{50} of 0.237 ± 0.110 mg/mL, 0.417 ± 0.090 mg/mL and 0.137 ± 0.092 mg/mL while *Mycoplasma capricolum* subspecies *capricolum* (6443-90) with IC_{50} of 0.05 mg/mL, 0.005 mg/mL and 0.05 mg/mL (Kama-Kama *et al.*, 2016). Aqueous extracts only exhibited bioactivity against B 237, Gladysdale, PG1 and V5 *Mycoplasma mycoides* subspecies *mycoides*.

In another investigation, ethanolic extracts of *A. coriaria* stem bark had no inhibitory effect on the growth of *Enterococcus faecium* EU-44, *S. aureus* UAMS-1, *Klebsiella pneumoniae* CDC-004 and *Enterobacter cloacae* CDC-0032 when tested at 256 µg/mL (Schultz *et al.*, 2020a). The ethanolic extract had IC_{50} and MIC values greater than 256 µg/mL for *Acinetobacter baumannii* CDC-0033, and 32 µg/mL and > 256 µg/mL for *P. aeruginosa* AH-71. Further, ethyl acetate and ethanolic extracts did not exhibit quorum sensing above 40% at 16 µg/mL in a quorum-sensing inhibition plant extract

library screen on *S. aureus* accessory gene regulator I reporter strain (Schultz *et al.*, 2020a).

An extension of the foregoing study (Schultz *et al.*, 2021b) determined the *in vitro* selective cyclooxygenases (COX-1 and COX-2) inhibitor, 15-Lipoxygenase (15-LOX) inhibition screening as well as the total phenolic content, antioxidant potential and antibacterial assay against multidrug-resistant *S. aureus*, *E. coli* K12 and *Listeria innocua* using 76 different plant extracts including ethyl acetate and ethanolic extracts of *A. coriaria* stem bark. Initial COX-2 extract library screen of *A. coriaria* stem bark extracts at 50 μg/mL indicated that only the ethanolic extract had 1-40% COX-2 inhibition. The extracts did not exhibit any 15-LOX inhibition at 10 μg/mL (Schultz *et al.*, 2021b). The total phenolic content of the ethyl acetate and ethanolic extracts were 28.36 ± 0.97 mg chlorogenic acid equivalent/g extract (mg CAE/ gE) and 28.37 ± 0.34 mg 1CAE/ gE while the antioxidant potential as half effective concentrations were 22.98 ± 2.47 μg/mL and 18.39 ± 2.23 μg/mL, respectively. In antibacterial activity assay, the extracts had MIC between 250-500 mg/mL and greater than 500 mg/mL for *S. aureus*, *E. coli* K12 and *L. innocua*, respectively (Schultz *et al.*, 2021b).

2.4.3 Antitumor (antiproliferative) activity

Coriarioside A (**1**) and gummiferaoside C (**3**) isolated by Note *et al.* (2009) exhibited good antitumor activity against two colorectal human cancer cells: HCT 116 (with IC_{50} of 4.2 μM for **1** and 2.7 μM for **3**) and HT-29 (with IC_{50} 6.7 μM for **1** and 7.9 μM for **3**). Crude extracts of *A. coriaria* have not been evaluated for antiproliferative activity.

2.4.4 Toxicity and mutagenicity profile of *A. coriaria*

Anywar *et al.* (2021) cited prolonged boiling of *A. coriaria* stem bark decoctions for up to 6 hours prior to administration by herbalists in Uganda (Anywar *et al.*, 2020a). The authors argued that it is because the extracts have side effects (notably vomiting, dizziness and weakness), which explains why it is contraindicated in pregnant women and weak patients (Anywar, 2020; Anywar *et al*., 2020a; Anywar *et al.*, 2020b).

Toxicity studies on aqueous extracts of *A. coriaria* stem bark revealed that it had a median lethal dose (LD_{50}) of 533.67 µg/mL, which is considered non-toxic (Akanga, 2008). Kigondu *et al.* (2009) also reported that the methanolic and aqueous extracts of *A. coriaria* stem bark exhibited low cytotoxicity against human embryonic lung fibroblast cells with median cytotoxic concentration greater than 500 µg/mL. These reports indicated that utilization of *A. coriaria* stem bark in traditional management of diseases may not have adverse health effects.

Interestingly, ethyl acetate and ethanolic extracts of *A. coriaria* stem bark were indicated to be non-mutagenic with mutagenicity indices of 0.7 to 1.6 without and with metabolic activation in a *Salmonella* reverse mutation assay at 500 µg/plate using *S. enterica* subspecies enterica *Typhimurium* strains TA98 and TA100 (Schultz *et al.*, 2021c). Therefore, further studies are warranted to generate sufficient evidence on the safety (toxicity) and other adverse effects of *A. coriaria* extracts from other parts of the plant to guarantee their safety when utilized in traditional medicine.

2.5 Oxidative stress and its role in pathophysiology of diseases: the role of plants as natural antioxidants

Oxidative stress is a phenomenon triggered by an imbalance between production and accumulation of reactive oxygen and nitrogen species (RONS) in cells and tissues, and the ability of the biological antioxidant system to detoxify these reactive products (Liguori *et al.*, 2018; Pizzino *et al*., 2017). Reactive oxygen species (ROS) or reactive oxygen intermediates are highly reactive chemical molecules that are formed due to the electron receptivity of oxygen. They include hydroxyl radical ($^{\bullet}OH$), superoxide radical ($^{\bullet}O_2^-$), singlet oxygen ($^1O_2$), alpha-oxygen (α-O) and hydrogen peroxide, H_2O_2 (Hayyan *et al.*, 2016). The reduction of molecular oxygen (O_2) produces superoxide radical which is the precursor of other ROS (Turrens, 2003).

$$O_2 + e^- \longrightarrow {}^{\bullet}O_2^- \quad \textbf{(Equation 2.5.1)}$$

Dismutation of superoxide radical yields hydrogen peroxide (H_2O_2).

$$2\,H^+ + {}^{\bullet}O_2^- + {}^{\bullet}O_2^- \longrightarrow H_2O_2 + O_2 \ \textbf{(Equation 2.5.2)}$$

The hydrogen peroxide may be partly reduced, yielding hydroxide ion (OH^-) and hydroxyl radical ($^{\bullet}OH$) or may get completely reduced to water.

$$H_2O_2 + e^- \longrightarrow HO^- + {}^{\bullet}OH \ \textbf{(Equation 2.5.3)}$$

$$2\,H^+ + 2\,e^- + H_2O_2 \longrightarrow 2\,H_2O \ \textbf{(Equation 2.5.4)}$$

Though ROS are formed as by-products of normal aerobic oxygen metabolism (respiration and photosynthesis), environmental stressors such as ultraviolet, ionizing radiations, pollutants, heavy metals and xenobiotics can enhance ROS production resulting into an imbalance that causes cell and tissue damage/oxidative stress (Borges

et al., 2022). At low and stationary levels in normal cells, ROS play important physiological roles in cell signaling and homeostasis (Edreva, 2005).

Reactive nitrogen species (RNS) on the other hand is a diverse group of reactive compounds which include the free radical nitric oxide and compounds originating from the reaction of nitric oxide and oxygen or oxygen-derived compounds. They include peroxynitrite and its reaction products such as nitric oxide (Ronzio, 2020).

Oxidative stress has been implicated in the etiology of various age-related conditions such as chronic obstructive pulmonary disease, cardiovascular diseases, diabetes, chronic kidney diseases, neurodegenerative diseases, cancers, ischemia-reperfusion (re-oxygenation) injury, sarcopenia and frailty (de Araújo *et al.*, 2016; García-Sánchez *et al.*, 2020; Liguori *et al.*, 2018; Pleńkowska *et al.*, 2020; Shiota, 2021). Antioxidants such as polyphenols, vitamin E and vitamin C have been used for a long time for their actual or supposed beneficial effect against oxidative stress. Plant materials such as seeds, fruits, vegetables and medicinal herbs houses free radical scavenging (antioxidant) molecules as phenolic and nitrogenous compounds, vitamins, terpenoids and other endogenous secondary metabolites (Dziurka *et al.*, 2021).

The *in vitro* antioxidant activity of plant extracts have been established using various methods which involves either hydrogen-atom transfer reactions or single electron transfer reactions i.e. compound reduction reactions through electron transfer from an antioxidant (Chaves *et al.*, 2020). The commonly used methods are based on single electron transfer reactions. These include radical generating systems such as 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, nitric oxide, ferric reducing power, trolox equivalent antioxidant capacity, copper reduction and reducing power

assays. The hydrogen atom transfer reaction assays include total peroxyl radical-trapping antioxidant parameter, crocin bleaching, total oxyradical scavenging capacity and the oxygen radical absorbance capacity assays (Chaves *et al.*, 2020).

2.6 Pathogenic bacteria and bacterial diseases

Bacteria are ubiquitous prokaryotic organisms which carry their genetic information in a double-stranded circular molecule of DNA (Rath *et al.*, 2020). They play an important role in maintaining the environment as well as normal human body functions. This gives them a unique position among prokaryotes in that many of them are normal flora that colonize the host without causing infections (Rath *et al.*, 2020). However, some known bacteria cause infections and diseases. Bacterial infections are becoming a global health burden due to the emergence of antimicrobial-resistant bacterial strains (Schultz *et al.*, 2020a). *Escherichia coli*, *S. aureus*, *P. aeruginosa* and *S. typhi* are some of the pathogenic bacteria that are responsible for the diseases treated using *A. coriaria* leaves in Uganda (**Table 2.2.1**). They are therefore further discussed in the penultimate subsections.

2.6.1 *Escherichia coli*

Escherichia coli belong to the large group of Gram-negative bacteria referred to as enterobacteria. It is a rod-shaped facultative anaerobe that inhabits the lower intestine of warm-blooded organisms where it symbiotically benefit the host by biosynthesizing menaquinone or vitamin K2 (Bentley & Meganathan, 1982). It has many harmless known strains but some serotypes such as enterotoxigenic *E. coli* and Shiga toxin-producing *E. coli* are implicated as the causative microorganisms of urinary tract

infections, hemolytic uremic syndrome, wound infections, Crohn's disease, sepsis, meningitis and diarrheal diseases (CDC, 2020; Lim *et al.*, 2010).

2.6.2 *Staphylococcus aureus*

Staphylococcus aureus is a round-shaped Gram-positive bacterium and a component of human microbiota. It is a facultative anaerobe and a commensal that may become an opportunistic pathogen, causing pleuropulmonary, osteoarticular, skin and soft tissue infections, bacteremia and infective endocarditis (Leung, 2014; Tong *et al.,* 2015). Pathogenic strains of *S. aureus* promote infections through production of virulence factors such as potent protein toxins and the expression of a cell-surface protein that binds to and inactivates antibodies (Tong *et al.*, 2015). The bacterium is known to possess a remarkable genetic diversity and can acquire new exogenous genes that allows it to adapt to various changing environmental conditions to modulate its pathogenicity (Abdelbary *et al.*, 2017). Antibiotic-resistant strains of *S. aureus* such as methicillin-resistant *S. aureus* (MRSA) are known to escape the antimicrobial effect of antibiotics such as β-lactam agents, aminoglycosides and fluoroquinolones (Greenwood *et al.*, 2002). The MRSA strains are reported to possess a *mec* gene on their bacterial chromosomes (CDC, 2003). This is part of the larger Staphylococcal chromosomal cassette *mec* (SCC*mec*) region which confer multi-antibiotic resistance which is contingent on the type of SCC*mec*. The *mec* gene is responsible for encoding penicillin-binding protein 2a (PBP-2a) which is an enzyme that catalyzes the production of peptidoglycan layer in bacterial cell walls. The penicillin-binding protein 2a has a lower affinity to bind to beta-lactams (and other penicillin-derived antibiotics) when compared to other penicillin-binding proteins, enabling it to catalyze the buildup of the bacterial cell walls in the presence of various antibiotics. Subsequently, all

MRSA strains are penicillin-binding protein 2a biosynthesizers (CDC, 2003; Rasigade & Vandenesch, 2014).

2.6.3 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is an encapsulated rod-shaped Gram-negative bacterium. Like *S. aureus*, it is a ubiquitous multidrug-resistant facultative anaerobe with complex efflux-mediated antibiotic resistance mechanisms (Poole, 2004). It exploits weaknesses in host defense to mount pneumonia, sepsis syndromes, gastrointestinal, urinary tract, skin and soft tissue infections (Todar, 2014; Wu & Li, 2015). *Pseudomonas aeruginosa* biosynthesizes fluorescent siderophores (pyoverdin and pyochelin) which are iron scavengers. Redox-active phenazines such as pyocyanin pigment which confers the characteristic blue color to the bacterium are known to play an important role in electron transport especially under microaerophilic conditions, thereby enhancing the bioavailability of iron and bacterial virulence through oxidative stress (Todar, 2014; Wu & Li, 2015).

2.6.4 *Salmonella typhi*

Salmonella enterica serotype *typhi* (*S. typhi*) is a rod-shaped flagellated Gram-negative bacterium whose only reservoir is the human body (Crump *et al.*, 2015; Wain *et al.*, 2015). It causes enteric fever (typhoid) and bacteremia (Ashurst *et al.*, 2020). *Salmonella typhi* infects the intestinal tract and the blood. Other strains (*S. paratyphi* A, B and C) cause a similar illness (paratyphoid fever) which is included under the typhoid heading. However, paratyphoid fever is generally milder and shorter in duration. *Salmonella typhi* and *S. paratyphi* are prevalent in developing nations in which sewage and water treatment systems are compromised (Crump *et al*., 2015).

2.7 Major plant secondary metabolites with antioxidant and antimicrobial activities

Phytochemicals are structurally diverse natural bioactive compounds biosynthesized by plants which need them for growth, pigmentation, reproduction and resistance to pathogens, stressors, competitors and predators (Breslin, 2019; Jurić *et al.*, 2020). They work in synergy with nutrients and fibers to form an integrated defense system against various diseases and stress conditions (Erba & Kliebenstein, 2020; Isah, 2019). Because of their immobility and inability to escape predation, plants produce a range of phytochemicals which are categorized according to their functions into primary metabolites, secondary metabolites and plant hormones (Taiz & Zeiger, 2003). Primary phytochemicals include sugars, chlorophyll, amino acids and proteins which are highly conserved and directly essential for plant growth and development (Fernie & Pichersky, 2015). Secondary plant metabolites include alkaloids, terpenes, flavonoids, saponins, tannins and phenolic compounds. They are solely responsible for defense of plants against pests, predators and pathogenic microorganisms (Jurić *et al.*, 2020). Plant hormones on the other hand are small compounds that regulate organismal processes, including the biosynthesis of primary and secondary metabolites (Jurić *et al.*, 2020). Some plant secondary metabolites with antioxidant and antimicrobial activity are discussed below.

2.7.1 Alkaloids

Alkaloids is a diverse group of secondary metabolites (compounds) which possess at least a nitrogen atom in their heterocycle which confer upon them alkalinity (Kurek, 2019). Other than carbon, hydrogen and nitrogen, alkaloids may also contain sulfur, oxygen, amino or amido groups and less commonly bromine, chlorine and phosphorus.

Alkaloids are classified based on their structures as indoles, quinolines, isoquinolines, pyrrolidines, pyridines, pyrrolizidines, tropanes, terpenoids and steroids (Kurek, 2019). Alkaloids from plants have been reported to possess antioxidant (Gan *et al.*, 2017) and antibacterial (Othman *et al.*, 2019; Uzor, 2020) activities. Most alkaloids are bactericidal compounds rather than being bacteriostatic agents (Thawabteh *et al.*, 2019).

2.7.2 Tannins

Tannins (also called tannoids) is a heterogeneous group of polyphenolic plant biomolecules that are capable of binding to and precipitating proteins, polysaccharides (such as cellulose, hemicelluloses, pectin), alkaloids and nucleic acids (Fraga-Corral *et al.*, 2020). They are widely distributed in most plant species but tend to be concentrated in the leaves and flowers. Tannins are known for their physiological activities, including antioxidant (Szczurek, 2021) and antimicrobial activities (Akiyama *et al.*, 2001; Kaczmarek, 2020).

2.7.3 Saponins

Saponins is a class of structurally similar amphiphilic molecules characterized by the presence of polycyclic aglycones (triterpenoid aglycone called sapogenin) or a steroid connected to one or more oligosaccharide (sugar) side chains (Mohan *et al.*, 2016). The aglycone part (sapogenin) is either a steroid (C27) or a triterpene (C30). Saponins possess several pharmacological activities such as antioxidant, antibacterial, antifungal, antiviral, anticancer, hepatoprotective, anti-inflammatory and antiulcer activities (Anwar & Hussain, 2017).

2.7.4 Flavonoids

Flavonoids are water-soluble polyphenolic molecules consisting of 15 carbon atoms. They are further subcategorized as flavonols, flavan-3-ols, anthocyanidins, flavanones, flavones and isoflavones (Linus Pauling Institute, 2020). Flavonoids have anti-allergic, anti-inflammatory, antioxidant, antibacterial, antifungal, antiviral, anticancer and antidiarrheal activities (Cushnie & Lamb, 2011; Manner *et al.*, 2013; Shaheen *et al.*, 2020).

2.7.5 Terpenoids

Terpenoids (isoprenoids) are phytochemicals biosynthesized from the 5-carbon compound isoprene and its polymers called terpenes (International Union of Pure and Applied Chemistry, 2019). It is a diverse class of heterocyclic compounds with oxygenated functional groups. Examples of common terpenoids are menthol and camphor (monoterpenes), farnesol and artemisin (sesquiterpenoids). Terpenoids are reported to possess antioxidant, antibacterial, antitumor, anti-inflammatory, antiviral and antimalarial activities (Yang *et al*., 2020).

2.8 Some analytical methods used in natural products research

Plant extracts have been considered as a veritable source of biologically active secondary metabolites (molecules) which can result in the discovery of new drug leads (Cieśla & Moaddel, 2016). For qualitative and quantitative studies of bioactive compounds in plant materials, selection of proper methods is important. In this section, some commonly used analytical methods and techniques in natural products research are discussed.

2.8.1 Extraction

Extraction is the first step in natural products research and it determines the final outcome of the study (Chemat *et al.*, 2019). Extraction methods, usually referred to as "sample preparation techniques'', involves separating the bioactive compounds from plant matrices. Though advancement in spectrometric and chromatographic techniques have increased the accuracy and speed in the analysis of phytochemicals, analytical success of such studies still relies on the appropriate choice of the extraction methods, input parameters and the exact nature of natural product being investigated (Altemimi *et al.*, 2017). Some of the factors that affect extraction include the extracting solvent(s) employed (their polarities), temperature, matrix properties of the plant part used and the extraction time (Nawaz *et al.*, 2020).

Various classical extraction techniques used in natural products research relies on the extracting power of solvents and the application of heat and/or mixing. For instance, multiple solvents mixed in different proportions have been employed to extract biomolecules from dried powder of plants which overcomes possible interference of water (Altemimi *et al.*, 2017). The choice of the solvents is dictated by the polarity of the solute of interest. Solvents with similar polarities to the solute of interest will efficiently dissolve it. In some instances, multiple solvents are employed sequentially in order to limit the number of analogous compounds in the desired yield. Some common solvents arranged according to their polarity (from the least polar to the most polar) are hexane < chloroform < ethyl acetate < acetone < methanol < ethanol < water (Altemimi *et al*., 2017). The commonly used extraction techniques include Soxhlet extraction, maceration and hydrodistillation which usually yield crude extracts that are further concentrated on an evaporator. Other techniques in use include ultrasonic-

assisted extraction, solid phase extraction, supercritical fluid extraction and solid-phase microextraction. Microwave-assisted extraction is an emerging extraction technique (Chemat *et al.*, 2019).

2.8.2 Phytochemical screening

This is a quick, cheap and simple classical method used to analyze secondary metabolites present in natural product extracts. The technique involves preparation of aqueous and organic extracts from sample parts (for example whole plant, leaves, stems, roots or bark) that are the reservoir of secondary metabolites. The extracts are then analyzed for the presence of secondary metabolites such as flavonoids, alkaloids, tannins, terpenes, glycosides, saponins, volatile oils and phenols. Standard tests are available in literature for each secondary metabolite (Suleiman & Ateeg, 2020).

2.8.3 Common analytical techniques used in natural products research

Several analytical techniques have been employed in natural products research and some have undergone further improvements or have been hyphenated with other techniques (Kafkas *et al.*, 2018). These are employed for both qualitative and quantitative analyses. The most used techniques are often spectrophotometric (such as mass spectrometry, ultraviolet spectroscopy, Fourier transform infrared spectroscopy, Nuclear magnetic resonance spectroscopy) or chromatographic (such as gas chromatography, thin layer chromatography and liquid column chromatography).

2.8.3.1 Column chromatography

Column chromatography is a solid-liquid biophysical technique that is used to separate, identify and purify components of a mixture for qualitative and quantitative analyses (Wilson & Walker, 2018). Here, a mobile phase (usually a liquid) carrying the mixture

of compounds is caused to move in contact with a packed absorbent solid stationary phase. There are two major types of column chromatography namely: liquid chromatography and gas chromatography. The most widely used forms of column chromatography are adsorption chromatography, partition chromatography, ion exchange chromatography and gel chromatography (Aryal, 2020). For liquid chromatography, advancements into high performance liquid chromatography and ultra-high-performance liquid chromatography has been the latest developments in this technique (Aryal, 2018). In these advanced techniques, the solvent is not allowed to drip through a column under gravity but rather it is forced through under high pressures of up to 400 atmospheres. Whereas a standard high performance liquid chromatography typically have column particles with sizes from 3 µm to 5 µm, ultrahigh performance liquid chromatography use specially designed columns with particles down to 1.7 µm in size, at pressures in excess of 1000 bars (Aryal, 2018).

In natural products research, the crude plant extract to be purified by column chromatography is fed at the top of the column. The liquid solvent (the eluent) is then passed through the column by gravity or with application of air pressure (Wilson & Walker, 2018). An equilibrium is established between the solute adsorbed on the adsorbent and the eluting solvent flowing down through the column. Due to the fact that the different components in the extract have different interaction potential with the stationary and mobile phases, they will be carried along with the mobile phase to varying degrees and a separation will be perfected (Aryal, 2020). The individual components (elutants) are obtained as solvent drips from the bottom of the column.

2.8.3.2 Thin layer chromatography

Thin layer chromatography (TLC) is used to analyze plant extract fractions obtained from column chromatography to determine if the fraction contains more than one component and if the fractions can be combined without compromising their chemical purity (Akash & Rehman, 2020; Kenkel, 2003). It is particularly useful for separation of non-volatile mixtures, identifying compounds, determining their purity and following the progress of a reaction. It also permits optimization of the solvent system for a given separation problem (Chepkorir *et al.*, 2018). Unlike in column chromatography, TLC only requires small quantities of the compound (about 1 nanogram), is much faster, cost-effective and easy to dispose its waste (Saldaña *et al.*, 2020).

The separation is performed on a sheet of glass, plastic or aluminium foil which is coated with a thin layer of an adsorbent material, usually silica gel, aluminium oxide (alumina) or cellulose as the stationary phase. Separation by TLC relies on the relative affinity of compounds towards the stationary and mobile phases (Tocher, 2003). The compounds under the influence of a mobile phase (driven by capillary action) travel over the surface of the stationary phase. During this movement, compounds having higher affinity to the stationary phase travels slowly while those with less affinity to the stationary phase travel faster, thereby separating the different components. The individual components are seen as spots on the plate following staining with iodine vapor (Akash & Rehman, 2020).

2.8.3.3 Fourier transform infrared and ultraviolet-visible spectroscopy

Fourier Transform Infrared (FTIR) spectroscopy is one of the most powerful tools used to obtain infrared spectrum of absorption, emission and photoconductivity of solids, liquids and gases. It uses the mathematical process (Fourier transform) to translate raw data (interferogram) into the actual spectrum. It is primarily used for identification of chemical bonds and/or functional groups in compounds (Deena *et al.*, 2019). Different functional groups absorb at different wavelengths of light and this can be seen in a spectrum. The chemical bonds in a molecule can be determined by interpreting the resultant signal (the samples' molecular fingerprint) recorded at the detector which is a spectrum from 4000 to 400 cm^{-1} (Deena *et al.*, 2019).

On the other hand, ultraviolet-visible (UV-Vis) spectroscopy is a technique that has found application in characterization of plant extracts both qualitatively and quantitatively. The technique uses absorption spectroscopy in the ultraviolet and visible wavelength ranges (i.e. 180 nm to 380 nm and 380 nm to 750 nm, respectively) to characterize molecules. It is one of the most basic and preliminary techniques that need to be used in natural products research, especially for major classes of biomolecules that contain certain light absorbing functional groups (chromophores). Upon absorbing UV-Vis light, the chromophores are excited from ground state to a higher energy levels and gives out characteristic spectra which are used in the identification of specific biomolecules (Kalaichelvi & Dhivya, 2017). For quantitative analysis, the scan is done at a fixed wavelength and the absorbances obtained are used in quantification of the compound or group of compounds under study.

2.8.3.4 Mass spectrometry

Mass spectrometry (MS) is a high-throughput analytical technique that is based on the measurement of mass-to-charge ratio (*m/z*) of ions from ionization and fragmentation of sample molecules in the gas or liquid phases. Because molecules fragment in a unique manner, the resulting ion fragmentation pattern can be used to identify and quantify known or unknown compounds within a sample as well as elucidate the structures and chemical properties of such molecules (Baghel *et al.*, 2017). In most cases, the mass spectrometer is coupled with either a gas chromatograph or liquid chromatograph system where it is used as a detector. The gas or liquid chromatograph is utilized to achieve separation of compounds in the sample which are then channeled to the mass spectrometer sequentially for ionization, separation and detection of generated ions.

Mass spectrometry is one of the rapidly evolving analytical techniques in natural products research. For example, there has been increasing evolution in the types of acquisition and ionization such as electrospray ionization (ESI), matrix-assisted laser desorption/ionization and mass selection (time-of-flight, quadrupole and orbitrap) (Cotter *et al.*, 2020). Further, the emergence of tandem mass spectrometry (MS/MS) which affords multiple stages of MS with fragmentation of precursor ions (MS1) to highly specific fragment ions (MS2) have enhanced its sensitivity and precision in identification of compounds.

2.8.3.5 Nuclear magnetic resonance spectroscopy

Nuclear magnetic resonance (NMR) spectroscopy is an advanced elucidative technique which relies on the absorption of energy when the nucleus of an atom is excited from its lowest energy spin state to the next higher one. Two common elements in organic

molecules (carbon and hydrogen) have isotopes (^{1}H and ^{13}C) capable of giving NMR spectra that are rich in structural information. A proton NMR (^{1}H NMR) spectrum gives information about the chemical environments of the hydrogen atoms in an organic molecule whereas a carbon-13 NMR (^{13}C NMR) spectrum does the same for carbon atoms. Used together, ^{1}H and ^{13}C NMR can determine the exact molecular structure of a compound. It is however usually employed after spectrometric techniques such as FTIR, UV-Vis and mass spectrometry. The emergence of NMR complimentary techniques such as Heteronuclear Multiple Bond Correlation (HMBC) spectroscopy, Heteronuclear Multiple Quantum Coherence (HMQC) spectroscopy, Nuclear Overhauser Effect Spectroscopy (NOESY) and Circular dichroism (CD) spectroscopy have made it more successful in elucidation of chemical structures of unknown compounds (Altemimi *et al*., 2017).

2.8.3.6 Hyphenated techniques

To achieve efficiency, instrumental sensitivity and lower detection limits, chromatographic and spectroscopic techniques are being used in tandem for phytochemical analyses (Rambo *et al.*, 2019; Trubetskaya *et al*., 2021). The common ones used include liquid chromatography-mass spectrometry, liquid chromatography-tandem mass spectrometry, gas chromatography-mass spectrometry, ultra-high-performance liquid chromatography-mass spectrometry, high performance thin layer chromatography-ultraviolet-visible spectrometry, high performance liquid chromatography-photodiode array detection, liquid chromatography nuclear magnetic resonance-mass spectrometry, gas chromatography nuclear magnetic resonance-mass spectrometry and high-resolution electron spray ionization-mass spectrometry (Altemimi *et al.*, 2017).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Research design

This research employed experimental (both qualitative and quantitative) research designs. Qualitative techniques included phytochemical screening, ultraviolet visible spectroscopy, Fourier transform infrared spectroscopy scanning and gas chromatography-mass spectrometry. Quantitative experiments included determination of total phenolic and total flavonoid contents, antioxidant and antibacterial activities.

3.2 Chemicals and reagents

Analytical grade ethyl acetate, ethanol, hexane, methanol and dimethyl sulfoxide were from Merck, Darmstadt, Germany. Extra pure (99.5%) gallic acid (Loba Chemie Pvt Ltd, India), quercetin hydrate (≥ 99.5%, Sigma Aldrich), Folin-Ciocalteu reagent, ascorbic acid, 1,1-diphenyl-2-picrylhydrazyl reagent, aluminium chloride, sodium hydroxide, concentrated sulphuric acid, iron (III) chloride, glacial acetic acid, concentrated hydrochloric acid, iodine and potassium iodide (for Wagner's reagent), acetic anhydride and chloroform were of analytical grade supplied by Centrihex Limited, Nairobi, Kenya. Muller Hinton agar (Oxoid, UK), Luria-Bertani agar, Miller (Sigma Aldrich, St. Louis, USA), Nutrient agar (HiMedia Laboratories Pvt. Ltd, Mumbai, India) and the positive control drug (ciprofloxacin, 5 µg disc) were supplied by Kobian Kenya Limited, Nairobi, Kenya. The bacterial strains: *Escherichia coli* ATCC 25922 (Gram negative), *Staphylococcus aureus* ATCC 25923 (Gram positive), *Pseudomonas aeruginosa* ATCC 27853 (Gram negative) and *Salmonella typhi* 14028 (Gram negative) were obtained from Kenya Medical Research Institute, Nairobi, Kenya.

3.3 Description of the study area, sample collection and preparation

3.3.1 Study area

The samples used in this study were obtained from Jinja, Kole and Mbarara districts of Uganda as indicated in **Figure 3.3.1.1**.

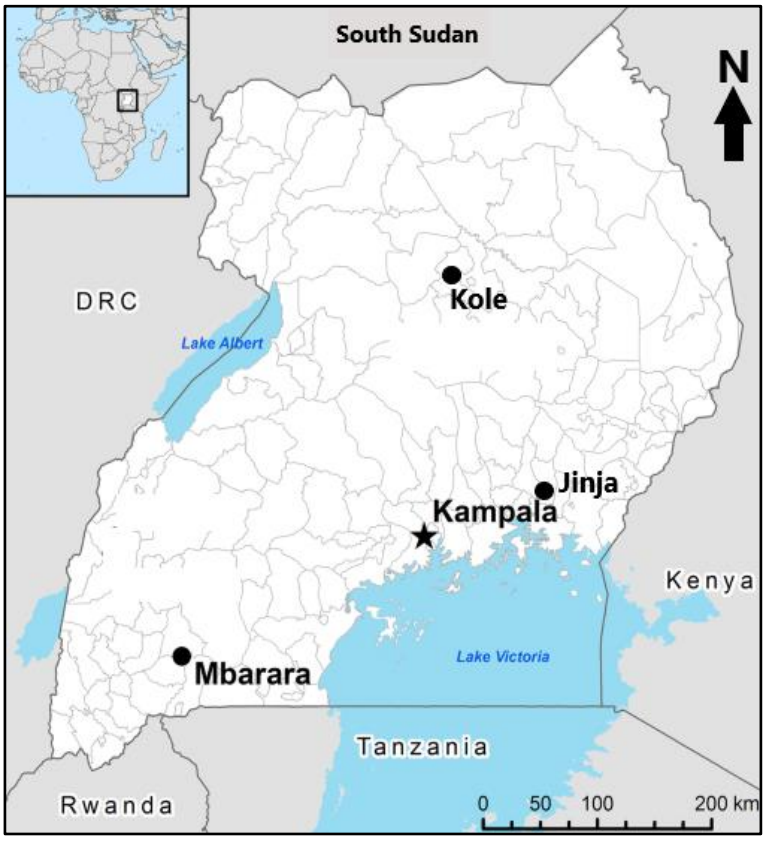


Figure 3.3.1.1. Map of Uganda showing location of sampling districts (Jinja, Kole and Mbarara). Inset is the location of Uganda in Africa (Adapted from Google Earth).

The prevalent climate in Uganda is equatorial, moderated by relatively high altitudes with a mean annual temperature of 20.5 ℃. Two distinct wet and dry seasons are experienced in many parts of the country, with the dry season occurring between December and March. Uganda is divided into 10 agro-ecological zones (**Appendix I**) primarily based on agro-climatic factors (rainfall totals and distribution) and soils i.e.

productivity and fertility (Kajobe *et al.*, 2016). Topography, temperature, moisture and vegetation cover are the secondary factors characterizing a zone but differs significantly between the different zones. Hence, the climate, geological formation, topography, soil types, rainfall and farming systems or practices are fairly homogeneous within zones (Okello *et al.*, 2018). Jinja, Kole and Mbarara districts which were chosen in this study represented the South East (moderately hot), Mid Northern (hot) and Southern drylands (cold) agroecological zones, respectively.

3.3.2 Collection, authentication and preparation of samples

Leaves (5 kg each) were sampled from *A. coriaria* trees in Jinja, Kole and Mbarara districts of Uganda (**Figure 3.3.1.1; Appendix I**). In Jinja, the samples were obtained from a tree in the periphery of AgroWays Uganda Limited, along Kyabazinga Way (0°26'34.3''N 33°12'27.3''E) on Monday 8th February 2021. In Kole, the samples were taken from Otangula village, Ilera Parish, Kole Subcounty (2°17'35.0''N 32°46'31.2''E) on Wednesday 20th January 2021. Samples from Mbarara were obtained from a tree in Ruharo ward, Ruhizi cell, Kamukuzi division, Mbarara municipality (0.6164° S 30.6186° E) on Thursday, 28th January 2021.

The samples were identified and authenticated by a taxonomist at the Department of Botany, Natural Chemotherapeutics Research Institute, Wandegeya, Kampala, Uganda. Samples from Jinja, Kole and Mbarara with voucher numbers 50994, 50995 and 50996 respectively were deposited at Makerere University Herbarium, Department of Botany, Makerere University, Kampala, Uganda. Because of hot weather in January 2021 and February 2021, the laboratory samples were washed under running tap water to remove dusts and shade-dried for two days in nearby laboratories (AgroWays Uganda Limited

Jinja and Mbarara branches for Jinja and Mbarara samples, and Otino Waa Secondary School Biology Laboratory for Kole samples). This was done to avoid their deterioration prior to transportation for further drying. Thereafter, they were packaged in sterilized polyethylene bags and transported to Moi University Chemistry Laboratory, Eldoret, Kenya where they were separately air-dried under shade at room temperature for 3 weeks.

The dry leaves were separately ground into fine powder using a NutriBullet® 600 Series electric grinder (Capbran Holdings, LLC Los Angeles, CA 90025, USA). The method of extraction was sequential. The powders (500 g) were separately weighed using a Mettler Toledo digital analytical balance (XS204 Delta Range, Switzerland). The weighed samples were transferred into 2000 mL conical flasks and then macerated at room temperature for 48 hours in 1000 mL of 99.5% ethyl acetate with occasional shaking. The extracts were filtered through cotton wool and subsequently Whatman No.1 filter paper while the residues (labelled R1) were used in the subsequent extraction.

Residues (R1) were air-dried for 48 hours at room temperature and macerated with 1000 mL of ethanol in 2000 mL conical flasks at room temperature for 48 hours with intermittent shaking. After 48 hours, the solutions were filtered through cotton wool and then Whatman No.1 filter paper. The second set of residues (labelled R2) were kept for further extraction. Residues (R2) were air-dried for 48 hours and thereafter macerated in 1000 mL of distilled water at room temperature in 2000 mL conical flasks for 48 hours. The solutions were filtered through cotton wool and then filter paper.

The organic solvent extracts were concentrated to dryness on a Hahnvapour HS-2005S vacuum rotary evaporator (Hahnshin S&T Limited, Korea) at 40 ℃ under reduced

pressure. The concentrated extracts were collected in pre-weighed labelled 10 mL sample vials (**Appendix II**) and kept in a desiccator containing anhydrous sodium sulphate to remove traces of water in them. The aqueous extracts were lyophilized at Kenya Industrial Research and Development Institute (KIRDI), Nairobi (Kenya) using an FD5-series freeze dryer (MRC Scientific Instruments, UK). The extraction process was replicated once, and the results obtained were used to compute the total extractable components (percentage yield) of each extract using **Equation 3.3.2**.

$$\text{Percentage yield} = \left(\frac{A}{A0}\right) \times 100 \qquad \textbf{(Equation 3.3.2)}$$

Where A = mass of dry crude extract, and A_0 = mass of the leaf powder macerated.

3.4 Phytochemical screening of *A. coriaria* leaf extracts

Dry extracts (1.0 g) were dissolved in their respective solvents of extraction (1:10, w/v). Standard phytochemical screening procedures (Trease & Evans, 1989) were followed to test for the presence of alkaloids, flavonoids, cardiac glycosides, phenols, saponins, quinones, steroids, tannins and terpenes in all the extracts. The results were reported in terms of relative abundance of the secondary metabolites i.e. quantifications as abundant, moderate and traces were based on colour/foam intensities observed (Gul *et al.*, 2017; Suleiman & Ateeg, 2020).

3.4.1 Test for alkaloids (Wagner's test)

To 2 mL of the extract, 2 mL of 1% hydrochloric acid was added and steamed. Three (3) drops of Wagner's reagent was added to the resultant solution. Formation of a brown or reddish brown precipitate indicates presence of alkaloids.

3.4.2 Test for cardiac glycosides

Glacial acetic acid (1 mL) was added to 2 mL of the extract in a test tube followed by 3 drops of ferric chloride solution and finally 1 mL of concentrated sulphuric acid. Presence of a brown ring at the interface indicates presence of cardiac glycosides.

3.4.3 Test for flavonoids (Alkaline reagent test)

To measured 2 mL of the extract in a test tube was added 2 drops of sodium hydroxide solution. Appearance of a yellow colour which disappeared or became colorless after addition of two drops of dilute sulphuric acid indicates presence of flavonoids.

3.4.4 Test for phenols (Ferric chloride test)

Measured 1 mL of 5% Iron (III) chloride solution was added to 2 mL of the extract in a test tube. A blue, green or dark green colour formation indicates presence of phenols.

3.4.5 Test for quinones

Concentrated sulphuric acid (1 mL) was added to 1 mL of the extract in a test tube. Formation of a red colour indicates presence of quinones.

3.4.6 Test for saponins/saponin glycosides (Froth formation test)

Distilled water (5 mL) was added to 1 mL of the extract in a test tube. The mixture was shaken vigorously for 2 minutes. Appearance of a stable foam lasting for 5 minutes indicates presence of saponins.

3.4.7 Test for steroids (Salkowski's test)

Acetic anhydride (2 mL) was added to the extract (1 mL) in a test tube followed by 2 mL of concentrated sulphuric acid. A blue or green reddish black coloration formed indicates presence of steroids.

3.4.8 Test for tannins (ferric chloride test)

In a test tube containing 2 mL of the extract, 2 mL of distilled water was added followed by three drops of ferric chloride solution. A blue or green precipitate formed indicates the presence of hydrolysable and condensed tannins, respectively.

3.4.9 Test for terpenes/terpenoids

To 5 mL of the extract, 2 mL of chloroform and 3 mL of concentrated hydrochloric acid were added. A reddish brown colour formed at the interface indicates presence of terpenes/terpenoids.

3.5 Ultraviolet-visible spectrometry and Fourier transform infrared spectroscopy scanning of *A. coriaria* leaf extracts

3.5.1 Ultraviolet visible spectrometry

The crude extracts were dissolved in a ratio of 1:10 with their solvents of extraction. They were separately filtered through cotton wool and then Whatman No. 1 (11 µm) filter paper. The extracts were centrifuged at 12,000 rpm for 10 minutes and again filtered through Whatman No.1 filter paper. The samples were further diluted in a ratio of 1:10 (v/v) with their solvents of extraction (Donkor *et al.*, 2019) and then scanned from 200 nm to 800 nm on a general-purpose (single beam) UV-Vis spectrophotometer (Beckham Coulter DU 720, Beckham Coulter Inc., USA) using the respective solvents of extraction as the blank.

3.5.2 Fourier transform infrared spectroscopy

Attenuated total reflection-Fourier transform infrared (ATR-FTIR) scanning of the extracts was performed at room temperature on a Nicolet 6700 FTIR spectrometer

(Thermo Scientific, USA). The spectral resolution was set at 4 cm^{-1} and the scanning was done from 500 cm^{-1} to 4000 cm^{-1}.

3.6 Quantification of total phenolic and total flavonoid contents of *A. coriaria* leaf extracts

3.6.1 Quantification of phenolics

Total phenolic content (TPC) of *A. coriaria* leaf extracts was determined spectrophotometrically using Folin-Ciocalteau method reported by Velioglu *et al*. (1998) with some modifications. The dry crude extracts (0.01 g) were dissolved in 10 mL of distilled water. From this, 0.01 mg/mL solutions were prepared by dissolving 0.1 mL of the resultant solutions in 10 mL of distilled water. A 0.5 mL volume of the resultant solutions were transferred into vials and mixed with 2.5 mL of Folin-Ciocalteau reagent. After 7 minutes, 2.5 mL of 6% sodium carbonate solution was added. The solutions were incubated in the dark at room temperature (25.0 ± 2.0 ℃) for 30 minutes after which their absorbances were measured at 725 nm using a UV-Vis spectrophotometer.

A calibration curve was prepared for quantitative analysis of TPC in the extracts using gallic acid as the standard. A 0.5 mL volume of prepared gallic acid solutions (10, 20, 40, 60 and 80 ppm) were transferred into vials and given the same treatment as the extracts. The TPC were determined as milligrams gallic acid equivalent per gram of dry weight (mg GAE / g DW) of extract from the calibration curve.

3.6.2 Quantification of flavonoids

Total flavonoid content (TFC) of the extracts were determined using the aluminium chloride colorimetric assay reported by Pękal and Pyrzynska (2014) with some specific modifications (Chandra *et al*., 2014). A mass of 0.01 g of the extracts were separately weighed and dissolved in 5 mL of methanol and used for TFC determination.

Standard quercetin solutions (5, 10, 25, 50, 75 and 100 ppm) were prepared in methanol. Micropipetted 0.6 mL of the extracts and quercetin solutions were mixed with 0.6 mL of 2% aluminum chloride solution in test tubes. After, the solutions were incubated for 1 hour at room temperature. The absorbance of the reaction mixtures was measured against methanol blank at 420 nm on a UV-Vis spectrometer. The TFC was determined as milligrams quercetin equivalent per gram of dry weight of the extracts (mg QE / g DW) from the quercetin calibration curve.

3.7 Determination of antioxidant activity of *A. coriaria* leaf extracts

The antioxidant potential of the extracts was evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay as described by Khiya *et al.* (2021) with modifications. A stock solution of DPPH (1.3 mg/mL in methanol) was prepared, while crude extracts were dissolved in methanol to produce solutions of 10, 15, 25, 50 and 60 µg/mL.

Measured 75 µL of freshly prepared DPPH solution was added in each of the test tubes containing 200 µL of the extracts and incubated in the dark for 30 minutes. Ascorbic acid was used as the reference standard but was dissolved in distilled water to make the sample solutions with the same concentrations as that of the extracts.

The absorbances of DPPH alone and the assay mixtures were read at 517 nm on a spectrophotometer using methanol and distilled water as the blank for DPPH alone,

extracts and ascorbic acid, respectively. The DPPH radical inhibition was calculated (**Equation 3.7.1**). The half-inhibitory concentration (IC_{50}) was determined from the percentage inhibition curve as a function of the concentrations (Khiya *et al*., 2021).

$$\text{Percent inhibition} = \frac{\mathbf{A_0 - A_S}}{\mathbf{A_o}} \times 100 \qquad \textbf{(Equation 3.7.1)}$$

Where A_o = the absorbance of the solution containing only DPPH and A_S = absorbance of the sample solution in the presence of DPPH.

3.8 Assessment of antibacterial activity of *A. coriaria* leaf extracts

The extracts were tested for their ability to inhibit growth of selected human pathogenic bacteria (*E. coli*, *S. aureus*, *P. aeruginosa* and *S. typhi*) in the Biological Sciences Laboratory, Department of Biological Sciences, Moi University, Kenya. Selection of the bacteria was based on the diseases for which the leaves are traditionally used for, the WHO priority list and availability (Makoye *et al.*, 2020; WHO, 2017).

Materials used in the assay were autoclaved at 121 ℃ and 15 pascals square inch pressure for 30 minutes in an All-American® Steam Sterilizer (Model No. 25X, Wisconsin Aluminium Foundry Co. Inc., USA). Bacterial inoculation was performed under a laminar flow cabinet disinfected with 70% ethanol (v/v). Ultraviolet radiation from the cabinet was used for sterilization prior to the bioassay.

3.8.1 Preparation of bacterial media and working culture

Exactly 7.5 g of nutrient agar was weighed and suspended in 250 mL of sterile distilled water in a sterilized 500 mL conical flask and swirled to dissolve the media. The media was then autoclaved before being used for resuscitation of the working bacterial culture.

Luria-Bertani and Muller Hinton broth were prepared by separately dissolving 14.0 g of Luria-Bertani and Muller Hinton agar in 500 mL of sterile distilled water in 1000 mL flask as per manufacturer's instructions. The broths were swirled to completely dissolve and then sterilized. After sterilization, they were transferred to the laminar flow cabinet to cool.

Nutrient broth was poured into six (6) sterilized 90 mm petri dishes so that the dishes were filled three-quarter way with the liquid media. *Escherichia coli, P. aeruginosa* and *S. typhi* were sub-cultured on nutrient broth in petri dishes in duplicate. *Staphylococcus aureus* was subcultured onto Luria-Bertani broth in two 90 mm petri dishes (Yang *et al.*, 2018). The plates were sealed using parafilm, inverted and maintained in a bacteriological incubator at 37 ℃ for 48 hours.

3.8.2 Agar disc diffusion assay for antibacterial activity screening

Determination of antibacterial activity of the *A. coriaria* leaf extracts was achieved using the agar disc diffusion method. The test extracts (500 µg/mL) were prepared by separately dissolving the crude extracts in 1% sterile dimethyl sulfoxide (Schultz *et al.*, 2020b). Four sterile paper discs (6.0 mm diameter) were saturated with each prepared test extract and dried (Suleiman & Ateeg, 2020).

A total of forty-two (42) plates (14 per bioassay) were used. The test bacteria (1×10^8 colony forming units/mL) were aseptically inoculated onto sterile Muller Hinton broth using sterile swabs. Using sterile forceps, extract-impregnated discs were gently placed on each test plate in a triangular formation. Separate agar plates were used to test the positive control (ciprofloxacin) and negative control (1% dimethyl sulfoxide). The

plates were sealed using parafilm, inverted and incubated at 37 ℃ for 24 hours. Using a metric ruler, the zone of inhibition diameter was measured to the nearest millimeter.

3.8.3 Determination of minimum inhibitory and minimum bactericidal concentrations of *A. coriaria* leaf extracts

Minimum inhibitory concentration (MIC) was determined using the broth dilution method for bacteria that exhibited the highest sensitivity to the extracts with inhibition diameter above 12 mm upon screening (Ganza, 2014). Pipetted 500 µL of bacteria were serially diluted from 2-fold to 4-fold dilution in sterile Muller Hinton broth. Five hundred microliters of the test bacteria was aseptically inoculated in each of the four tubes containing the extract in order of increasing dilution (500, 250, 125 and 62.5 µg/mL). Thereafter, the tubes were incubated at 37 ℃ for 24 hours. After incubation, the tube next to the one showing no microorganism turbidity was considered as containing the MIC of the extract.

Following MIC determination, 4 mL of the test extracts in order of increasing dilution (500, 250, 125 and 62.5 µg/mL) were placed on Muller Hinton broth and incubated at 37 ℃ for 24 hours. The highest dilution (lowest concentration) which showed growth of the bacteria was considered as the minimum bactericidal concentration.

3.9 Isolation and characterization of compounds in *A. coriaria* leaf extracts

Isolation and characterization was done for the most bioactive (ethanolic) extracts.

3.9.1 Thin layer chromatography

Thin layer chromatography (TLC) is fast and its elution patterns usually carry over to column chromatography. Thus, the ethanolic extracts were subjected to one-

dimensional TLC to establish the best solvent system to be used for column elution (Chepkorir *et al.*, 2018). The extracts were dissolved in ethanol in a ratio of 1: 20 (w/v). A spot of each solution was carefully applied onto TLC plates (TLC sheets ALUGRAM® Xtra SIL G/UV_{254}, Macherey-Nagel GmbH & Co. KG, Germany) and left to dry. The plates were developed in solvent systems of varying ratios of hexane: ethyl acetate and ethyl acetate: ethanol (10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9, 0: 10). The positions of the compounds were observed under ultraviolet light at 254 nm and 365 nm. Solvent ratios giving the best separation of compounds were used for column chromatography.

3.9.2 Column chromatography

Using the established solvent ratios i.e. hexane: ethyl acetate (9:1) for Mbarara leaves extracts, hexane: ethyl acetate (4:6) for Kole and Jinja leaves extracts and ethyl acetate: ethanol (3:7) for all the extracts, the crude extracts were subjected to silica gel (60-120 mesh, Griffchem™ Fine Chemicals) column chromatography. Silica gel (100 g) was mixed with hexane: ethyl acetate solvent system to form a homogenous suspension (slurry) and stirred using a glass rod to remove bubbles. The silica gel slurry was then poured into the column. The sample was prepared by mixing and grinding 5 g of the extract with 5 g of silica gel into fine green powder. It was then introduced into the column and eluted with the solvent systems established (Chepkorir *et al.*, 2018). For each eluent system, fractions were collected and those with similar TLC profiles were pooled together and concentrated to dryness by rotary evaporation (**Appendix II**).

3.9.3 Gas chromatography-mass spectrometry analysis

The fractions obtained were reconstituted in dichloromethane: methanol (1:1, v/v) mixture, filtered through 0.45 μm filters and transferred to 2 mL vials one at a time for gas chromatography-mass spectrometry (GC-MS) analysis.

A gas chromatograph interfaced with mass spectrometer triple-quad system (Agilent 8890A GC and Agilent 5977 GC/MSD) with an Agilent 7693A automatic liquid sampler, a National Institute of Standards and Technology (NIST) library and installed with Mass Hunter Workstation software was used. An HP-5MS ultra-inert column (30 m × 0.25 mm × 0.25 μm) was used with an electron ionization system of 70 eV. Helium (99.999%) carrier gas flow rate was 1 mL/minute, with a splitless injection volume of 1 μL. Injector temperature was 250 ºC while the ion source temperature was 280 ºC. Oven temperature was programmed from 110 ºC with an increase of 10 ºC to 200 ºC, and then 5 ºC to 280 ºC. The compounds were identified from gas chromatograms based on their elution times. The mass spectra of the compounds were matched with those of the NIST 11 spectral library and/or compared with published literature.

3.10 Statistical Analysis

All experiments were done in triplicate except extraction which was replicated once. Quantitative data were expressed as means ± standard deviations of replicates. The means were separated by One-Way Analysis of Variance (ANOVA) with Tukey post hoc test. Correlations among TPC, TFC and antioxidant activity (IC_{50}) of the extracts were assessed using Pearson's bivariate correlation (Suleiman & Ateeg, 2020). All analyses were done using GraphPad Prism for Windows (v9.1.0, GraphPad Software Inc., San Diego) with statistical significance set at $p < 0.05$.

The bacteriostatic efficacy of the extracts against the selected bacteria were determined by comparing their zone of inhibition diameters with that of the standard drug (ciprofloxacin). The zones of inhibition measured were categorized as ‘susceptible’, ‘intermediate’ and ‘resistant’ according to Clinical and Laboratory Standards Institute Interpretive Criteria (CLSI, 2017).

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1 Extraction yields of *Albizia coriaria* leaves

In natural products research, extraction yield measures a solvent's efficiency to extract compounds from matrices. The yields obtained in this study were expressed as percentages of the initial mass of the macerated dry leaf powders (**Figure 4.1.1**). Extraction using ethanol gave the highest yields in comparison to ethyl acetate and distilled water with *A. coriaria* leaves from Mbarara having the highest yield of 10.88 ± 0.12%. However, ANOVA results (**Appendix V**) indicated that the yields were not significantly influenced by the extraction solvent used ($p > 0.05$). Thus, the null hypothesis was accepted.

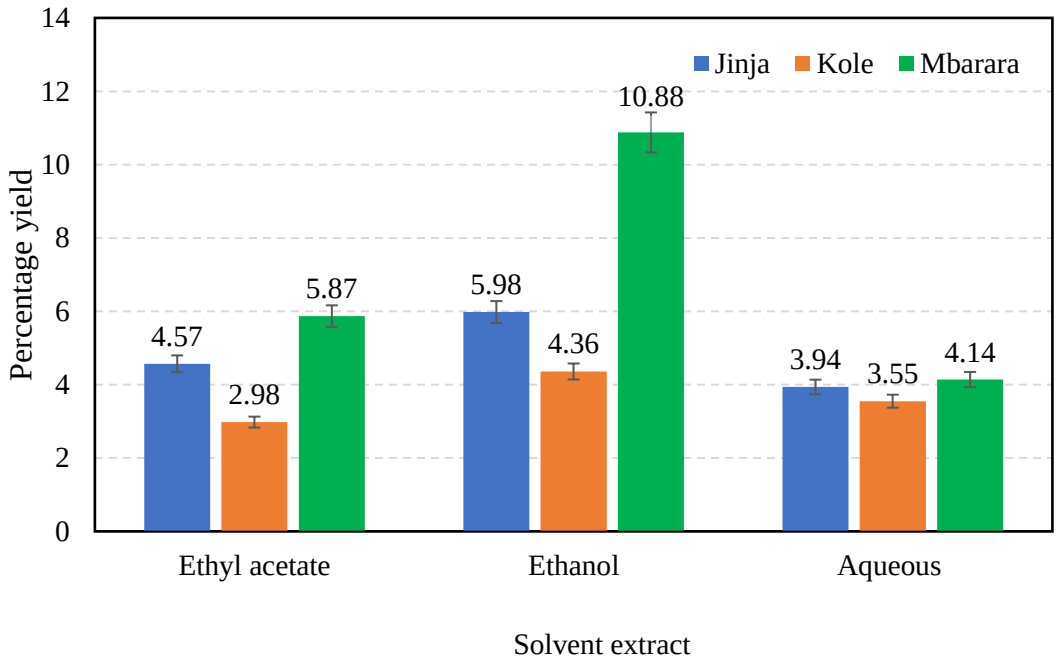


Figure 4.1.1. Percentage yield of the different solvent extracts of *A. coriaria* leaves.

The yields recorded for *A. coriaria* leaves in this study are comparable to yields of 8.3% and 12.7% for ethyl acetate and methanolic extracts of *A. coriaria* stem bark from Uganda reported by Ganza (2014). Owuor *et al.* (2012) also reported a comparable yield of 11.9% for dichloromethane extract of *A. coriaria* stem bark from Kenya.

From the obtained results, it can be inferred that ethanol was a good solvent for extraction of phytochemicals in *A. coriaria* leaves as compared to ethyl acetate probably because most compounds in *A. coriaria* leaves are polar. Thus, the phytochemicals were able to dissolve in ethanol than ethyl acetate used initially in the sequential extraction. Aqueous extracts had the lowest yields because most of the polar phytochemicals had already been extracted by the moderately polar ethanol before the leaf residues (R2) were macerated in distilled water. Differences in polarities of solvents employed in serial extraction of phytochemicals is known to play a key role in increasing the solubility of phytochemicals in plant matrices (Abd Aziz *et al.*, 2021; Altemimi *et al.*, 2017). Further, differences in the structure (functional groups) of phytochemicals, time, temperature and polarity also influence the solubility of phytochemicals in either organic and polar solvents or solvents of different polarities (Aissani *et al.*, 2021; Nawaz *et al.*, 2020). The three solvents used for serial extraction in this study had different polarity indices, arranged as ethyl acetate (4.4) < ethanol (5.2) < water (9.0) (Abarca-Vargas *et al.*, 2016; Nawaz *et al*., 2020). Therefore, the results of this study confirmed the richness of *A. coriaria* leaves in polar phytochemicals as previously indicated for its stem bark (Ganza, 2014; Schultz *et al.*, 2021b).

Among the three districts, the yield for ethanolic extract of *A. coriaria* leaves from Mbarara was nearly two times higher than that of leaves from Jinja and Kole despite

using the same extraction solvent and method (**Figure 4.1.1**). A similar pattern was noted for yield of ethyl acetate extract of leaves from Mbarara being almost twice that of leaves from Kole.

The intraspecific variation in yields of *A. coriaria* leaf extracts in the three different agroecological zones could be due to extrinsic factors such as differences in soil chemistry, season, topography and climate which are known to affect the phytochemical yields of plant organs (Chepkwony *et al.*, 2020; Ghavam, 2021; Muraina *et al.,* 2008; Zargoosh *et al.*, 2019).

Mbarara receives an average rainfall of 1,200 mm per annum with two distinct rainy seasons with the first rainy season starting from February to May and the second rainy season from September to December. The temperature ranges from 17 ℃-30 ℃, corresponding to a humidity range of 80-90% (Mbarara District Local Government, 2021). The topography is a mixture of fairly rolling and sharp hills and mountains, shallow valleys and flat land lying at about 1483 mm above sea level. The soils are sandy, clay and slightly laterite loams (Mbarara District Local Government, 2021).

The sampling area in Jinja on the other hand is on the shores of Lake Victoria (near the source of River Nile). The area has warm temperatures ranging between 23 ℃ to 32 ℃ and a bi-modal rainfall pattern averaging 1260 mm annually (Namuhani & Kimumwe, 2015). The soils in this area are primarily made of granites and granitoid gneisses, with patches of shales, phillites and schists (Namuhani & Kimumwe, 2015). It is located about 1,187 m above sea level, with valleys in some parts of the district.

Kole district is located in the Northern part of Uganda at an elevation of 1,073 m (Weather atlas, 2021). It was formed out of Apac district in 2010 (The New Vision,

2010). This area has a unimodal season with a total annual rainfall of 1,330 mm from April to November (Atube *et al.*, 2021). The dry season is from December to March, with average minimum and maximum temperatures are 17 °C and 29 °C.

Thus, samples from Mbarara recorded the highest yields possibly because of the good soils in this district than in the other two districts (Minai, 2015). The district is also the coldest of the three studied districts. Previous studies have indicated that plant materials sampled from plants growing in cooler areas (at higher altitudes) tend to give higher extraction yields compared to those in warm climatic conditions and lowland areas (Muraina *et al.*, 2008; Zargoosh *et al.*, 2019). In addition, the sampling season could have contributed to the disparities in the yields obtained (Machumi *et al.*, 2021). For example, Kole and Jinja districts were in a dry season when the leaves were sampled while Mbarara district was at the beginning of the rainy season when the samples were taken despite the same time of sampling.

4.2 Phytochemical screening results

Screening of *A. coriaria* leaf extracts revealed the presence of several secondary metabolites including alkaloids, flavonoids, cardiac glycosides, phenols, saponins, tannins and terpenes in different quantities (**Table 4.2.1**). Quinones and steroids were however not detected. Alkaloids were absent in the ethyl extract of *A. coriaria* leaves from Mbarara, and in ethanolic extracts from Jinja. Flavonoids were absent in the ethyl acetate extract from Jinja while tannins were also not detected in ethanolic extract of leaves from Jinja. The results of this study agreed with previous studies on *A. coriaria* stem bark which reported tannins, saponins, alkaloids, flavonoids, terpenes and cardiac glycosides as the major secondary metabolites (Agroforestry Database, 2009; Akanga,

2008; India, 2015; Langat, 2013; Mengesha *et al.*, 1997; Owuor *et al.*, 2012; Wanyama *et al.*, 2011).

Table 4.2.1. Secondary metabolites identified in *A. coriaria* leaves from Jinja, Kole and Mbarara districts of Uganda.

Phytochemicals	Extract	Jinja	Kole	Mbarara
Alkaloids	Ethyl acetate	++	++	-
	Ethanolic	-	+++	++
	Aqueous	+	+	+
Cardiac glycosides	Ethyl acetate	++	+	++
	Ethanolic	+	+	+
	Aqueous	+	+	+
Flavonoids	Ethyl acetate	-	+	++
	Ethanolic	+	++	++
	Aqueous	++	++	++
Phenols	Ethyl acetate	++	++	++
	Ethanolic	++	++	+++
	Aqueous	+	+	+
Quinones	Ethyl acetate	-	-	-
	Ethanolic	-	-	-
	Aqueous	-	-	-
Saponins	Ethyl acetate	+	+	+
	Ethanolic	++	++	+++
	Aqueous	+	+	+
Steroids	Ethyl acetate	-	-	-
	Ethanolic	-	-	-
	Aqueous	-	-	-
Tannins	Ethyl acetate	+	+	+
	Ethanolic	-	+	+++
	Aqueous	++	++	++
Terpenes/terpenoids	Ethyl acetate	++	++	+
	Ethanolic	++	++	+++
	Aqueous	+	+	+

Note: $^{+++}$ means **abundant**, $^{++}$ means **moderate**, $^{+}$ means **traces**, and $^{-}$ means **absent**. These quantifications were based on colour/foam intensities observed (Gul *et al.*, 2017; Suleiman & Ateeg, 2020).

The identified secondary metabolites were also reported in the leaf extracts of other *Albizia* species such as *Albizia lebbeck* (Lawan *et al.*, 2017), *Albizia multiflora* (María *et al.*, 2018), *Albizia harveyi* (Makoye *et al.*, 2020) and *Albizia tulearensis* (Aurélie *et*

al., 2020). The absence of alkaloids was previously reported in leaf extracts of *Albizia lebbeck* (Lawan *et al.*, 2017; Rahul *et al.*, 2010), *Albizia zygia* (Uwaya *et al.*, 2017), *Albizia tulearensis* (Aurélie *et al.*, 2020) and *Albizia harveyi* (Makoye *et al.*, 2020). The absence of steroids in *A. coriaria* leaves corroborates a previous observation in which steroids were absent in extracts of *Albizia lebbeck* leaves (Rahul *et al.*, 2010).

The observed variation of secondary metabolites in the leaf extracts could be due to differences in soil chemistry, rainfall, topography and climate that affects the interaction between plants and the environment, and ultimately the depot of bioactive compounds in plant leaves (Holopainen *et al*., 2018; Sandeep *et al.*, 2017).

4.3 Confirmation of the presence of secondary metabolites identified by phytochemical screening

4.3.1 Ultraviolet-visible spectra of *A. coriaria* extracts

The secondary metabolites identified by phytochemical screening were confirmed by spectroscopic scanning for characteristic absorptions in the ultraviolet and visible regions of the electromagnetic spectrum. All the extracts were scanned from 210 nm to 800 nm to establish if compounds containing σ-bonds, π-bonds, lone pair of electrons, chromophores and aromatic rings are present in them (Bashyam *et al.*, 2015; Karpagasundari & Kulothungan, 2014). The spectra for the ethyl acetate, ethanolic and

aqueous extracts of *A. coriaria* leaves are shown in **Figure 4.3.1.1**, **Figure 4.3.1.2** and **Figure 4.3.1.3** respectively (**Table 4.3.1.1**).

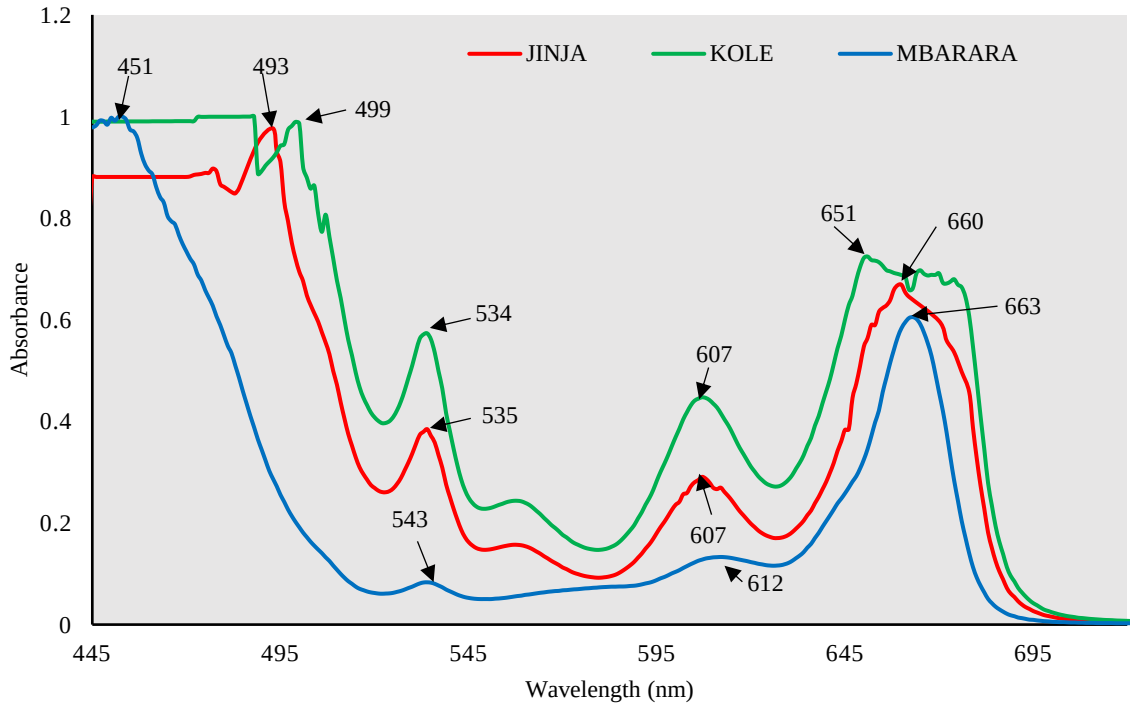


Figure 4.3.1.1. UV-Vis spectra of ethyl acetate extracts of *A. coriaria* leaves

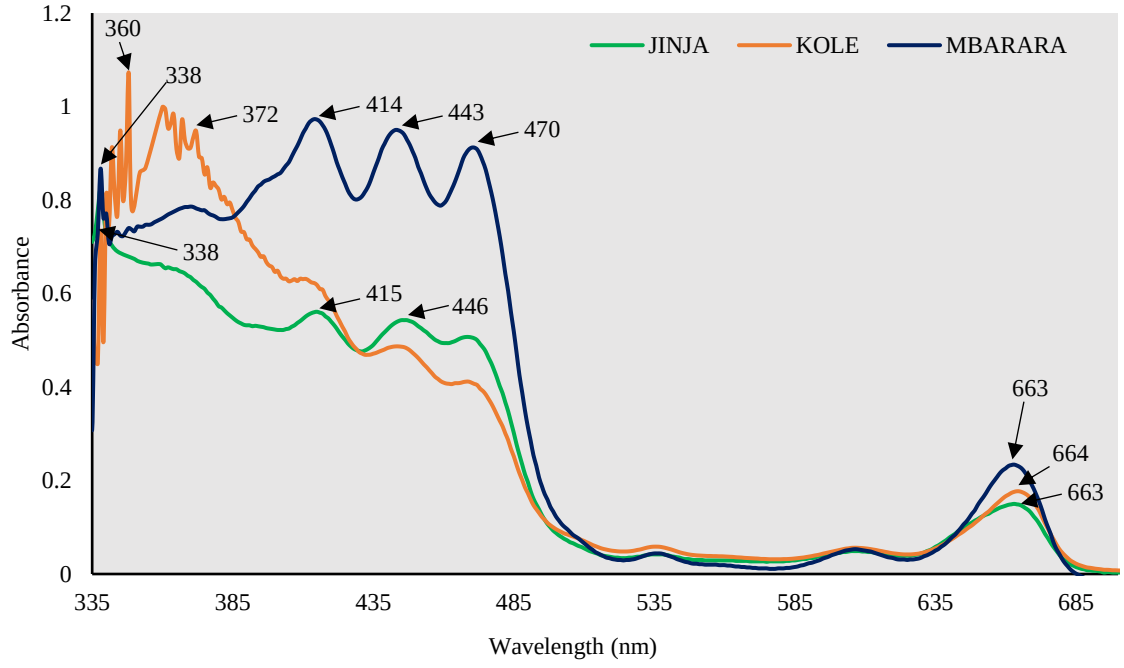


Figure 4.3.1.2. UV-Vis spectra of ethanolic extracts of *A. coriaria* leaves

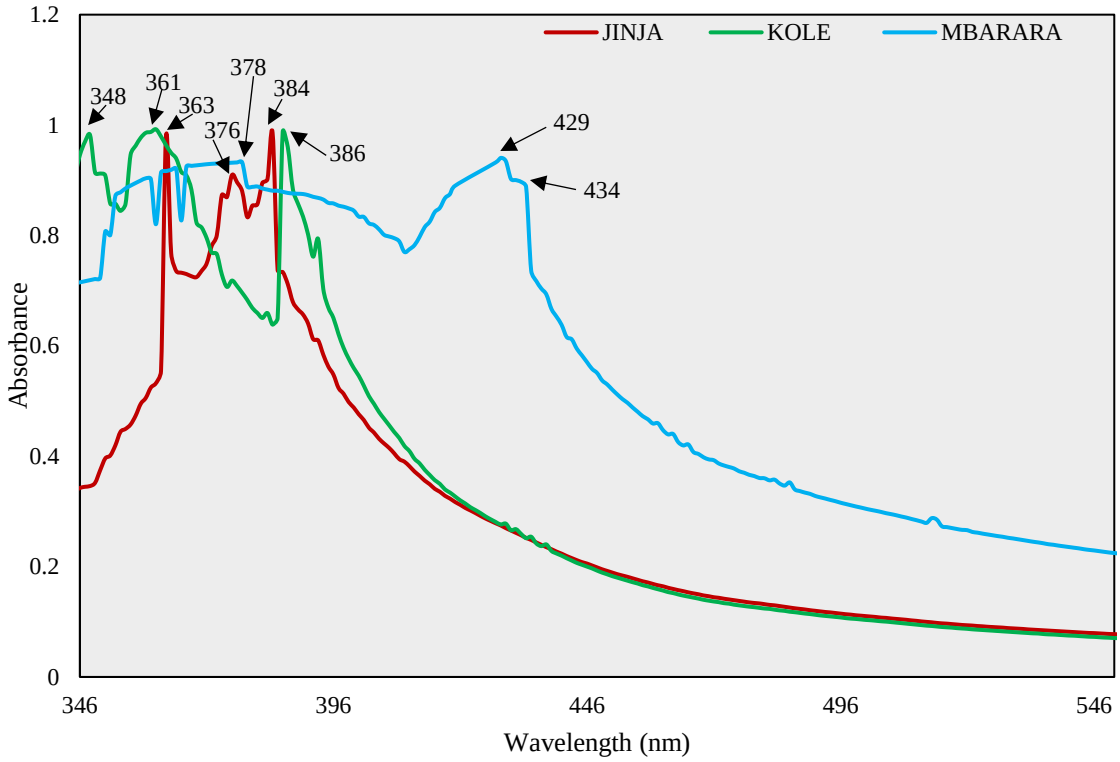


Figure 4.3.1.3. UV-Vis spectra of aqueous extracts of *A. coriaria* leaves

Table 4.3.1.1. UV-Vis absorption peaks for *A. coriaria* leaf extracts

District	Extract	Wavelength max (nm)	Absorbance of the sample
Jinja	Ethyl acetate	493, 535, 607, 660	0.977, 0.384, 0.290, 0.669
	Ethanolic	338, 415, 446, 663	0.825, 0.561, 0.543, 0.150
	Aqueous	363, 376, 384	0.980, 0.909, 0.986
Kole	Ethyl acetate	499, 534, 607, 651	0.989, 0.573, 0.447, 0.724
	Ethanolic	360, 367, 372, 664	0.998, 0.972, 0.947, 0.177
	Aqueous	348, 361, 386	0.982, 0.992, 0.987
Mbarara	Ethyl acetate	451, 534, 612, 663	0.991, 0.083, 0.133, 0.605
	Ethanolic	338, 414, 443, 470, 663	0.867, 0.973, 0.950, 0.912, 0.234
	Aqueous	360, 365, 378, 429, 434	0.902, 0.9208, 0.9325, 0.9405, 0.888

The absorption peaks at 338 nm, 363 nm and 338 nm are due to the presence of alkaloids (Sangster & Stuart, 1965; Zahari *et al*., 2016). Absorptions at 660, 663, 651, 665, 664, 663 and 664 nm indicate the presence of flavonoids (Dhivya & Kalaichelvi, 2017; Donkor *et al.*, 2019; Rani *et al.*, 2016) while 372 nm and 376 nm indicated the presence of terpenoids (Malik *et al.*, 2018).

4.3.2 Fourier transform infrared spectra of *A. coriaria* leaf extracts

The Fourier transform infrared spectra of crude leaf extracts of *A. coriaria* indicated the presence of O−H, C=O, N−H and aromatic−C=C which confirmed the presence of alkaloids, alcohols, phenolics, carboxylic acids, amines and aromatics in the extracts (**Table 4.3.2.1; Appendix III**). These agreed with the groups of secondary metabolites identified by phytochemical screening and UV-Vis spectroscopy.

Table 4.3.2.1. Functional groups identified in *A. coriaria* leaf extracts

Extract	Wave numbers (cm^{-1})	Functional group	Reference(s)
Ethyl acetate (Jinja)	3370.27	O−H stretch	Santiago *et al.* (2014)
	2954.90, 2926.42	C−H stretch	Bautista-Hernández *et al.* (2021)
	2852.88	C-H stretch	Santiago *et al.* (2014)
	1739.70	C=O (aromatic, conjugated), cyclic C=O, saturated C=O	
	1454.48	Ar−C (aromatic ring)	Abbas *et al.* (2017)
	1370.00	$-CH_3$ (for isopropyl group)	Bacher (2002)
Ethyl acetate (Kole)	3295.75	O−H stretch	Santiago *et al.* (2014)
	2930.66	C−H	
	1750.80	C=O (for esters)	Nandiyanto *et al.* (2019)
	1636.81	C=C (olefinic band)	Invernizzi *et al.* (2018); Santiago *et al.* (2014)
	1454.48	C–H bending /aromatic –C=C stretching vibrations	
	1030.14	Aliphatic C−O stretching	Md Salim *et al.* (2021)
Ethyl acetate (Mbarara)	3261.46	N−H	Balan *et al.* (2019)
	2775.09	C−H stretching vibrations	D'Souza *et al.* (2008)
	2420.15	Aliphatic C-H stretch	Reddy *et al.* (2018)

Extract	Wave numbers (cm^{-1})	Functional group	Reference(s)
	1615.30	Aromatic (C=C) stretching	Khobragade *et al.* (2017)
Ethanolic (Jinja)	3273.66	O−H stretching of carboxylic acids & phenolics	Kiran *et al.* (2017); Oliveira *et al*. (2016)
	2919.94	C−H stretching	Jain *et al.* (2016)
	2341.57	C−H stretching	Ashokkumar *et al.* (2014)
	1632.74, 1541.19	C=C stretching	Jain *et al.* (2016); Oliveira *et al*. (2016)
	1219.24, 1037.98	C−O stretching	
Ethanolic (Kole)	3362.30	O−H stretching (phenolics)	Das *et al.* (2016)
	2358.93	O =C=O stretching	Ferreira *et al.* (2020)
	1660.08	C=O (conjugated, aromatic)	Gómez (2021)
	1369.46, 1319.00	C−O stretching	Netala *et al.* (2015)
	1072.44	C−N stretch	Janakiraman and Johnson (2015)
	824.91	C–Cl stretch (alkyl halides)	
Ethanolic (Mbarara)	3365.73	O−H	Das *et al.* (2016)
	2912.89	C−H stretching	Ashokkumar *et al.* (2014)
	1659.42	C=O	Nandiyanto *et al.* (2019)
	1631.91	C=C	Oliveira *et al*. (2016)
	1370.12	C−O stretching	Netala *et al.* (2015)
	1319.88	C−H, N=O	Kavitha *et al.* (2019)
	1065.87	C−N stretch	Janakiraman and Johnson (2015)
	825.40	C–Cl stretch	
Aqueous (Jinja)	3574.60	O−H	Santiago *et al.* (2014)
	3468.38	C=O (ester)	Nandiyanto *et al.* (2019)
	2840.00	C−H stretching	Ashokkumar *et al.* (2014)
	2210.90	C−N vibration	Pongpiachan (2014)
	1552.42	Ar−N=O	Lindblom (2014)
Aqueous (Kole)	3362.85	O−H	Das *et al.* (2016)
	2859.40, 2297.16	C−H stretching	Ashokkumar *et al.* (2014); Fei *et al.* (2017)
	1783.92	C=O	
	1140.06	C−O	Nandiyanto *et al.* (2019)
Aqueous (Mbarara)	3364.09	O−H	Das *et al.* (2016)
	2796.00	C=O	Santiago *et al.* (2014)
	1998.26	C-H (aromatic)	
	1730.97	C=O	
	1606.11	C=C (aromatic) stretching	Khobragade *et al.* (2017); Santiago *et al.* (2014)

4.4. Total phenolic and total flavonoid contents of *A. coriaria* leaf extracts

4.4.1 Total phenolic content

In this study, a calibration curve (**Figure 4.4.1.1**) for TPC quantification was prepared and the linearity for gallic acid standard was established from 10 ppm to 80 ppm which was fitted on the line $y = 0.0126x - 0.0107$ with $R^2 = 0.9992$.

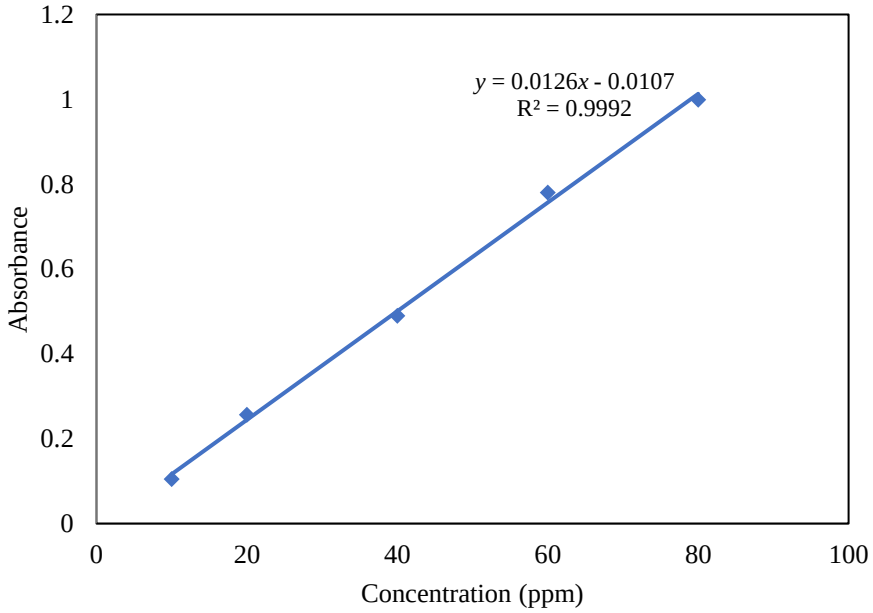


Figure 4.4.1.1. Calibration curve for total phenolic content quantification.

As shown in **Table 4.4.1.1**, TPC was highest for ethanolic extracts compared to the ethyl acetate and aqueous extracts. Ethanolic extracts from Mbarara showed the highest TPC of 101.72 ± 0.22 mg GAE /g DW. In comparison to extracts from Jinja and Kole, all extracts of *A. coriaria* leaves from Mbarara had the highest TPC (**Table 4.4.1.1**). One-Way ANOVA results showed that there were significant differences ($p = 0.002208$) between the mean TPC of the different solvent extracts of *A. coriaria* leaves (**Appendix V**). Hence, the null hypothesis of no difference in the TPC of the extracts

of *A. coriaria* leaves from the different districts was rejected. This could be because Mbarara has the most fertile soils than the other two studied districts (Minai, 2015). Mbarara is also the coldest of the three districts chosen for this study, which could explain the higher accumulation of phenolics in samples taken from this district. As reported by Cansev *et al.* (2012) and Król *et al.* (2015), cooler climates are associated with increased phenolics production, probably for plants' response to environmental stress and self-defense.

Table 4.4.1.1. Total phenolic content of *A. coriaria* leaf extracts from selected districts of Uganda.

District	Ethyl acetate extract (mg GAE / g DW)	Ethanolic extract (mg GAE / g DW)	Aqueous extract (mg GAE / g DW)
Jinja	16.88 ± 0.11	67.04 ± 0.19	5.29 ± 0.13
Kole	10.93 ± 0.13	77.99 ± 0.17	20.69 ± 0.27
Mbarara	60.69 ± 0.23	101.72 ± 0.22	61.25 ± 0.13

Note: Results are presented as means ± standard deviations of triplicates.

Schultz *et al.* (2021b) reported that the TPC of ethanolic extracts of *A. coriaria* stem bark (28.37 ± 0.34 milligram chlorogenic acid equivalent per gram of extract, mg CAE / gE) was only slightly higher than that of its ethyl acetate extract (28.36 ± 0.97 mg CAE / gE). A plausible explanation for this is that ethanol is a polar protic solvent. Thus, it extracted more polyphenols which are inherently polar through hydrogen bond formation (Felhi *et al.*, 2017; Sripad *et al.*, 1982). This also explains why aqueous extracts had lower TPC, because most of the phenolics were already extracted by ethanol. Further, water is known to extract even non-active compounds (such as

proteins and sugars) from matrices which do not contribute to the TPC of extracts (Altemimi *et al.*, 2017).

4.4.2 Total flavonoid content

In this study, a calibration curve prepared using quercetin as a standard was used to quantify TFC of the extracts (**Figure 4.4.2.1**). Linearity of the standard was established from 5 ppm to 100 ppm which was fitted on a straight line that gave the equation $y = 0.0109x + 0.0851$ with $R^2 = 0.9987$.

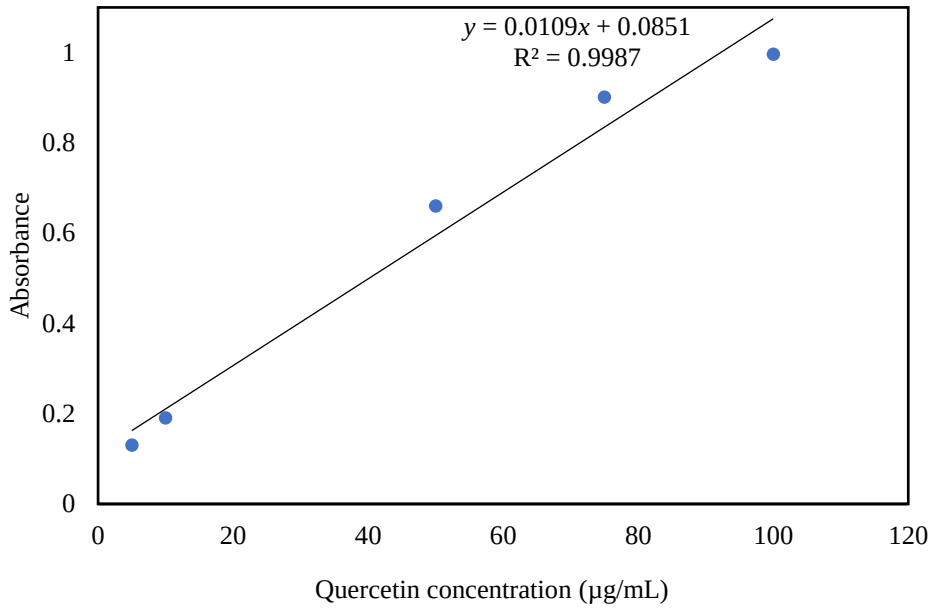


Figure 4.4.2.1. Calibration curve for total flavonoid content quantification.

It was found that TFC were highest for ethanolic extracts when compared with ethyl acetate and aqueous extracts (**Table 4.4.2.1**). Ethanolic extract of leaves from Mbarara district had the highest mean TFC of 13.23 ± 0.03 mg QE / g DW. This could have been because ethanol with a higher polarity than ethyl acetate was able to extract much of the flavonoids which are relatively polar (Nawaz *et al.*, 2020). This indicates that the

A. coriaria leaves contain more flavonoid heterosides than aglicones (Felhi *et al*., 2017). In addition, all extracts from Mbarara district had the highest TFC when compared to the extracts of leaves from Jinja and Kole districts (**Table 4.4.2.1**). As explained earlier for the yields and TPC, this could be because Mbarara is the coldest of the studied districts and also variations in the sampling seasons (Bouderias *et al.*, 2020). One-Way ANOVA performed (**Appendix V**) indicated that there were significant differences (p = 0.023560) between mean TFC of the different solvent extracts. Since the p-value was less than 0.05, the null hypothesis was rejected. Further, the mean TFC obtained for the different extracts were lower than their corresponding mean TPC values. High TPC of plant extracts than TFC supports the chemistry that most flavonoids are also phenolics (Ahmed *et al.*, 2015).

Table 4.4.2.1. Total flavonoid content of *A. coriaria* leaves from the selected districts of Uganda

District	Ethyl acetate extract (mg QE / g DW)	Ethanolic extract (mg QE / g DW)	Aqueous extract (mg QE / g DW)
Jinja	0.55 ± 0.01	8.63 ± 0.02	2.74 ± 0.02
Kole	2.50 ± 0.04	11.58 ± 0.04	2.35 ± 0.05
Mbarara	9.66 ± 0.01	13.23 ± 0.03	3.36 ± 0.04

Note: Results are presented as means ± standard deviations of triplicates.

4.5 Antioxidant activity of *A. coriaria* leaf extracts

The results of *in vitro* antioxidant activity assay (**Table 4.5.1**) indicated ethanolic extracts of *A. coriaria* leaves had the lowest minimum inhibitory concentration (IC_{50}) values. However, these were higher than that of ascorbic acid (0.17 ± 0.01 mg/mL). There were significant differences (p = 0.007548) between the antioxidant activity of ethyl acetate, ethanolic and aqueous extracts (**Appendix V**). With a p-value less than

0.05, the null hypothesis was rejected. These differences could be explained by the differences in the phytochemical composition of the different extracts, possibly due to geographical, soil, climate and genetic variations experienced in the different agro-ecological zones (Holopainen *et al.*, 2018; Sandeep *et al.*, 2017; Zargoosh *et al.*, 2019).

Table 4.5.1. Minimum inhibitory concentration (IC_{50}) of *A. coriaria* leaf extracts

District	Ethyl acetate extract (mg/mL)	Ethanolic extract (mg/mL)	Aqueous extract (mg/mL)
Jinja	23.99 ± 0.05	23.41 ± 0.13	29.80 ± 0.26
Kole	26.34 ± 0.09	23.18 ± 0.09	29.66 ± 0.21
Mbarara	23.73 ± 0.16	18.65 ± 0.06	25.51 ± 0.14

Note: Results are presented as means ± standard deviations of triplicates.

Further, it was noted that ethanolic extracts had the highest antioxidant activity (lower IC_{50} values). This could be because most phenolic compounds which accounts for the antioxidant activity of plant extracts possess polar functional groups and are therefore easily dissolved in polar protic solvents like ethanol (Widyawati *et al.*, 2014). The results of antioxidant activity in this study were higher than those reported for ethyl acetate and ethanolic extracts of *A. coriaria* stem bark harvested from Mpigi district of Uganda. The extracts were reported to have half-effective concentration (EC_{50}) of 0.02298 ± 0.00247 mg/mL and 0.01839 ± 0.00223 mg/mL, respectively (Schultz *et al.*, 2021b).

4.6 Correlation between total phenolics, total flavonoids and antioxidant activity

A strong positive correlation ($R = 0.898$, $p = 0.001$) between TPC and TFC was observed (**Appendix V**). This is usually expected because total phenolic compounds comprise of both flavonoids and non-flavonoid polyphenols (Suleiman & Ateeg, 2020).

Thus, a strong positive correlation indicates that TFC of the extracts contributes significantly to the TPC of the extracts (Kim & Lee, 2020).

Pearson's bivariate correlation coefficient between TPC and antioxidant activity of the extracts revealed that TPC exhibited high negative correlation with the IC_{50} values obtained in DPPH assay ($R = -0.831$, $p = 0.006$). In the same way, TFC negatively and highly correlated ($R = -0.755$, $p = 0.019$) with IC_{50} values obtained in DPPH assay. Schultz *et al.* (2021b) observed a poor correlation between TPC and antioxidant activity (EC_{50} values) of ethyl acetate and ethanolic extracts of *A. coriaria* stem bark. The negative correlation between DPPH radical quenching activity, TPC and TFC is because the radical content decreases as the activity of the extract increases. This therefore implies that the phenolics played a significant role in increasing DPPH scavenging activity of the extracts (Kim & Lee, 2020; Suleiman & Ateeg, 2020; Zengin *et al.*, 2019).

4.7 Antibacterial activity of *A. coriaria* leaf extracts

4.7.1 Antibacterial screening results

Antibacterial screening indicated that all ethanolic extracts and ethyl acetate extracts of leaves from Kole and Mbarara had inhibitory effect on the growth of *E. coli*, *S. aureus*, *P. aeruginosa* and *S. typhi* while the aqueous extracts were inactive (**Table 4.7.1.1; Appendix IV**). The antibacterial activity was higher for ethanolic extracts. The negative control had no inhibitory activity on the tested bacteria. Ethanolic extracts had comparable antibacterial activity to that of ciprofloxacin which was used as positive control. For example, ethanolic extract of leaves from Kole had a marked antibacterial activity against *S. typhi* with inhibition diameter of 16.00 ± 1.73 mm which was comparable to that of ciprofloxacin (20.00 ± 1.53 mm).

Table 4.7.1.1. Zone of inhibition (mm) of *A. coriaria* extracts

District	Extract	*E. coli*	*S. aureus*	*P. aeruginosa*	*S. typhi*
Jinja	Ethyl acetate	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Ethanolic	6.00 ± 1.73	5.00 ± 1.00	**18.00 ± 2.65**	9.00 ± 1.73
	Aqueous	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Kole	Ethyl acetate	3.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Ethanolic	7.00 ± 1.00	6.00 ± 0.00	**17.00 ± 0.00**	16.00 ± 1.73
	Aqueous	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mbarara	Ethyl acetate	4.00 ± 2.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Ethanolic	10.00 ± 1.73	12.30 ± 1.53	**25.00 ± 2.65**	10.00 ± 0.00
	Aqueous	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Ciprofloxacin (Positive control)		14.00 ± 2.10	12.00 ± 0.01	31.00 ± 0.11	20.00 ± 1.53
Dimethyl sulfoxide (Negative control)		0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Note: Results are expressed as means ± standard deviations of triplicates. Zones in **bold** are for bacteria that were susceptible to ethanolic extracts as per Clinical and Laboratory Standards Institute Interpretive Criteria (CLSI, 2017).

Only ethanolic extracts had inhibition zones greater than 12 mm after screening. These were Jinja extract against *P. aeruginosa*, Kole extract against *P. aeruginosa* and *S. typhi*, and Mbarara extract against *S. aureus* and *P. aeruginosa*. Thus, *P. aeruginosa* was regarded as susceptible to the ethanolic extracts as the respective zones of inhibition were within the zone diameter breakpoints for standard antibiotics such as ampicillin (inhibition diameter ≥ 17 mm), doxycycline (inhibition diameter 18-24 mm) and tetracycline (inhibition diameter 18-25 mm), as reported by the Clinical and Laboratory Standards Institute (CLSI, 2017).

Gram-negative bacteria (*E. coli*, *S. typhi* and *P. aeruginosa*) were more inhibited than Gram-positive bacterium (*S. aureus*). One-Way ANOVA (**Appendix V**) indicated that there were no significant differences between the mean zones of inhibition of the extracts against the tested bacteria ($p = 0.5881$). Thus, the null hypothesis was accepted.

The antibacterial activity results indicated that more bioactive compounds were extracted by ethanol than ethyl acetate and distilled water. This observation is comparable to the report by Schultz *et al.* (2021b) who found that ethanolic extracts of *A. coriaria* stem bark harvested from Mpigi district of Uganda were more active against pathogenic bacteria than the ethyl acetate extract. A plausible explanation for this could be that the phytochemicals in *A. coriaria* leaves are more soluble in ethanol than ethyl acetate and were extracted before they were macerated in distilled water. Byamukama *et al.* (2015) reported that ethyl acetate extracts of *A. coriaria* stem bark from Mukono district of Uganda had the highest antibacterial activity compared to methanolic and aqueous extracts. It was previously reported that aqueous extracts of *A. coriaria* stem bark obtained by serial extraction using ethyl acetate, methanol and distilled water did not elicit any antibacterial activity (Byamukama *et al*., 2015) which is similar to the results obtained for aqueous leaf extracts in this study.

The inactivity of non-polar solvent extracts of this species was previously observed where hexane extracts of *A. coriaria* stem bark did not inhibit bacterial growth when tested against *S. aureus*, *E. coli* isolate, clinical *S. aureus* and *E. coli* ATCC 25922 (Akanga, 2008). Similarly, aqueous and ether extracts of *A. coriaria* stem bark were inactive when tested against *E. coli* and *S. typhi* (Nalubega *et al.*, 2011). Ethanolic extracts of *A. coriaria* stem bark at 256 μg/mL was recently reported to have no

inhibitory effect on *S. aureus* UAMS-1 among other multi-drug resistant bacteria (Schultz *et al*., 2020a).

The identified secondary metabolites in the ethanolic and ethyl acetate extracts could be responsible for the observed antibacterial activities of the *A. coriaria* leaves. For example, alkaloids, saponins, tannins and polyphenols (flavonoids and phenols) have been reported to have antibacterial activities (Adeeyo *et al.,* 2021) which are attributed to both their direct action against microorganisms or suppression of microbial virulence factors (Daglia, 2012). Alkaloids function by penetrating cells, intercalating microbial DNA and targeting several nucleic acid enzymes, resulting into irreversible damages to microbial cells (Yi *et al.,* 2007). Tannins and saponins inhibit microbial growth through precipitation of microbial proteins, rendering such nutritional proteins unavailable to the microorganisms (Panda & Tripathy, 2009). Tannins may also disrupt bacterial enzymes, cell envelope, adhesins and transport proteins. Their high affinity for iron in microbial cell membranes inactivates membrane-bound proteins, making extracts of gallotannin-rich plants to exhibit antibacterial activities (Engels *et al.*, 2011).

The observed antibacterial activities agreed with the abundances of the secondary metabolites identified in the extracts as well as the TPC and TFC of the extracts. For example, ethanolic extracts had higher quantities of phenols and saponins compared to ethyl acetate and aqueous extracts (**Table 4.2.1**). Ethanolic extract of leaves from Mbarara which had the highest bioactivity had the highest concentration of phenols, saponins and tannins. There was a slight correlation between the yield of the extracts and their antibacterial activities i.e. Mbarara ethanolic extracts which had the highest yield also gave the highest inhibition diameters.

4.7.2 Minimum inhibitory and bactericidal concentrations of the extracts

Ethanolic extracts were the most active and were thus considered for further tests for minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against *S. aureus*, *P. aeruginosa* and *S. typhi* (**Table 4.7.2.1**). Extract of leaves from Mbarara had the lowest MIC of 62.5 µg/mL against *P. aeruginosa* followed by *S. aureus* with MIC of 125 µg/mL. This showed that the extract was more effective against *P. aeruginosa* compared to *S. typhi* and *S. aureus.*

Table 4.7.2.1. Minimum inhibitory and bactericidal concentrations of *A. coriaria* extracts

District	**Minimum inhibitory concentration (µg/mL)**			**Minimum bactericidal concentration (µg/mL)**		
	S. aureus	*P. aeruginosa*	*S. typhi*	*S. aureus*	*P. aeruginosa*	*S. typhi*
Jinja	ND	125.0	ND	ND	250.0	ND
Kole	ND	250.0	250.0	ND	250.0	250.0
Mbarara	125.0	62.5	ND	250.0	125.0	ND

***Note**: ND = Not determined, as the bacteria had inhibition diameter less than 12 mm. All values reported are from assays performed in triplicate.

A previous study utilizing ethyl acetate extract of *A. coriaria* stem bark indicated a higher MIC of 125 mg/mL (125,000 µg/mL) for *E. coli* and 250 mg/mL (250,000 µg/mL) for *P. aeruginosa* (Byamukama *et al*., 2015). In another investigation, ethanolic extracts of *A. coriaria* stem bark had MIC greater than 256 µg/mL for *P. aeruginosa* AH-71 (Schultz *et al*., 2020a). The authors further reported that ethanolic and ethyl acetate extracts of *A. coriaria* stem bark had MIC of 250, 500 and > 500 µg/mL against *S. aureus* ATCC 25923, *E. coli* K12 ATCC 23716 and *Listeria innocua* ATCC 33090 (Schultz *et al*., 2021b) which are comparable to MIC values obtained for ethanolic extracts of *A. coriaria* leaves from Kole against *P. aeruginosa* and *S. typhi* in this study.

The MBC of 125 µg/mL of the extracts against *P. aeruginosa* showed that the extract had a stronger activity towards *P. aeruginosa* than the rest of the bacteria (**Table 4.7.2.1**). A contrastingly higher MBC of 125 mg/mL (125,000 µg/mL) against *E. coli* was reported for ethyl acetate extract of *A. coriaria* stem bark while *P. aeruginosa* was not affected at the tested concentrations of 62.5, 125, 250 and 500 mg/mL (Byamukama *et al*., 2015).

Overall, previous studies using single-solvent extraction systems reported higher MIC and MBC values than those obtained in this study. For example, Luvonga (2007) reported MIC of 12.5 mg/mL (1,250 µg/mL) for *S. aureus* and 25 mg/mL (25,000 µg/mL) for *P. aeruginosa* when they were treated with aqueous extracts of *A. coriaria* stem bark. The corresponding MBC values were 12.5 mg/mL (12,500 µg/mL) and 50 mg/mL (50,000 µg/mL). In another study (Nalubega *et al*., 2011), the authors reported MIC of 0.5 g/mL (500,000 µg/mL) for aqueous *A. coriaria* stem bark extracts on *S. aureus*. The strikingly high MBC of aqueous extracts observed by previous studies on this species could be explained by the fact that water usually extracts even inactive compounds (such as carbohydrates and proteins) which do not contribute to the antibacterial activity of plant extracts but may act as matrix interferences (Altemimi *et al.*, 2017). It should be emphasized that high MIC and MBC values is indicative that higher doses of the plant extracts are required for effective treatment. These results could explain why the posology of 3-4 cups (500 mL three times) and three spoons per day of *A. coriaria* extracts are recommended by Ugandan traditional healers for adults and children, respectively (Asiimwe *et al.*, 2013; Ganza, 2014; Namukobe *et al.*, 2011).

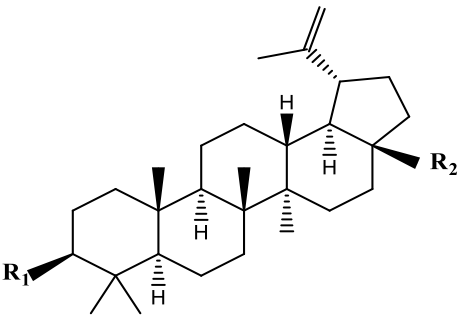
4.8 Compounds identified in fractions of ethanolic extracts of *A. coriaria* leaves

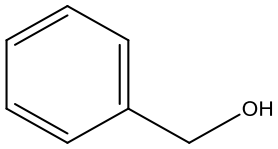
Nine known compounds were isolated and characterized in the different fractions of *A. coriaria* ethanolic leaf extracts (**Table 4.8.1**). These included lupeol (**7**), lupenone (**8**), betulinic acid (**9**), benzyl alcohol (**12**), betulin (**13**), oleanolic acid (**14**), oleanolic acid acetate (**15**), undecanol (**16**) and pterin-6-carboxylic acid (**17**) (**Figure 4.8.1**). The compounds were tentatively identified basing on their mass spectra analysis and matching with those in NIST 11 spectral library, and comparison with published spectroscopic data. Among the identified compounds, five compounds (**13-17**) are being reported for the first time in this species.

Table 4.8.1. Best solvent ratios used for elution of ethanolic extracts of *A. coriaria* leaves and compounds identified

Extract	Solvent system	Compounds identified
Jinja	Hexane : Ethyl acetate (4:6)	Oleanolic acid (R_f = 0.570), Oleanolic acid acetate (R_f = 0.533), Undecanol (R_f = 0.440)
Kole	Hexane : Ethyl acetate (4:6)	Oleanolic acid (R_f = 0.570), Oleanolic acid acetate (R_f = 0.533), Pterin-6-carboxylic acid (R_f = 0.490), Undecanol (R_f = 0.440)
Mbarara	Hexane : Ethyl acetate (9:1)	Lupeol (R_f = 0.745), Lupenone (R_f = 0.620)
Jinja, Kole, Mbarara	Ethyl acetate: Ethanol (3:7)	Betulinic acid (R_f = 0.596), Betulin (R_f = 0.500), Benzyl alcohol (R_f = 0.480)

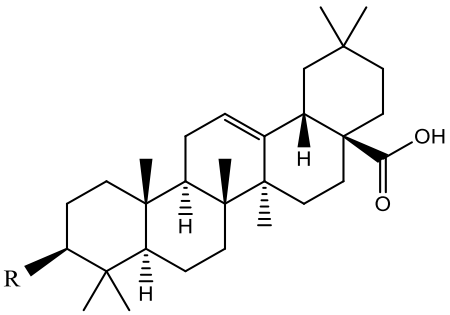
Note: R_f = retention factor on thin layer chromatography (TLC) plates. The TLC plates were visualized under ultraviolet lamp at 254 nm.





(**12**)

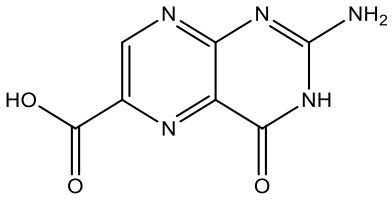
(**7**): R_1 = OH, R_2 = CH_3
(**8**): R_1 = O, R_2 = CH_3
(**9**): R_1 = OH, R_2 = COOH
(**13**): R_1 = OH, R_2 = CH_2OH



(**14**): R = OH

(**15**): R = 

(**16**)



(**17**)

Figure 4.8.1. Structure of compounds identified in fractions of ethanolic extracts of *A. coriaria* leaves.

The molecular ion of the compounds were identified using Nitrogen rule (Yetayih & Ravichandran, 2020). This rule indicates that if a molecular ion has an odd mass, it must have an odd number of nitrogen atoms and that a molecular ion with an even mass must either lack nitrogen atoms or contain an even number of them. As most fragments (excluding rearrangements) results from breaking a single bond, the nitrogen rule indicates that when a molecule with an even mass produces a fragment by breaking a single bond, the fragment will have an odd mass. When the sample's mass is odd, fragmentation via a similar pathway will give an even fragment as long as the nitrogen

is still contained in the observed fragment. Since this is the observed trend, analyzing the major fragments can help determine if the molecular ion should be even or odd (Yetayih & Ravichandran, 2020). Practically, if the major fragments are mostly odd, the molecular ion is likely even and contains no nitrogen. If the major fragments are even, the molecular ion is likely odd and contains one nitrogen atom.

For compound **7**, most peaks observed were odd numbers which implied that the molecular ion should be even. The molecular ion could not be assigned to be *m/z* 442 because the next fragment (*m/z* 426) is even. Thus, *m/z* 426 was identified to be the molecular ion peak as the next fragment (*m/z* 411) is odd. The ion *m/z* 426 was also analyzed to be capable of yielding important ions in the high mass region by logical neutral losses (Müller & Volmer, 2017). This procedure was used to identify the molecular ions of compounds **7-9** and **12-14**. For compounds **15-17**, the molecular ions were identified by comparison with published spectroscopic data.

As investigation of the bioactivities of the isolates were beyond the scope of this study, previous reports on antioxidant and antibacterial activities are discussed to establish if the compounds identified in the fractions of ethanolic extracts could have been the phytochemicals responsible for the observed bioactivities.

4.8.1 Compound 7

Compound **7** was obtained as colorless crystals which were soluble in hexane. It was eluted as the first fraction of ethanolic extracts of *A. coriaria* leaves from Mbarara using hexane: ethyl acetate (9:1) solvent system. The compound had a GC retention time of 36.943 minutes. The mass spectrum of compound **7** (**Figure 4.8.1.1**) had a molecular ion at *m/z* 426 which suggested a molecular formula $C_{30}H_{50}O$.

Fragmentation of the molecular ion by removal of a methyl group gave the fragment peak at *m/z* 411 (**Figure 4.8.1.2**). This peak is characteristic of a pentacyclic triterpene with an isopropenyl group (Pereira *et al.*, 1996). When it fragments by losing CH_2=CH_2 group, the fragment at *m/z* 383 is produced (Carvalho *et al*., 2010). Fragment *m/z* 383 further loses -$C_{13}H_{22}$ and -H_2O (or -$C_{13}H_{24}O$), which gives fragments *m/z* 207 (allocates the hydroxyl group at C-3 position) and *m/z* 189 (El Sayed, 2016). These fragments may also arise from the cleavage between C-8/C-14 and C-12/C-13 bonds (with proton transfer), and are confirmatory that such a compound possess a lupane or hopane skeleton (Budzikiewicz *et al.*, 1963; Ogunkoya, 1981).

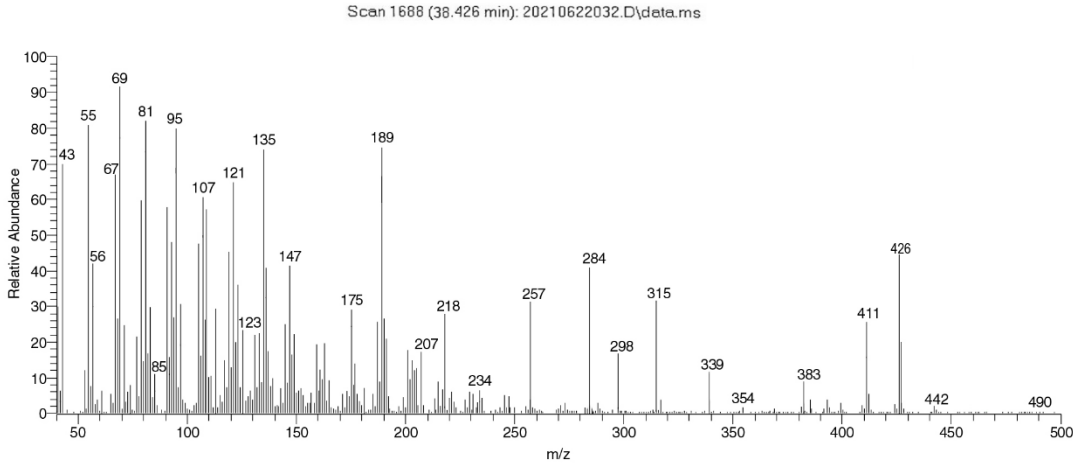


Figure 4.8.1.1. Mass spectrum of compound **7** (Lupeol)

The fragments at *m/z* 189 and *m/z* 218 indicates that compound **7** is a pentacyclic triterpene (Ahmad *et al.*, 2015; Cîntă-Pînzaru *et al.*, 2012; Cordeiro *et al.*, 1999; Galbraith *et al.*, 1965; Pereira *et al.*, 1996; Schmidt & Huneck, 1979). The other fragments at *m/z* 43, 55, 59, 81, 85, 91, 95, 107, 121, 135, 147, 161, 175, 218, 234, 257 and 315 are often associated with lupeol (Baek *et al.*, 2010; Byamukama *et al.*, 2015; Cîntă-Pînzaru *et al.*, 2012; Doshi *et al.*, 2015; Kiria, 2018; Leite *et al.*, 2020;

Wahyuono, 1985). By comparison with the foregoing literature and following NIST 11 library matching, compound **7** was concluded to be lup-20(29)-en-3β-ol (lupeol).

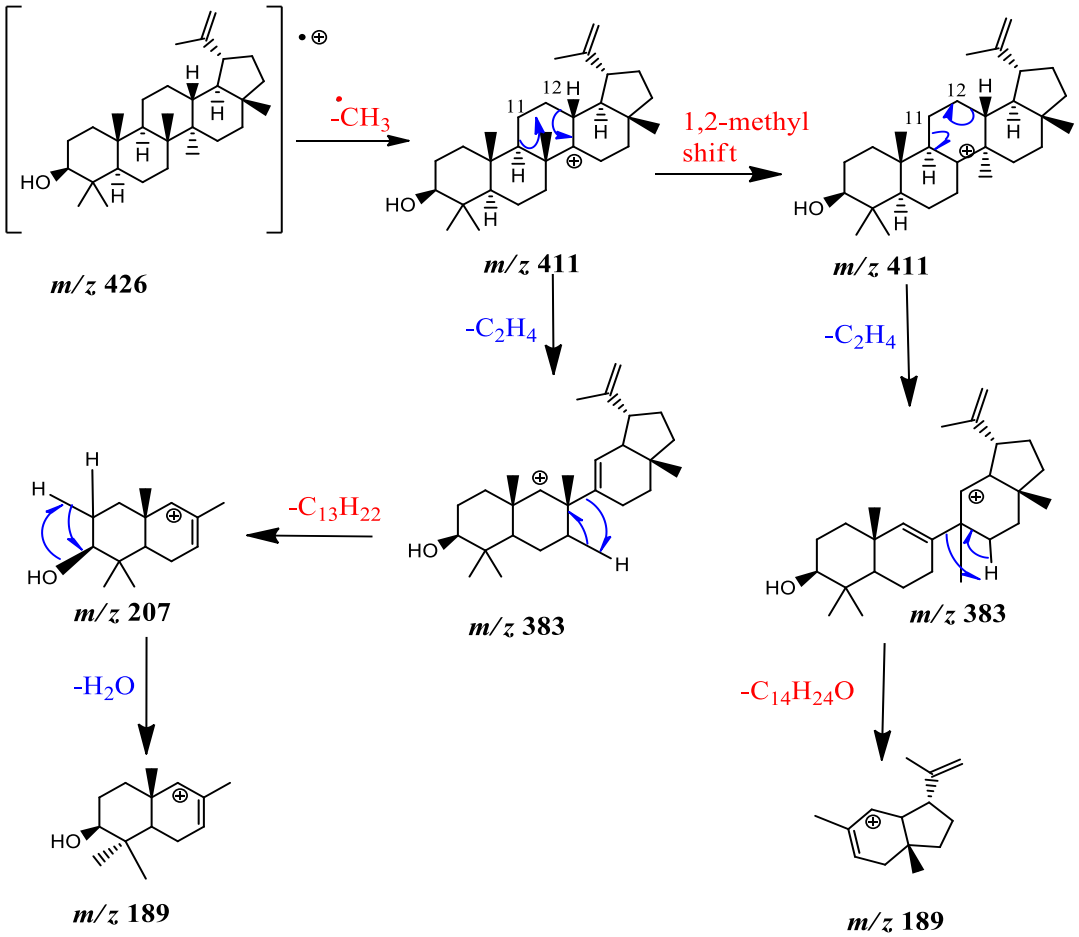


Figure 4.8.1.2. Fragmentation pattern of compound **7** (Lupeol).

Byamukama *et al.* (2015) previously reported the presence of lupeol in ethyl acetate extract of *A. coriaria* stem bark. Abd El-Ghany *et al.* (2015) and Tamokou *et al.* (2012) identified lupeol in the stem bark extract of other *Albizia* species (*A. lebbeck* and *A. adianthifolia*) using GC-MS. Lupeol was also reported in the root bark extract of *A. glaberrima* (Fotso *et al.*, 2017), wood extract of *A. myriophylla* (Thammavong, 2012) and stem bark extracts of *A. zygia* (Oloyede *et al.*, 2019), *A. julibrissin* (Baek *et al.*,

2010), *A. gummifera* (Rukunga & Waterman, 2001a), *A. versicolor*, *A. schimperana* (Rukunga & Waterman, 2001b) and *A. lebeckioides* (Arifnuryadin & Affandi, 1998).

The triterpene lupeol has excellent antioxidant and antibacterial activities (Amoussa *et al.*, 2016; Beserra *et al.*, 2019; Siddique & Saleem, 2011; Tchimene *et al.*, 2016). Its antioxidant properties is mediated through scavenging of free radicals, decreasing lipid peroxidation and increasing endogenous blood antioxidant enzymes levels (Tchimene *et al.*, 2016). As an antibacterial agent, it is effective against *E. coli*, *S. aureus*, *P. aeruginosa*, *S. typhi*, *Bacillus cereus*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Staphylococcus lutea*, *Shigella boydi*, *Shigella dysentriae* and *Vibrio mimicus* (Siddique & Saleem, 2011). These reports support the use of *A. coriaria* leaves in Uganda for treating cough, diarrhoea and typhoid.

4.8.2 Compound 8

Compound **8** was obtained as colourless needle-shaped crystals (soluble in hexane), having been eluted with hexane: ethyl acetate (9:1) solvent system as the second fraction of ethanolic extracts of *A. coriaria* leaves from Mbarara. Its GC retention time was 36.550 minutes. The mass spectrum of compound **8** (**Figure 4.8.2.1**) had a molecular ion peak at *m/z* 424, which suggested a molecular formula $C_{30}H_{48}O$. Compound **8** was closely related to compound **7** as its spectrum gave a molecular ion at *m/z* 424, corresponding to two fewer hydrogen atoms than the latter (Arifnuryadin & Affandi, 1998).

Fragmentation of the molecular ion (*m/z* 424) by removal of a methyl (CH_3) and C_6H_{10} group respectively yields the fragment ions at *m/z* 409 and *m/z* 342. The fragment at *m/z* 409 further loses $CH_2{=}CH_2$ to yield fragment *m/z* 381 (Suttiarporn *et al.*, 2015)(**Figure 4.8.2.2**). The fragment *m/z* 342 loses -C_4H_{10}, forming fragment *m/z* 271.

This fragmentation pattern agrees with the one suggested for lupenone in literature (Ahmad *et al.*, 2015; Branco *et al.*, 2004; Budzikiewicz *et al.*, 1963; Cordeiro *et al.*, 1999; Heinzen *et al.*, 1996; Suttiarporn *et al.*, 2015).

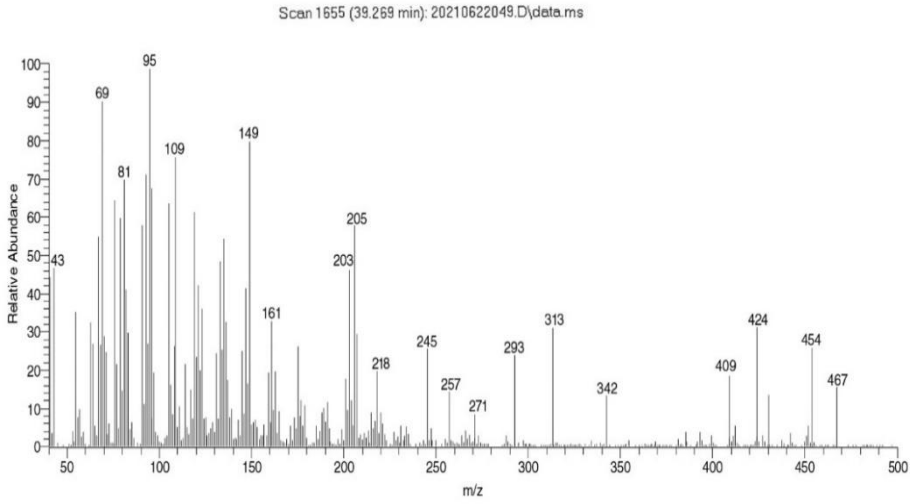


Figure 4.8.2.1. Mass spectrum of compound **8** (Lupenone)

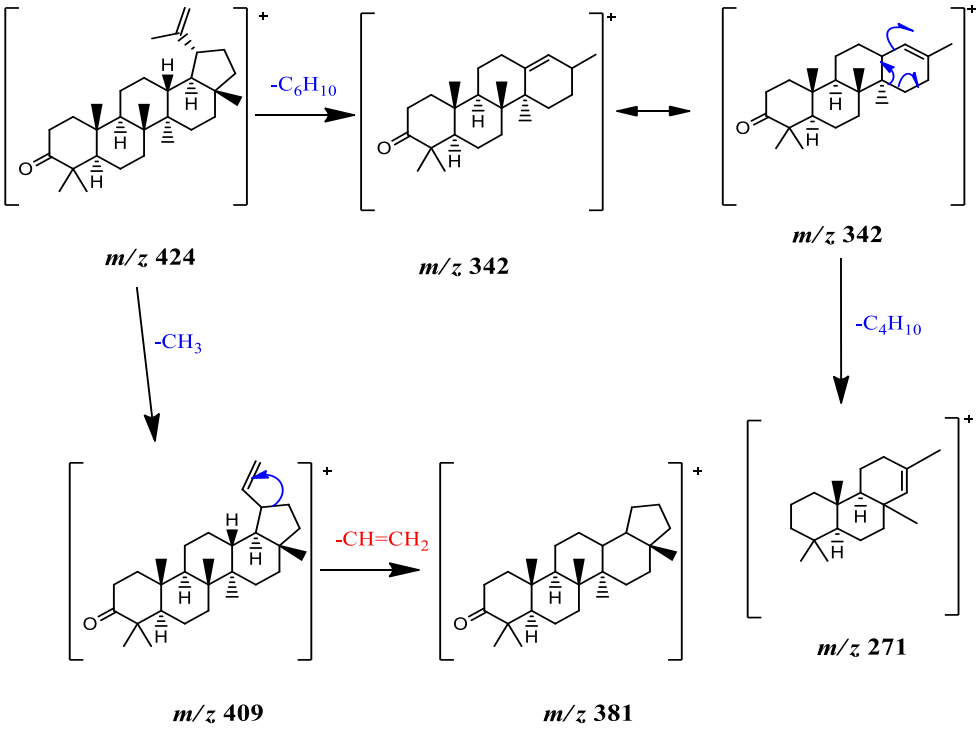


Figure 4.8.2.2. Fragmentation pattern for major ions of compound **8** (Lupenone).

Other fragments occurred at *m/z* 203, *m/z* 205 and *m/z* 218, which indicated that compound **8** possess a lupane-triterpene skeleton (Pereira *et al.*, 1996; Prashant & Krupadanam, 1993; Yam-Puc *et al.,* 2019). The mass spectral data also suggested the presence of a carboxylic acid group (at *m/z* 205), with the fragment at *m/z* 409 indicating that it is either attached to ring A or B of the pentacyclic ring (Pereira *et al.*, 1996). The other fragments (*m/z* 43, 81, 95, 105, 120 and 149) are usually observed in the mass spectrum of lupenone (Byamukama *et al.*, 2015; Kiria, 2018; Ogukwe *et al.*, 2018). These literature confirmed the NIST 11 spectral library matching that compound **8** is lup-20(29)-en-3-one (lupenone).

Lupenone was earlier identified in ethyl acetate extract of *A. coriaria* stem bark (Byamukama *et al.*, 2015). It was also isolated from petroleum ether leaf extract of a sister species: *Albizia inundata* (Andrade *et al.*, 2020) as well as stem bark extracts of *A. julibrissin* (Baek *et al*., 2010), *A. gummifera* (Rukunga & Waterman, 2001a), *A. versicolor* and *A. schimperana* (Rukunga & Waterman, 2001b), and *A. falcataria* (Arifnuryadin & Affandi, 1998).

Lupenone is a lupane-type triterpenoid with reported antioxidant activity (Çulhaoğlu *et al.*, 2015; Jeong *et al.*, 2013) and antibacterial activity against *E. coli, S. aureus, P. aeruginosa* and *Bacillus subtilis* (Prachayasittikul *et al.*, 2010). The identification of compounds **7** and **8** in the same extract in this study aligns well with previous reports which indicated that lupeol and lupenone often co-occur in plants (Gutierrez-Lugo *et al.*, 2004; Rukunga & Waterman, 2001a; 2001b; Xu *et al.*, 2018), including *A. coriaria* (Byamukama *et al.*, 2015).

4.8.3 Compound 9

Compound **9** was obtained as a yellowish-white powder, soluble in ethyl acetate. It was eluted with ethyl acetate: ethanol (3:7) as the first fraction of ethanolic extracts of leaves from Jinja, Kole and Mbarara. The GC retention time of compound **9** was 31.259 minutes. It showed a molecular ion at *m/z* 456, suggesting a molecular formula $C_{30}H_{48}O_3$ (**Figure 4.8.3.1**). The other prominent fragments were at *m/z* 455 (base peak) and *m/z* 457, which corresponded to the deprotonated (pseudo-molecular negative) ion $[M-H]^-$ and protonated ion $[M+H]^+$ for betulinic acid (Cichewicz & Kouzi, 2004; Koma & Sani, 2014; Räsänen *et al.*, 2019; Shin *et al*., 1999). Fragments *m/z* 437 $[M^+-H_2O]$ and *m/z* 411 $[M^+-COOH]$ were also observed, indicating the presence of a carboxylic acid group (Ayatollahia *et al.*, 2011).

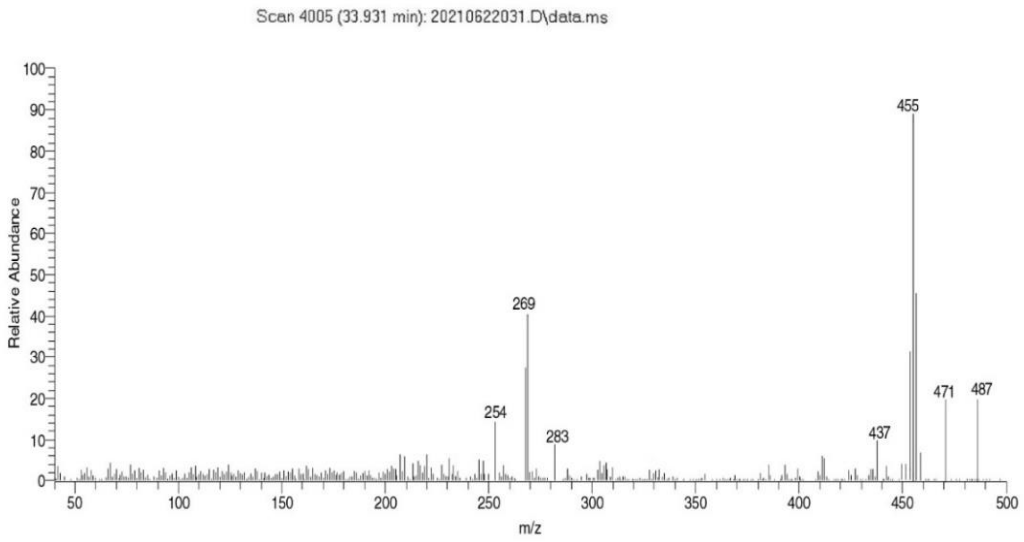


Figure 4.8.3.1. Mass spectrum of compound **9** (Betulinic acid)

The spectrum showed other peaks at *m/z* 254 and *m/z* 283, which are associated with pentacyclic triterpenoids with lupane skeleton (Joshi *et al.*, 2013; Koma & Sani, 2014; Nambooze, 2019). Another small but informative fragment was *m/z* 248, which usually

appears following ring C cleavage with charge retention either on ring A/B or D/E moiety (Wahyuono, 1985). Other fragments occurred at *m/z* 207 and 220, which are characteristic for betulinic acid (Baek *et al.*, 2010; Choi *et al.*, 2006; Lee *et al.*, 2009; Ogunmoye *et al.*, 2018). The other prominent fragment peaks were at *m/z* 437 $[M-CH_2]^+$ and 471 $[M-O]^+$ which are characteristic of a pentacyclic triterpene with an isopropenyl group (Nambooze, 2019). The other fragments (*m/z* 254, 269 and 283) are due to loss of H_2O, $-CH_3$ or $-CH_3OH$ from the enhanced ions, and are usually observed in the mass spectrum of betulinic acid (Peng *et al.*, 2017; Srivastava & Chaturvedi, 2010). These spectral characteristics supported that compound **9** is 3β-hydroxy-lup-20(29)-en-28-oic acid (betulinic acid).

Moreover, betulinic acid was previously isolated from *A. coriaria* stem bark extract (Byamukama *et al.*, 2015). It was also identified in ethanolic extract of *A. julibrissin* stem bark (Baek *et al*., 2010) as well as stem bark extract of *A. lebbeck* (Thube *et al.*, 2014). Betulinic acid is a widely distributed phenolic compound (pentacyclic lupane-type triterpene) in kingdom plantae and has been indicated to elicit intriguing pharmacological activities, including antioxidant and antibacterial activities (Karan *et al.*, 2019; Sulaiman *et al*., 2020). Specifically, it exhibited antibacterial activity against *E. coli*, *S. aureus*, *P. aeruginosa*, *S. typhi*, *Shigella dysenteriae* and *Bacillus subtilis* (Koma & Sani, 2014; Nambooze, 2019; Sallau *et al.*, 2016; Shai *et al.*, 2008).

4.8.4 Compound 12

Compound **12** was obtained as a brown powder, soluble in ethanol. It was eluted with ethyl acetate: ethanol (3:7) from the column as the third fraction of Jinja, Kole and Mbarara ethanolic leaf extracts. The compound had a GC retention time of 9.686 minutes, suggesting a molecular formula C_7H_8O. Its mass spectrum (**Figure 4.8.4.1**)

showed distinct peaks at *m/z* 108, 91 and 77, characteristic of benzylic alcohols (Dasgupta & Steinagel, 1997; Tedankara, 2021). In fact, the molecular ion of benzylic alcohols occur at *m/z* 108 (**Figure 4.8.4.2**). Loss of a proton from the molecular ion (*m/z* 108) gives the fragment at *m/z* 107. Rearrangement and loss of carbon monoxide (CO) from the latter peak gives the peak at *m/z* 79, which may lose two protons to yield the fragment ion at *m/z* 77 (Yetayih & Ravichandran, 2020). The fragment ion *m/z* 77 (phenyl cation, $C_6H_5^+$) may also be formed through alpha cleavage i.e. direct loss of -CH_2OH (Tedankara, 2021). Loss of acetylene (C_2H_2) from *m/z* 77 leads to the formation of *m/z* 51 (cyclobutenyl cation)(Nicolescu, 2017).

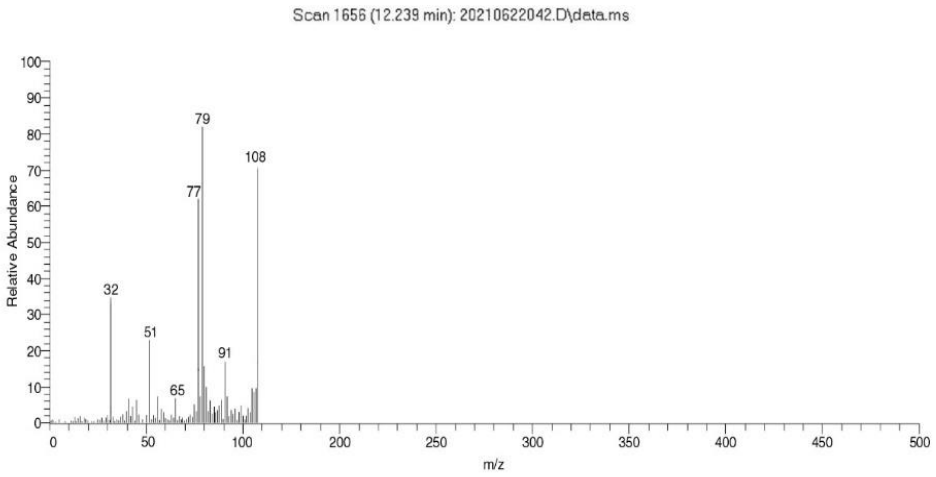


Figure 4.8.4.1. Mass spectrum of compound **12** (Benzyl alcohol)

Fragmentation (inductive cleavage) of the molecular ion through loss of a hydroxyl (mass = 17) yields the common benzyl cation fragment (intense ion) for alkylbenzenes at *m/z* 91 due to benzylic cleavage of the highly stable aromatic ring (Müller & Volmer, 2017; Yetayih & Ravichandran, 2020). This ion, called the tropylium ion or cycloheptatrienyl cation ($C_7H_7^+$), may undergo further fragmentation through successive loss of acetylene molecule to give the peak of cyclopentadienyl cation observed at *m/z* 65 (Müller & Volmer, 2017; Nicolescu, 2017; Yetayih &

Ravichandran, 2020). The other small fragments (*m/z* 57 and *m/z* 80) are usually observed in the mass spectrum of benzyl alcohol (Yetayih & Ravichandran, 2020) due to the presence of small amounts of carbon-13 in the sample (Tedankara, 2021). Thus, compound **12** was deduced to be benzyl alcohol as suggested by NIST 11 spectral library matching.

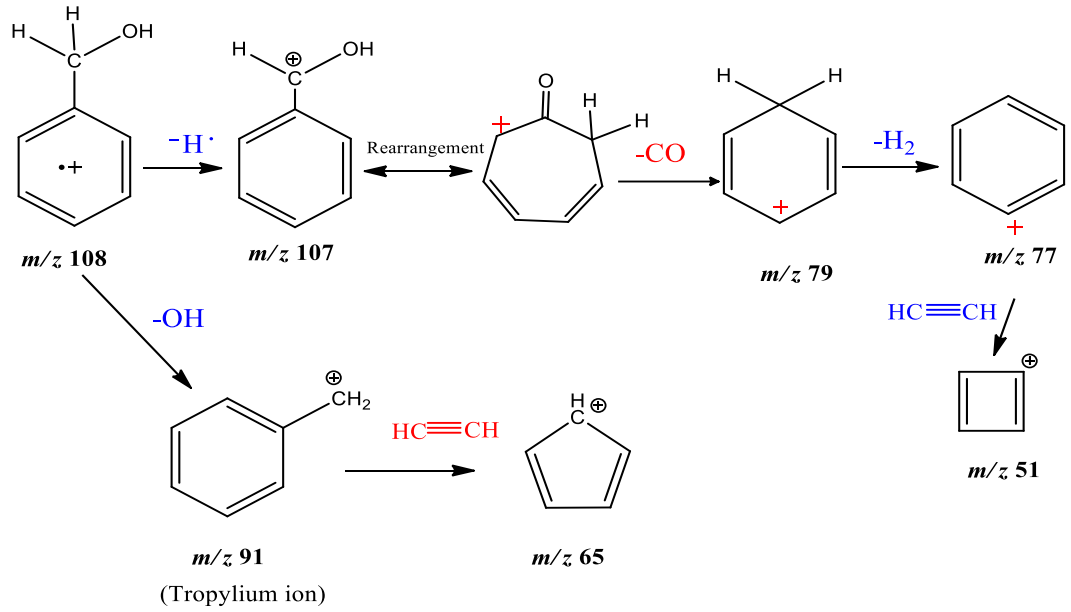


Figure 4.8.4.2. Fragmentation pattern for compound **12** (Benzyl alcohol)

Benzyl alcohol was previously identified in ethyl acetate extract of *A. coriaria* stem bark (Byamukama *et al.*, 2015). It has been indicated to possess *in vitro* antioxidant activity (Seung-Joo *et al.*, 2005), bacteriostatic and bactericidal activity against *E. coli*, *S. aureus*, *P. aeruginosa* and *Enterococcus faecium* (Lucchini *et al.*, 1990).

4.8.5 Compound 13

Compound **13** was obtained as colourless crystals, soluble in ethyl acetate. It was eluted with ethyl acetate: ethanol (3:7) from the column as the second fraction of Jinja, Kole and Mbarara ethanolic leaf extracts. Its GC retention time was 24.690 minutes,

corresponding to the molecular formula $C_{30}H_{50}O_2$. The mass spectrum of compound **13** (**Figure 4.8.5.1**) revealed the presence of a prominent fragment at *m/z* 442, suggesting that it is a lupeol-type triterpene (Cîntă-Pînzaru *et al.*, 2012; Tijjani *et al.*, 2012). The molecular ion peak at *m/z* 442 is characteristic of the molecular ion $[M]^+$ for the triterpenoid betulin, which matched the suggested molecular formula $C_{30}H_{50}O_2$ (Heinzen *et al*., 1996; Räsänen *et al*., 2019).

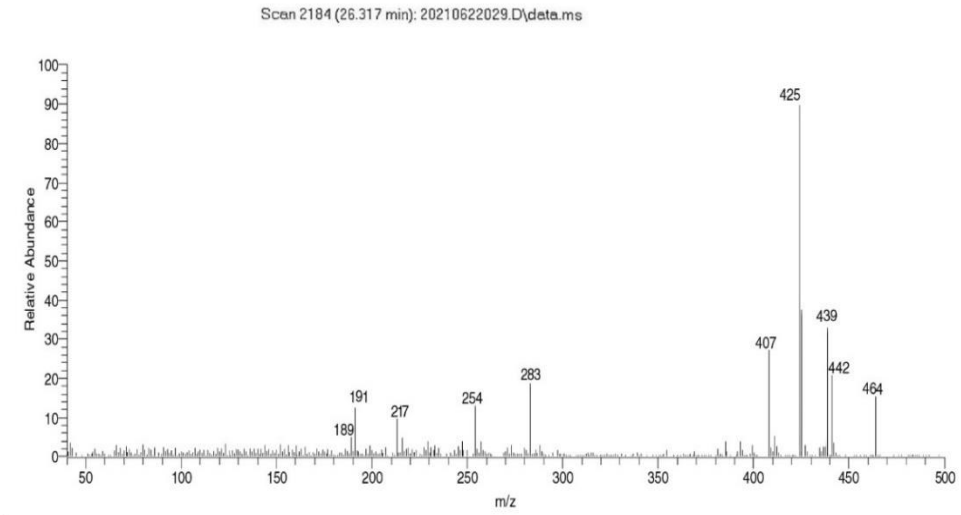


Figure 4.8.5.1. Mass spectrum of compound **13** (Betulin)

Another fragment occurred at *m/z* 428, formed following loss of $-CH_3$ from the protonated molecular ion, *m/z* 443 (Zhang *et al.*, 2019). Other fragments (*m/z* 411, 407, 395 and 393) are due to loss of H_2O, $-CH_3$ or $-CH_3OH$ from *m/z* 425 or *m/z* 410 (**Figure 4.8.5.2**). The peaks observed at *m/z* 217 and *m/z* 189 are due to ring opening of the protonated molecular ion, followed by loss of water (Zhang *et al.*, 2019). These spectroscopic data supported that compound **13** is the known compound (lup-20(29)-ene-3β,28-diol), commonly called betulin (Heinzen *et al.*, 1996).

Though being reported for the first time in *A. coriaria*, betulin was identified by GC-MS in the pericarp extract of a sister species (*A. lebbeck*) in Egypt (El-Hawary *et al.*,

2011). Oloyede *et al.* (2019) also identified betulin in the stem bark extract of *A. zygia*. Betulin possess a range of biological activities, including antioxidant and antibacterial activities (Šiman *et al.*, 2016). It was shown to have antibacterial activity against *Streptococcus pyogenes* with MIC of 85 μg/mL (Prachayasittikul *et al.*, 2010).

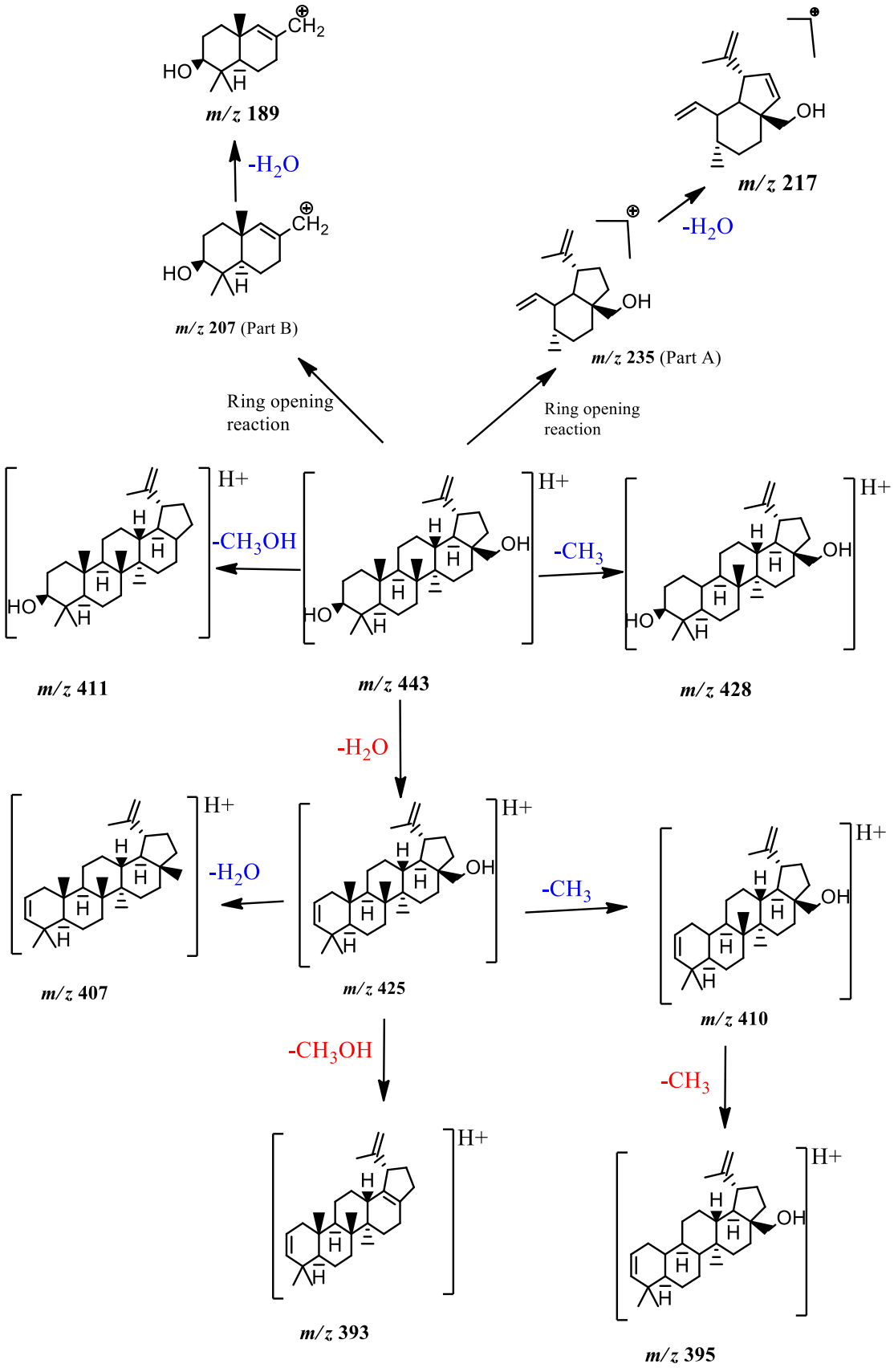


Figure 4.8.5.2. Major peaks from fragmentation of compound **13** (Betulin)

4.8.6 Compound 14

Compound **14** was obtained as a white solid, soluble in ethanol. It was eluted with ethyl acetate: ethanol (3:7) from the column as the first fraction of ethanolic extracts of *A. coriaria* leaves from Jinja and Kole. Its GC retention time was 31.875 minutes. The molecular ion at *m/z* 456 suggested a molecular formula $C_{30}H_{48}O_3$. The mass spectrum of compound **14** (**Figure 4.8.6.1)** was very similar to that of compound **9** (**Figure 4.8.3.1**). In compound **14**, the peak at *m/z* 455 suggested a deprotonated oleanolic acid molecule (Eunyoung *et al.*, 2016; Song *et al*., 2006; Sut *et al.*, 2018). The spectral data also showed presence of a carboxylic acid at *m/z* 407, that strongly suggested a C-12 unsaturated pentacyclic triterpene containing a carboxylic acid group in either ring D or E (Wahyuono, 1985). Another peak was observed at *m/z* 391 due to loss of $-CH_2$ group from the *m/z* 407 fragment (**Figure 4.8.6.2**)(Chen *et al.*, 2011; Hu *et al*., 2018). The other peaks observed at *m/z* 255, 269, 283 and 297 are fragments of the enhanced products from the molecular ion (Bugeja, 2012). Thus, compound **14** was suggested to be oleanic acid/oleanolic acid.

Free oleanolic acid is being reported for the first time in *A. coriaria*. However, it was previously identified in the ethanolic extracts of *A. julibrissin* stem bark (Baek *et al*., 2010). Oleanolic acid is one of the best known bioactive pentacyclic triterpenoids with widespread occurrence throughout the plant kingdom in the form of free acid or aglycones for triterpenoid saponins (Jesus *et al.*, 2015; Song *et al.*, 2006).

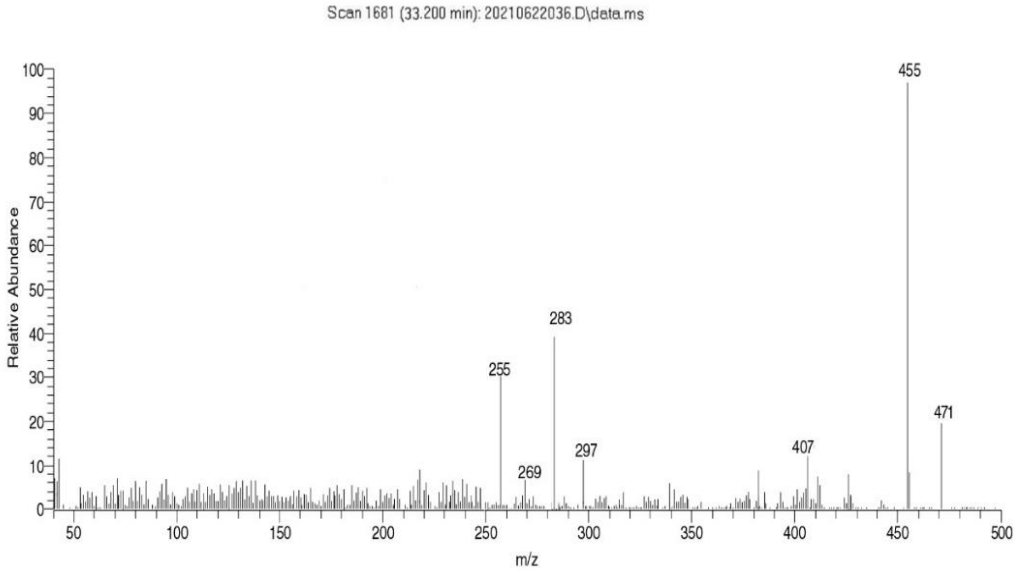


Figure 4.8.6.1. Mass spectrum of compound **14** (Oleanolic acid)

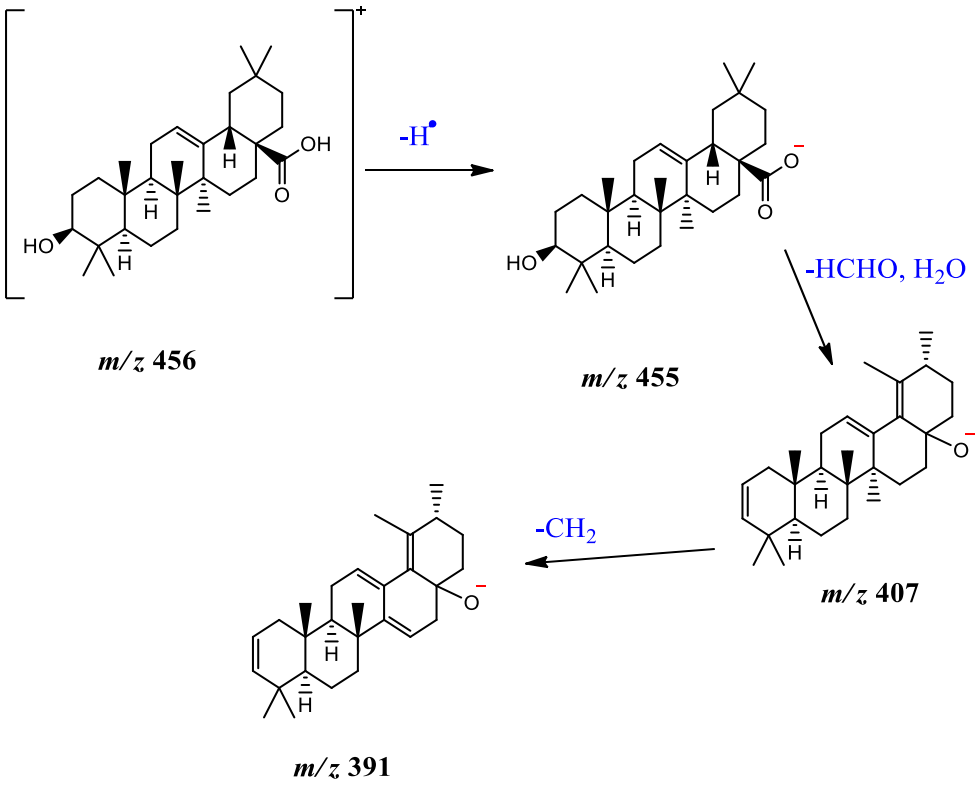


Figure 4.8.6.2. Fragmentation pattern of compound **14** (Oleanolic acid)

Oleanolic acid possesses several pharmacological activities, including antioxidant and antibacterial activities (Ayeleso *et al.*, 2017). For example, oleanolic acid isolated from the plant *Ligustrum lucidum* was indicated to decrease malonaldehyde level and increase superoxide dismutase and gluthatione peroxidase activities in alloxan-induced diabetic rats (Gao *et al*., 2009). It has also been reported to inhibit the growth of Gram-positive and Gram-negative bacteria such as *E. coli, S. aureus, P. aeruginosa, S. typhi, Bacillus cereus, Bacillus megaterium, Bacillus subtilis, Klebsiella pneumoniae, Mycobacterium tuberculosis, Staphylococcus lutea, Salmonella paratyphi, Shigella boydi, Shigella dysentriae, Streptococcus mutans, Streptococcus sobrinus* and *Vibrio mimicus* (Horiuchi *et al.*, 2007; Jesus *et al.*, 2015; Nambooze, 2019).

4.8.7 Compound 15

Compound **15** was eluted with ethyl acetate: ethanol (3:7) from the column as the second fraction of ethanolic extracts of *A. coriaria* leaves from Jinja and Kole. Its GC retention time was 30.992 minutes. The mass spectrum of compound **15** (**Figure 4.8.7.1**) had a molecular ion at *m/z* 497, suggesting a molecular formula $C_{32}H_{50}O_4$. Fragments at *m/z* 269 and 423, characteristic of pentacyclic triterpenes possessing carboxylic acid groups were present (Joshi *et al.*, 2013; Nambooze, 2019). The spectrum further suggested the presence of a carboxylic acid group at *m/z* 301, $[M\text{-}COOH]^+$ (Joshi *et al*., 2013; Nambooze, 2019). The ion peak observed at *m/z* 469 could have been due to loss of HCHO from the molecular ion (**Figure 4.8.7.2**). Further loss of HCHO and H_2O molecules as in oleanolic acid could have given the peak observed at *m/z* 423 (Nambooze, 2019). These spectral characteristics supported the NIST library suggestion that compound **15** is 3-*O*-acetyloleanolic acid (oleanolic acid acetate) (Oh *et al.,* 2014).

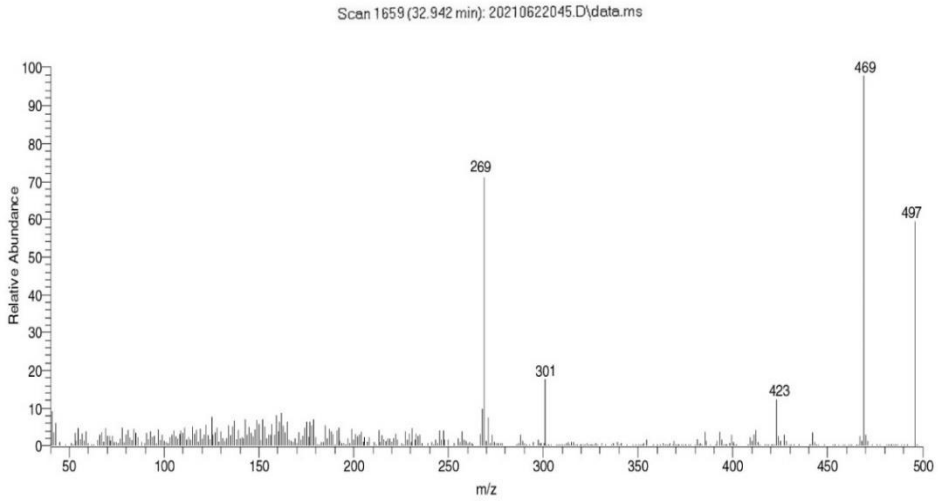


Figure 4.8.7.1. Mass spectrum of compound **15** (Oleanolic acid acetate)

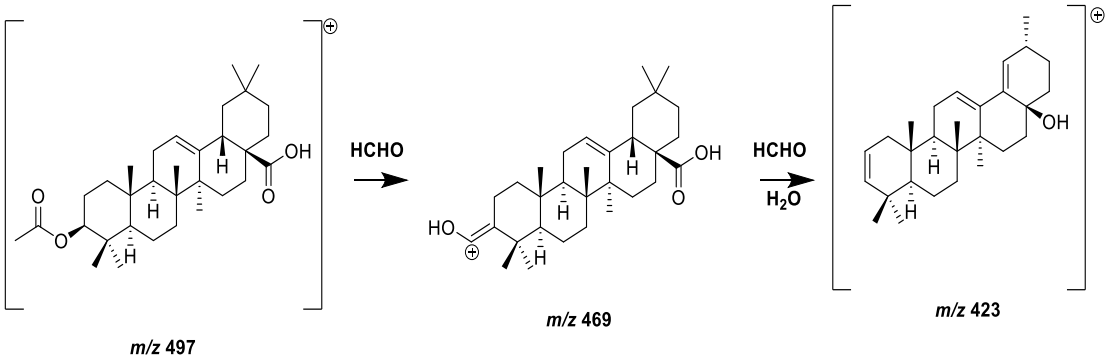


Figure 4.8.7.2. Proposed fragmentation pattern for major ions of compound **15** (Oleanolic acid acetate).

Oleanolic acid acetate was reported to elicit antibacterial activity against *S. aureus* and *P. aeruginosa* with inhibition zones of 6.5 mm and 8.1 mm respectively (Nambooze, 2019). No report was retrieved on the antioxidant potential of oleanolic acid acetate. However, most derivatives of oleanolic acid are known to have antioxidant activity (Ayeleso *et al*., 2017).

4.8.8 Compound 16

Compound **16** was obtained as a pale greenish liquid with mild odor, soluble in ethanol. It was isolated as the third and fourth fractions of ethanolic extract of *A. coriaria* leaves from Jinja and Kole, respectively after elution of the column with hexane: ethyl acetate (4:6) solvent system. Its retention time was 15.307 minutes. The mass spectrum of compound **16** (**Figure 4.8.8.1**) had a base peak at *m/z* 55 and a molecular ion at *m/z* 172 which suggested a molecular formula $C_{11}H_{24}O$. The fragment observed at *m/z* 154 is due to loss of water from the molecular ion (Hashem, 2008; Nicolescu, 2017). The fragment at *m/z* 125 is due to loss of water and vinyl (CH_2 = CH) group from the molecular ion (Dunnivant & Ginsbach, 2008; Hashem, 2008). Loss of ethene (CH_2=CH_2) molecule from *m/z* 125 gave the fragment at *m/z* 97 (**Figure 4.8.8.2**).

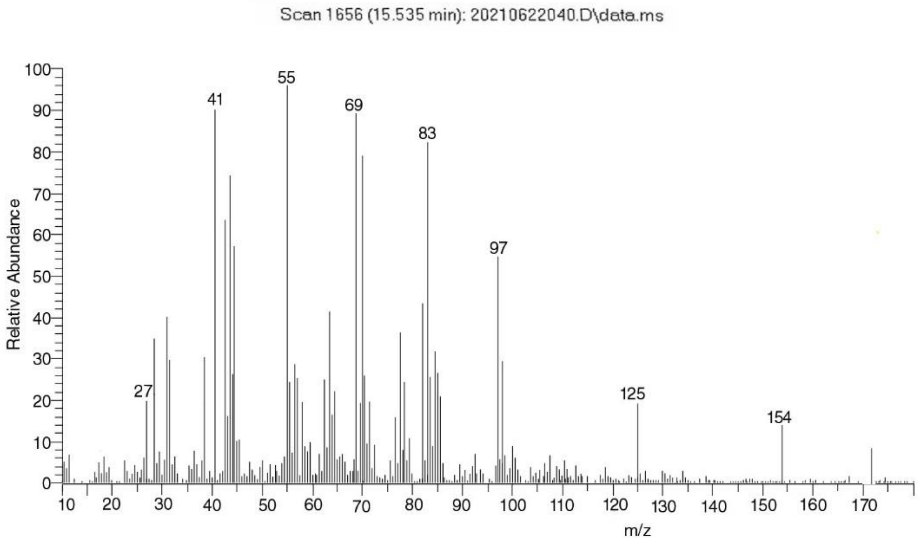


Figure 4.8.8.1. Mass spectrum of compound **16** (Undecanol)

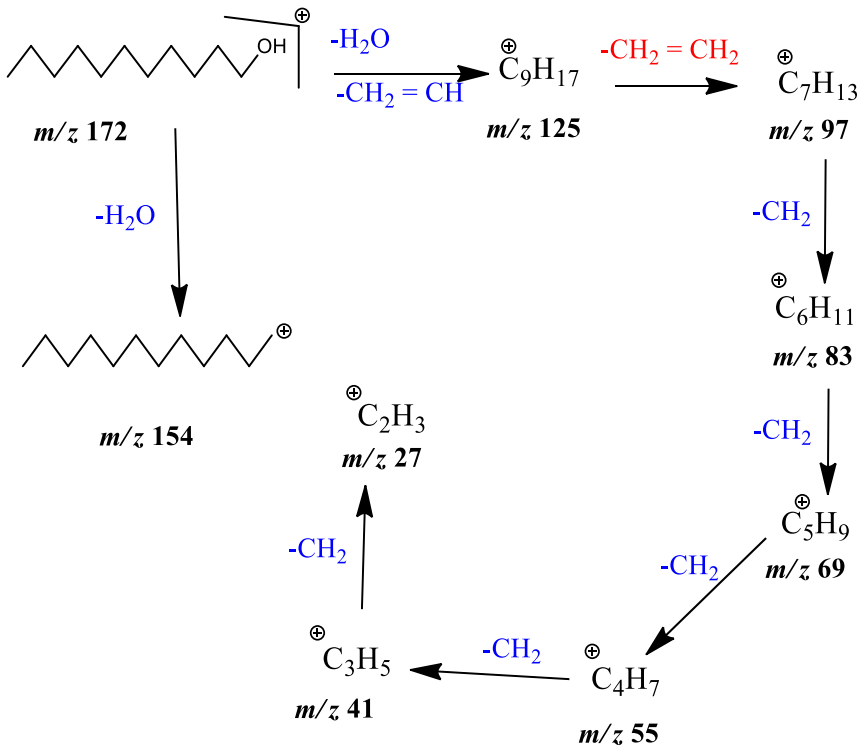


Figure 4.8.8.2. Proposed fragmentation pattern of compound **16** (Undecanol)

The other fragments observed at *m/z* at 83, 69, 55, 41 and 27 are due to loss of -CH_2 from the preceding fragments (97, 83, 69, 55 and 41, respectively)(Habib *et al.*, 2017). These spectral patterns confirmed that compound **16** is undecan-1-ol (undecanol), also known as 1-undacanol.

Though it has never been reported before in *A. coriaria*, undecanol was earlier reported in *A. zygia* stem bark fixed oil analyzed by GC-MS (Oloyede *et al.*, 2019). Previous reports indicate that undecanol (a long-chain fatty acid) had antibacterial activity against *S. aureus* with MIC and MBC of 32 µg/mL and 40 µg/mL, respectively (Togashi *et al.*, 2007). However, there is no report on the antioxidant activity of undecanol or other such polysaturated fatty alcohols.

4.8.9 Compound 17

Compound **17** was eluted with hexane: ethyl acetate (4:6) from the column as the third fraction of ethanolic extracts of *A. coriaria* leaves from Kole. It had a GC retention time of 21.579 minutes. The molecular ion of compound **17** occurred at *m/z* 207, suggesting a molecular formula $C_7H_5N_5O_3$ (**Figure 4.8.9.1**).

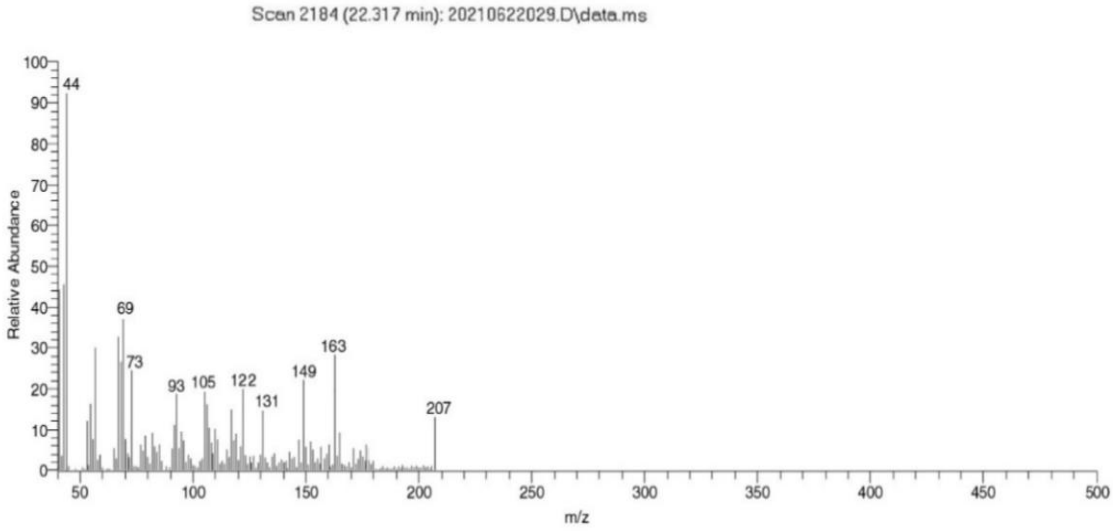


Figure 4.8.9.1. Mass spectrum of compound **17** (pterin-6-carboxylic acid)

The fragment observed at *m/z* 163 is due to loss of carbon dioxide (CO_2, mass = 44) from the molecular ion (Kuse *et al.*, 2010). Loss of N_2HCHO (mass = 58) from *m/z* 163 (**Figure 4.8.9.2**) could have yielded the fragment at *m/z* 105 as observed in the fragmentation of pterins (Allegri *et al*., 2012). The fragment at *m/z* 149 is due to loss of glycolaldehyde gas ($HOCH_2CHO$, mass = 59) from the molecular ion (Allegri *et al*., 2012). Loss of H_2O from *m/z* 149 gives the fragment at *m/z* 131. The other peaks at *m/z* 57, 69, 105 and 122 are usually observed in the spectrum of pterin-6-carbozylic acid (Hussein *et al.* 2016a; Hussein *et al*., 2016b; Mohammed *et al.*, 2021; Shenta & Al-Maliki, 2013). Thus, compound **17** was suggested to be 2-amino-4-hydroxy-6-

pteridinecarboxylic acid (pterin-6-carboxylic acid) as initially indicated by NIST 11 library matching.

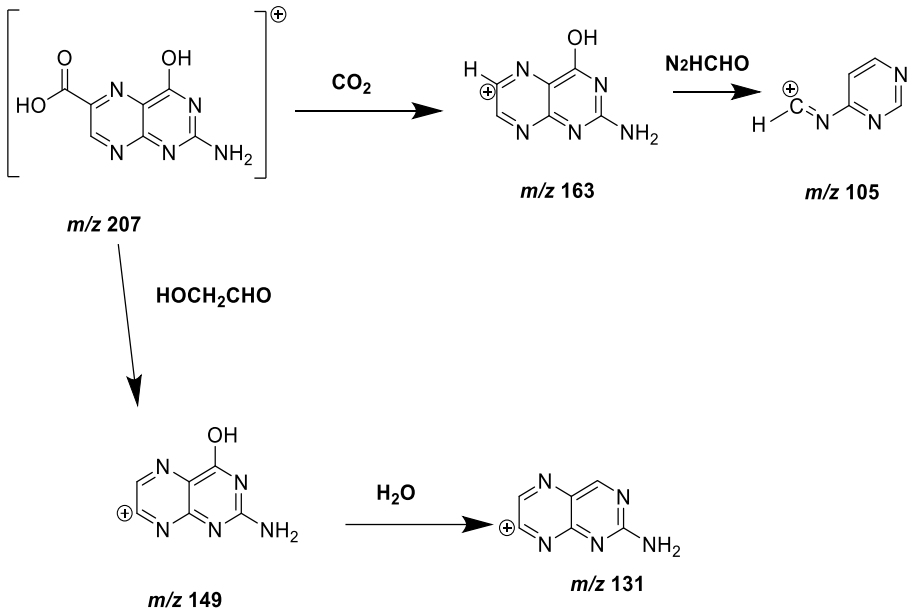


Figure 4.8.9.2. Proposed fragmentation pattern of compound **17** (pterin-6-carboxylic acid).

Pterin-6-carboxylic acid (an alkaloid) was previously characterized in *A. lebbeck* leaf extracts by column chromatography and GC-MS analysis (Shenta & Al-Maliki, 2013). Though there is no report on its antioxidant activity, a fraction containing it elicited antibacterial activity against *E. coli, S. aureus, P. aeruginosa, Klebsiella* and *Proteus* species at 150 mg/mL (Shenta & Al-Maliki, 2013).

Overall, the compounds identified agreed with results of classical phytochemical screening (**Table 4.2.1**) which indicated the presence of alkaloids and terpenes (triterpenoids) in ethanolic extracts of *A. coriaria* leaves. Compounds **7-9** and **13-15** are terpenoids, **12** and **16** are alcohols while **17** is an alkaloid.

Most compounds identified in the ethanolic extracts of *A. coriaria* leaves have reported antioxidant and antibacterial activities, which supports the use of the leaves in treatment of oxidative stress-induced conditions and bacterial diseases in Uganda. The null hypothesis was rejected because the compounds identified were not the same for all the extracts. Two compounds: lupeol (**7**) and lupenone (**8**) were only identified in ethanolic extracts of *A. coriaria* leaves from Mbarara. These compounds have reported significant bioactivities, which could explain the higher antioxidant and antibacterial activities of this extract. On the other hand, oleanolic acid (**14**), oleanolic acid acetate (**15**) and undecanol (**16**) were not present in ethanolic extract of *A. coriaria* leaves from Mbarara. Pterin-6-carboxylic acid (**17**) was only reported in ethanolic extract of leaves from Kole. Because it is known to elicit antibacterial activity (Shenta & Al-Maliki, 2013), it could be responsible for the high antibacterial activity of the ethanolic extract of leaves from Kole against *S. typhi* (with inhibition diameter of 16.00 ± 1.73 mm) which was comparable to 20.00 ± 1.53 mm for ciprofloxacin (**Table 4.7.1.1**).

CHAPTER FIVE: CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The following conclusions have been drawn from the results obtained.

1. Phenols, alkaloids, saponins, flavonoids, cardiac glycosides and tannins are the major secondary metabolites in extracts of *Albizia coriaria* (*A. coriaria*) leaves from Jinja, Kole and Mbarara districts of Uganda.
2. Extracts of *A. coriaria* leaves from Jinja, Kole and Mbarara districts have significant intraspecific variation of total phenolic and total flavonoid contents.
3. *A. coriaria* leaves extracts from the studied districts exhibited significant but varying free radical scavenging potentials. This result lends credence to the traditional use of *A. coriaria* leaves in the treatment of oxidative stress-induced conditions in Uganda.
4. All ethanolic extracts, and ethyl acetate extracts of *A. coriaria* leaves from Kole and Mbarara districts of Uganda exhibited antibacterial activity against *Escherichia coli, Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella typhi*. This result justifies the traditional use of *A. coriaria* leaves extracts in Uganda for treatment of some diseases caused by these pathogenic bacteria.
5. Isolation and characterization of the most bioactive (ethanolic) extracts of *A. coriaria* leaves led to the identification of nine known compounds namely: lupeol (**7**), lupenone (**8**), betulinic acid (**9**), benzyl alcohol (**12**), betulin (**13**), oleanolic acid (**14**), oleanolic acid acetate (**15**), undecanol (**16**) and pterin-6-carboxylic acid (**17**). Among these compounds, five compounds (**13-17**) are being reported for the first time in this species.

5.2 Recommendations

From this study, the following are recommended.

1. Toxicity studies of the crude leaf extracts and compounds **15-17** are recommended, so as to establish their safety when utilized in traditional medicine.
2. *In vivo* studies and clinical trials using the active extracts of *A. coriaria* leaves and the identified compounds should be done.
3. Ethyl acetate extract of *A. coriaria* leaves from Kole and Mbarara should be subjected to structural elucidation to establish the compounds responsible for their antibacterial activity against *Escherichia coli.*
4. Extracting the leaves using water only should be performed to assess whether traditional maceration of the leaves could give higher total phenolic and total flavonoid contents, antioxidant and antibacterial activities.
5. Phytochemical analysis of *A. coriaria* flowers and seeds should be done to identify the if the compounds identified in this study are also present in them.

REFERENCES

Abarca-Vargas, R., Malacara, C. F. P., & Petricevic, V. L. (2016). Characterization of Chemical Compounds with Antioxidant and Cytotoxic Activities in *Bougainvillea x buttiana* Holttum and Standl, (var. Rose) Extracts. *Antioxidants, 5*, 45.

Abbas, O., Compère, G., Larondelle, Y., Pompeu, D., Rogez, H., & Baeten, V. (2017). Phenolic compound explorer: A mid-infrared spectroscopy database. *Vibrational Spectroscopy, 92*, 111–118.

Abd-El-Raouf, R., Ouf, S. A., Gabr, M. M., Zakaria, M. M., El-Yasergy, K. F., & Ali-El-Dein, B. (2020). *Escherichia coli* foster bladder cancer cell line progression via epithelial mesenchymal transition, stemness and metabolic reprogramming. *Scientific Reports, 10*, 18024.

Abd Aziz, N. A., Hasham, R., Sarmidi, M. R., Suhaimi, S. H., & Idris, M. (2021). A review on extraction techniques and therapeutic value of polar bioactives from Asian medicinal herbs: Case study on *Orthosiphon aristatus*, *Eurycoma longifolia* and *Andrographis paniculata*. *Saudi Pharmaceutical Journal, 29*, 143–165.

Abd El-Ghany, A. E.-S., Dora, G., Abdallah, R. H., Hassan, W. H. B., & Abd El-Salam, E. (2015). Phytochemical and biological study of *Albizia lebbeck* stem bark. *Journal of Chemical and Pharmaceutical Research, 7*, 29-43.

Abdel-Kader, M., Hoch, J., Berger, J. M., Evans, R., Miller, J. S., Wisse, J. H., . . . Kingston, D. G. I. (2001). Two bioactive saponins from *Albizia subdimidiata* from the Suriname rainforest. *Journal of Natural Products, 64*, 536–539.

Abdelbary, M. M. H., Basset, P., Blanc, D. S., & Feil, E. J. (2017). The Evolution and Dynamics of Methicillin-Resistant *Staphylococcus aureus*. *Genetics and Evolution of Infectious Diseases (2nd Edn), 24*, 553-572.

Adeeyo, A. O., Edokpayi, J. N., Alabi, M. A., Msagati, T. A. M., & Odiyo, J. O. (2021). Plant active products and emerging interventions in water potabilisation: disinfection and multi-drug resistant pathogen treatment. *Clinical Phytoscience, 7*, 31.

Adia, M. M., Anywar, G., Byamukama, R., Kamatenesi-Mugisha, M., Sekagya, Y., Kakudidi, E. K., & Kiremire, B. T. (2014). Medicinal plants used in malaria treatment by Prometra herbalists in Uganda. *Journal of Ethnopharmacology, 155*, 580-588.

Agroforestry Database. (2009). *Albizia coriaria*. Retrieved January 20th, 2020 from http://apps.worldagroforestry.org/treedb/AFTPDFS/Albizia_coriaria.PDF.

Ahmad, S., Sukari, M. A., Ismail, N., Ismail, I. S., Abdul, A. B., Bakar, M. F. A., . . . Ee, G. C. L. (2015). Phytochemicals from *Mangifera pajang* Kosterm and their biological activities. *BMC Complementary and Alternative Medicine, 15*, 83.

Ahmed, D., Khan, M. M., & Saeed, R. (2015). Comparative Analysis of Phenolics, Flavonoids, and Antioxidant and Antibacterial Potential of Methanolic, Hexanic and Aqueous Extracts from *Adiantum caudatum* Leaves. *Antioxidants, 4*, 394-409.

Aissani, F., Grara, N., Bensouici, C., Bousbia, A., Ayed, H., Md Idris, M. H., & Teh, L. K. (2021). Algerian *Sonchus oleraceus* L.: a comparison of different extraction solvent on phytochemical composition, antioxidant properties and anti-cholinesterase activity. *Advances in Traditional Medicine, 2*, 1-12.

Akanga, J. O. (2008). Screening of antidiarrhoea medicinal plants for *in vitro* antimicrobial activity against clinical and environmental enteropathogens. *MSc Thesis, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya*

Akash, M. S. H., & Rehman, K. (2020). Thin Layer Chromatography. In: *Essentials of Pharmaceutical Analysis*. Springer, Singapore. pp. 157-165.

Akiyama, H., Fujii, K., Yamasaki, O., Oono, T., & Iwatsuki, K. (2001). Antibacterial action of several tannins against *Staphylococcus aureus*. *Journal of Antimicrobial Chemotherapy, 48*, 487-491.

Allegri, G., Costa Netto, H. J., Ferreira Gomes, L. N., Costa de Oliveira, M. L., Scalco, F. B., & de Aquino Neto, F. R. (2012). Determination of six pterins in urine by LC-MS/MS. *Bioanalysis, 4*, 1739–1746.

Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D. G., & Lightfoot, D. A. (2017). Phytochemicals: Extraction, Isolation, and Identification of Bioactive Compounds from Plant Extracts. *Plants, 6*, 42.

Amoussa, A. M. O., Lagnika, L., Bourjot, M., Vonthron-Senecheau, C., & Sanni, A. (2016). Triterpenoids from *Acacia ataxacantha* DC: antimicrobial and antioxidant activities. *BMC Complementary and Alternative Medicine, 16*, 284.

Andrade, E. M. J., Teixeira, J. S., Silva, D. K. C., dos Santos, T. B., Korn, M. G. A., Brandão, H. N., . . . Júnior, A. F. S. (2020). Phytochemical Analysis, Multi-Element Composition and Biological Activities of Extracts and Lupenone from *Albizia inundata* (Mart.) Barneby & J.W.Grimes. *Journal of the Brazilian Chemical Society, 31*, 1825-1837.

Anwar, Z., & Hussain, F. (2017). Steroidal Saponins: An Overview of Medicinal Uses. *International Journal of Chemical and Biochemical Sciences, 11*, 20-24.

Anywar, G. (2020). Ethnopharmacology, cytotoxicity, antiviral and immunomodulatory profiles of medicinal plant species used by herbalists in treating people living with HIV/AIDS in Uganda. *PhD thesis, Makerere University, Kampala, Uganda*.

Anywar, G., Kakudidi, E., Byamukama, R., Mukonzo, J., Schubert, A., & Oryem-Origa, H. (2020a). Indigenous traditional knowledge of medicinal plants used

by herbalists in treating opportunistic infections among people living with HIV/AIDS in Uganda. *Journal of Ethnopharmacology, 246*, 112205.

Anywar, G., Kakudidi, E., Byamukama, R., Mukonzo, J., Schubert, A., & Oryem-Origa, H. (2020b). Medicinal plants used by traditional medicine practitioners to boost the immune system in people living with HIV/AIDS in Uganda. *European Journal of Integrative Medicine, 35*, 101011.

Anywar, G., Kakudidi, E., Byamukama, R., Mukonzo, J., Schubert, A., Oryem-Origa, H., & Jassoy, C. (2021). A Review of the Toxicity and Phytochemistry of Medicinal Plant Species Used by Herbalists in Treating People Living With HIV/AIDS in Uganda. *Frontiers in Pharmacology, 12*, 615147.

Araya, Y. N. (2007). Contribution of Trees for Oral Hygiene in East Africa. *Ethnobotanical Leaflets, 11*, 38-44.

Arifnuryadin, H. A., & Affandi, H. (1998). Studies on Natural Products of *Albizia* sp. *Biotropia, 11*, 1-8.

Aryal, S. (2018). High-Performance Liquid Chromatography (HPLC). Retrieved September 14th, 2020 from https://microbenotes.com/high-performance-liquid-chromatography-hplc/.

Aryal, S. (2020). Column Chromatography. Retrieved September 14th, 2020 from https://microbenotes.com/column-chromatography/#:~:text=Column%20chromatography%20is%20a%20technique,and%20are%20usually%20collected%20in.

Ashokkumar, R., & Ramaswamy, M. (2014). Phytochemical screening by FTIR spectroscopic analysis of leaf extracts of selected Indian medicinal plants. *International Journal of Current Microbiology and Applied Sciences, 3*, 395-406.

Ashurst, J. V., Truong, J., & Woodbury, B. (2020). *Salmonella typhi*. In: StatPearls. Treasure Island (FL): StatPearls Publishing.

Asiimwe, S., Kamatenesi-Mugisha, M., Namutebi, A., Borg-Karlsson, A.-K., & Musiimenta, P. (2013). Ethnobotanical study of nutri-medicinal plants used for the management of HIV/AIDS opportunistic ailments among the local communities of western Uganda. *Journal of Ethnopharmacology, 150*, 639–648.

Asiimwe, S., Namukobe, J., Byamukama, R., & Imalingat, B. (2021). Ethnobotanical survey of medicinal plant species used by communities around Mabira and Mpanga Central Forest Reserves, Uganda. *Tropical Medicine and Health, 49*, 52.

Atube, F., Malinga, G. M., Nyeko, M., Okello, D. M., Alarakol, S. P., & Okello-Uma, I. (2021). Determinants of smallholder farmers' adaptation strategies to the

effects of climate change: Evidence from northern Uganda. *Agriculture & Food Security, 10*, 6.

Aurélie, R. L., Judicael, R. L., Ranjana, R. H., Doll, R. D. A., Thomas, P., & Louis, J. V. (2020). Antimicrobial activity of *Albizia tulearensis*, an endemic Fabaceae from Madagascar. *World Journal of Biology Pharmacy and Health Sciences, 02*, 030–043.

Avoseh, O. N., Mtunzi, F. M., Ogunwande, I. A., Ascrizzi, R., & Guido, F. (2021). *Albizia lebbeck* and *Albizia zygia* volatile oils exhibit anti-nociceptive and anti-inflammatory properties in pain models. *Journal of Ethnopharmacology, 268*, 113676.

Ayatollahia, A. M., Ghanadian, M., Afsharypour, S., Abdella, O. M., Mirzai, M., & Askari, G. (2011). Pentacyclic Triterpenes in *Euphorbia microsciadia* with Their T-cell Proliferation Activity. *Iranian Journal of Pharmaceutical Research, 10*, 287-294.

Ayeleso, T. B., Matumba, M. G., & Mukwevho, E. (2017). Oleanolic Acid and Its Derivatives: Biological Activities and Therapeutic Potential in Chronic Diseases. *Molecules, 22*, 1915.

Bacher, A. (2002). Infrared Spectroscopy. Retrieved August 14th, 2021 from https://www.chem.ucla.edu/~bacher/spectrocopy/IR1.html.

Baek, M.-Y., Cho, J.-G., Lee, D.-Y., Ann, E.-M., Jeong, T.-S., & Baek, N.-I. (2010). Isolation of triterpenoids from the stem bark of *Albizia julibrissin* and their inhibition activity on ACAT-1 and ACAT-2. *Journal of the Korean Society for Applied Biological Chemistry, 53*, 310–315

Baghel, U. S., Singh, A., Singh, D., & Sinha, M. (2017). Application of Mass Spectroscopy in Pharmaceutical and Biomedical Analysis. In: *Spectroscopic Analyses* - Developments and Applications. *IntechOpen.* pp. 105-121.

Balan, V., Mihai, C. T., Cojocaru, F. D., Uritu, C. M., Dodi, G., Botezat, D., & Gardikiotis, I. (2019). Vibrational Spectroscopy Fingerprinting in Medicine: from Molecular to Clinical Practice. *Materials, 12*, 2884.

Bashyam, R., Thekkumalai, M., & Sivanandham, V. (2015). Evaluation of Phytoconstituents of *Bryonopsis laciniosa* fruit by UV-Visible Spectroscopy and FTIR analysis. *Pharmacognosy Journal, 7*, 165-170.

Bautista-Hernández, I., Aranda-Ledesma, N. E., Rojas, R., Tafolla-Arellano, J. C., & Martínez-Ávila, G. (2021). Antioxidant activity of polyphenolic compounds obtained from *Euphorbia antisyphilitica* by-products. *Heliyon, 7*, e06734.

Bentley, R., & Meganathan, R. (1982). Biosynthesis of vitamin K (menaquinone) in bacteria. *Microbiological Reviews, 46*, 241-280.

Beserra, F. P., Vieira, A. J., Gushiken, L. F. S., de Souza, E. O., Hussni, M. F., Hussni, C. A., . . . Pellizzon, C. H. (2019). Lupeol, a Dietary Triterpene, Enhances Wound Healing in Streptozotocin-Induced Hyperglycemic Rats with Modulatory Effects on Inflammation, Oxidative Stress, and Angiogenesis. *Oxidative Medicine and Cellular Longevity, 2019*, 3182627.

Borges, A. C. P., Piassão, J. F. G., Albani, S. M., Albertoni, E. F., Martins, M. C., Cansian, R. L., . . . Mielniczki-Pereira, A. A. (2022). Multiple metals and agricultural use affects oxidative stress biomarkers in freshwater aegla crabs. *Brazilian Journal of Biology, 82*, e230147.

Bossard, E. (1993). Angolan medicinal plants used also as piscicides and/or soaps. *Journal of Ethnopharmacology, 40*, 1-19.

Bouderias, S., Teszlák, P., Jakab, G., & Kőrösi, L. (2020). Age- and season-dependent pattern of flavonol glycosides in Cabernet Sauvignon grapevine leaves. *Scientific Reports, 10*, 14241.

Branco, A., Pinto, A. C., & Braz-Filho, R. (2004). Chemical constituents of *Vellozia graminifolia* (Velloziaceae). *Anais da Academia Brasileira de Ciências, 76*, 505–518.

Breslin, A. (2019). The Chemical Composition of Green Plants. Retrieved September 10th, 2020 from https://sciencing.com/chemical-composition-green-plants-8336363.html.

Budzikiewicz, H., Wilson, J. M., & Djerassi, C. (1963). Mass spectrometry in structural and stereochemical problems. XXXII. Pentacylic triterpenes. *Journal of American Chemical Society, 85*, 3688-3699.

Bugeja, M. L. (2012). Comparison of gas chromatography-mass spectrometry and liquid chromatography-mass spectrometry for discrimination of *Salvia divinorum* from related *Salvia* species using chemometric procedures. *MSc Thesis, Michigan State University, East Lansing, Michigan*.

Bunalema, L., Obakiro, S., Tabuti, J. R. S., & Waako, P. (2014). Knowledge on plants used traditionally in the treatment of tuberculosis in Uganda. *Journal of Ethnopharmacology, 151*, 999-1004.

Byamukama, R., Ganza, B., Namukobe, J., Heydenreich, M., & Kiremire, B. T. (2015). Bioactive compounds in the stem bark of *Albizia coriaria* (Welw. ex Oliver). *International Journal of Biological and Chemical Sciences, 9*, 1013-1024.

Cansev, A., Gulen, H., Celik, G., & Eris, A. (2012). Alterations in total phenolic content and antioxidant capacity in response to low temperatures in olive (*Olea europaea* L. "Gemlik"). *Plant Archives, 12*, 489-494.

Cao, S., Norris, A., Miller, J. S., Ratovoson, F., Razafitsalama, J., Andriantsiferana, R., . . . Kingston, D. G. (2007). Cytotoxic triterpenoid saponins of *Albizia*

gummifera from the Madagascar rain forest. *Journal of Natural Products Research, 70*, 361-366.

Carvalho, T. C. d., Polizeli, A. M., Turatti, I. C. C., Severiano, M. E., Carvalho, C. E. d., Ambrósio, S. R., . . . Furtado, N. A. J. C. (2010). Screening of Filamentous Fungi to Identify Biocatalysts for Lupeol Biotransformation. *Molecules, 15*, 6140-6151.

CDC. (2003). Centers for Disease Control and Prevention. Outbreaks of community-associated methicillin-resistant *Staphylococcus aureus* skin infections-Los Angeles County, California, 2002-2003. *Morbidity and mortality weekly report, 52*, 88.

CDC. (2020). Centers for Disease Control and Prevention: *E. coli* and Food Safety. Retrieved on September 15th, 2020 from https://www.cdc.gov/foodsafety/communication/ecoli-and-food-safety.html?CDC_AA_refVal=https%3A%2F%2Fwww.cdc.gov%2Ffeatures%2Fecoliinfection%2Findex.html.

Chandra, S., Khan, S., Avula, B., Lata, H., Yang, M. H., ElSohly, M. A., & Khan, I. A. (2014). Assessment of Total Phenolic and Flavonoid Content, Antioxidant Properties, and Yield of Aeroponically and Conventionally Grown Leafy Vegetables and Fruit Crops: A Comparative Study. *Evidence-Based Complementary and Alternative Medicine, 2014*, 253875.

Chaves, N., Santiago, A., & Alías, J. C. (2020). Quantification of the Antioxidant Activity of Plant Extracts: Analysis of Sensitivity and Hierarchization Based on the Method Used. *Antioxidants, 9*, 76.

Chemat, F., Abert Vian, M., Ravi, H. K., Khadhraoui, B., Hilali, S., Perino, S., & Tixier, A. F. (2019). Review of Alternative Solvents for Green Extraction of Food and Natural Products: Panorama, Principles, Applications and Prospects. *Molecules, 24*, 3007.

Chen, Q., Zhang, Y., Zhang, W., & Chen, Z. (2011). Identification and quantification of oleanolic acid and ursolic acid in Chinese herbs by liquid chromatography-ion trap mass spectrometry. *Biomedical chromatography, 25*, 1381–1388.

Chepkorir, R., Matasyoh, J. C., & Wagara, I. N. (2018). Two withanolides from *Withania somnifera* (solanaceae) and activity of methanolic extracts against fungal and bacterial pathogens that affects food crops. *African Journal of Food Science, 12*, 115-125.

Chepkwony, S. C., Dumarçay, S., Chapuis, H., Kiprop, A., Gerardin, P., & Gerardin-Charbonnier, C. (2020). Geographic and intraspecific variability of mesquitol amounts in *Prosopis juliflora* trees from Kenya. *European Journal of Wood and Wood Products, 78*, 801–809.

Choi, S. Z., Yang, M. C., Choi, S. U., & Lee, K. R. (2006). Cytotoxic terpenes and lignans from the roots of *Ainsliaea acerifolia*. *Archives of Pharmacal Research, 29*, 203–208.

Cichewicz, R. H., & Kouzi, S. A. (2004). Chemistry, biological activity, and chemotherapeutic potential of betulinic acid for the prevention and treatment of cancer and HIV infection. *Medicinal Research Reviews, 24*, 90–114.

Cieśla, Ł., & Moaddel, R. (2016). Comparison of analytical techniques for the identification of bioactive compounds from natural products. *Natural product reports, 33*, 1131-1145.

Cîntă-Pînzaru, S., Dehelean, C. A., Soica, C., Culea, M., & Borcan, F. (2012). Evaluation and differentiation of the Betulaceae birch bark species and their bioactive triterpene content using analytical FT-vibrational spectroscopy and GC-MS. *Chemistry Central Journal, 6*, 67.

CLSI. (2017). Performance Standards for Antimicrobial Susceptibility Testing. 27th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute.

Cordeiro, P. J. M., Vilegas, J. H. Y., & Lanças, F. M. (1999). HRGC-MS Analysis of Terpenoids from *Maytenus ilicifolia* and *Maytenus aquifolium* ("Espinheira Santa"). *Journal of Brazilian Chemical Society, 10*, 523-526.

Cotter, D. R., Sabherwala, S., & Schuber, K. O. (2020). Proteomics for diagnostic and therapeutic blood biomarker discovery in schizophrenia and other psychotic disorders. *Personalized Psychiatry, 25*, 307-317.

Crump, J. A., Sjölund-Karlsson, M., Gordon, M. A., & Parry, C. M. (2015). Epidemiology, Clinical Presentation, Laboratory Diagnosis, Antimicrobial Resistance, and Antimicrobial Management of Invasive *Salmonella* Infections. *Clinical Microbiology Reviews, 28*, 901-937.

Çulhaoğlu, B., Hatipoğlu, S. D., Dönmez, A. A., & Topçu, G. (2015). Antioxidant and anticholinesterase activities of lupane triterpenoids and other constituents of *Salvia trichoclada*. *Medicinal Chemistry Research, 24*, 3831–3837.

Cushnie, T. P., & Lamb, A. J. (2011). Recent advances in understanding the antibacterial properties of flavonoids. *International Journal of Antimicrobial Agents, 38*, 99-107.

D'Souza, L., Devi, P., Shridhar, D. M., & Naik, C. G. (2008). Use of Fourier Transform Infrared (FTIR) spectroscopy to study cadmium-induced changes in *Padina tetrastromatica* (Hauck). *Analytical Chemistry Insights, 3*, 135–143.

Daglia, M. (2012). Polyphenols as antimicrobial agents. *Current Opinion in Biotechnology, 23*, 174–181.

Das, A. J., Khawas, P., Miyaji, T., & Deka, S. C. (2016). Phytochemical Constituents, Attenuated Total Reflectance Fourier Transform Infrared Analysis and Antimicrobial Activity of Four Plant Leaves Used for Preparing Rice Beer in Assam, India. *International Journal of Food Properties, 19*, 2087-2101.

Dasgupta, A., & Steinagel, G. (1997). Gas chromatographic-mass spectrometric identification and quantitation of benzyl alcohol from human serum and postmortem blood after derivatization with 4-carbethoxy hexafluorobutyryl chloride: a novel derivative. *Journal of Forensic Sciences, 42*, 697–700.

de Araújo, R. F. F., Martins, D. B. G., & Borba, M. A. C. S. M. (2016). Oxidative Stress and Disease. In: A Master Regulator of Oxidative Stress - The Transcription Factor Nrf2, IntechOpen, pp. 185-199.

de Martel, C., Georges, D., Bray, F., Ferlay, J., & Clifford, G. M. (2020). Global burden of cancer attributable to infections in 2018: a worldwide incidence analysis. *Lancet Global Health, 8*, e180–190.

Deena, T., Jebaseelan, E. J. S., & Roopan, S. M. (2019). Green Synthesis, Characterization and Applications of Nanoparticles. *Micro and Nano Technologies, 12*, 303-319.

Dharani, N., Yenesew, A., Aynekulu, E., Tuei, B., & Jamnadass, R. (2015). Traditional ethnoveterinary medicine in East Africa: a manual on the use of medicinal plants. *Dawson IK ed. The World Agroforestry Centre (ICRAF), Nairobi, Kenya*, 199 pages.

Dhivya, S. M., & Kalaichelvi, K. (2017). UV-Vis spectroscopic and FTIR analysis of *Sarcostemma brevistigma*, wight. and arn. *International Journal of Herbal Medicine, 9*, 46–49.

Domínguez, A. V., Algaba, R. A., Canturri, A. M., Villodres, A. R., & Smani, Y. (2020). Antibacterial Activity of Colloidal Silver against Gram-Negative and Gram-Positive Bacteria. *Antibiotics, 9*, 36.

Donkor, S., Larbie, C., Komlaga, G., & Emikpe, B. O. (2019). Phytochemical, Antimicrobial, and Antioxidant Profiles of *Duranta erecta* L. Parts. *Biochemistry Research International, 2019*, 8731595.

Doshi, G. M., Nalawade, V. V., Mukadam, A. S., Chaskar, P. K., Zine, S. P., Somani, R. R., & Une, H. D. (2015). Structural elucidation of chemical constituents from Benincasa hispida seeds and Carissa congesta roots by gas chromatography: Mass spectroscopy. *Pharmacognosy Research, 7*, 282–293.

Dunnivant, & Ginsbach. (2008). Fragmentation of Alcohols. Retrieved on 20th July, 2021 from http://people.whitman.edu/~dunnivfm/C_MS_Ebook/CH6/6_10.html.

Dziurka, M., Kubica, P., Kwiecień, I., Biesaga-Kościelniak, J., Ekiert, H., Abdelmohsen, S. A. M., . . . Szopa, A. (2021). *In vitro* Cultures of Some

Medicinal Plant Species (*Cistus* × incanus, *Verbena officinalis*, *Scutellaria lateriflora*, and *Scutellaria baicalensis*) as a Rich Potential Source of Antioxidants—Evaluation by CUPRAC and QUENCHER-CUPRAC Assays. *Plants, 10,* 454.

Edreva, A. (2005). Generation and scavenging of reactive oxygen species in chloroplasts: a submolecular approach. *Agriculture, Ecosystems & Environment, 106*,, 119–133.

El-Hawary, S., El-Fouly, K., Sokkar, N. M., & Talaat, Z. (2011). A Phytochemical Profile of *Albizia lebbeck* (L.) Benth. Cultivated in Egypt. *Asian Journal of Biochemistry, 6*, 122-141.

El Sayed, A. M. (2016). Leaves of *Schinus polygamous* (Cav.) Cabrera (Anacardiaceae) are a potential source of hepatoprotective and antioxidant phytochemicals. *Journal of Medicinal Plants Research, 10*, 223-231.

Engels, C., Schieber, A., & Gänzle, M. G. (2011). Inhibitory spectra and modes of antimicrobial action of gallotannins from mango kernels (*Mangifera indica* L.). *Applied and Environmental Microbiology, 77*, 2215–2223.

Erba, M., & Kliebenstein, D. J. (2020). Plant secondary metabolites as defenses, regulators, and primary metabolites: the blurred functional trichotomy. *Plant Physiology Preview*, 1-43.

Eunyoung, K., Noh, K., Lee, S. J., Shin, B., Hwang, J. T., Lee, S. W., . . . Kang, W. (2016). Simultaneous determination of 3-O-acetyloleanolic acid and oleanolic acid in rat plasma using liquid chromatography coupled to tandem mass spectrometry. *Journal of pharmaceutical and biomedical analysis, 118*, 96–100.

Fei, G., Lujia, H., & Xian, L. (2017). Vibration spectroscopic technique for species identification based on lipid characteristics. *International Journal of Agricultural and Biological Engineering, 10*, 255–268.

Felhi, S., Daoud, A., Hajlaoui, H., Mnafgui, K., Gharsallah, N., & Kadri, A. (2017). Solvent extraction effects on phytochemical constituents profiles, antioxidant and antimicrobial activities and functional group analysis of *Ecballium elaterium* seeds and peels fruits. *Journal of Chemical and Pharmaceutical Sciences, 37*, 483-492.

Fernie, A. R., & Pichersky, E. (2015). Focus Issue on Metabolism: Metabolites, Metabolites Everywhere. *Plant Physiology, 169*, 1421-1423.

Ferreira, I. C. C., Aguiar, E. M. G., Silva, A. T. F., Santos, L. L. D., Cardoso-Sousa, L., Araújo, T. G., . . . Maia, Y. C. P. (2020). Attenuated Total Reflection-Fourier Transform Infrared (ATR-FTIR) Spectroscopy Analysis of Saliva for Breast Cancer Diagnosis. *Journal of Oncology, 2020*, 4343590.

Fotso, G. W., Kamga, J., Ngameni, B., Uesugi, S., Ohno, M., Kimura, K. I., . . . Ngadjui, B. T. (2017). Secondary metabolites with antiproliferative effects from *Albizia*

glaberrima var glabrescens Oliv. (Mimosoideae). *Natural Product Research, 31*, 1981-1198.

Fraga-Corral, M., García-Oliveira, P., Pereira, A. G., Lourenço-Lopes, C., Jimenez-Lopez, C., Prieto, M. A., & Simal-Gandara, J. (2020). Technological Application of Tannin-Based Extracts. *Molecules, 25*, 614.

Galbraith, M., Miller, C., Rawson, J., Ritchie, E., Shannon, J., & Taylor, W. (1965). Moretenol and other triterpenes from *Ficus macrophylla* Desf. *Australian Journal of Chemistry, 18*, 226.

Gan, J., Feng, Y., He, Z., Li, X., & Zhang, H. (2017). Correlations between Antioxidant Activity and Alkaloids and Phenols of Maca (*Lepidium meyenii*). *Journal of Food Quality, 2017*, 3185945.

Ganza, B. (2014). Isolation and Characterization of the bioactive compounds in the stem bark of *Albizia coriaria.* MSc Thesis, Makerere University, Kampala, Uganda.

Gao, D., Li, Q., Li, Y., Liu, Z., Fan, Y., Liu, Z., . . . Han, Z. (2009). Antidiabetic and antioxidant effects of oleanolic acid from *Ligustrum lucidum* Ait in alloxan-induced diabetic rats. *Phytotherapy Research, 23*, 1257–1262.

García-Sánchez, A., Miranda-Díaz, A. G., & Cardona-Muñoz, E. G. (2020). The Role of Oxidative Stress in Physiopathology and Pharmacological Treatment with Pro- and Antioxidant Properties in Chronic Diseases. *Oxidative Medicine and Cellular Longevity, 2020*, 2082145.

Geissler, P. W., Harris, S. A., Prince, R. J., Olsen, A., Odhiambo, R. A., Oketch-Rabah, H., . . . Mølgaard, P. (2002). Medicinal plants used by Luo mothers and children in Bondo district, Kenya. *Journal of Ethnopharmacology, 83*, 39-54.

Ghavam, M. (2021). Relationships of irrigation water and soil physical and chemical characteristics with yield, chemical composition and antimicrobial activity of Damask rose essential oil. *PLoS ONE, 16*, e0249363.

Gómez, H. G. (2021). Determinación De Estructuras Orgánicas (Organic Spectroscopy): IR Spectroscopy. Retrieved July 5th, 2021 from http://www.upv.es/herme/files/4a-ir-spectroscopy.pdf.

Gradé, J. T., Tabuti, J. R. S., & Van Damme, P. (2009). Ethnoveterinary knowledge in pastoral Karamoja, Uganda. *Journal of Ethnopharmacology, 122*, 273-293.

Greenwood, D., Slack, B. C., & Peutherer, F. J. (2002). Medical Microbiology: A Guide to Microbial Infections: Pathogensis, Immunity, Laboratory Diagnosis and Control. 16th Edn. *Churchill livingstone*, London, UK, pp. 49-55.

Gul, R., Jan, S. U., Faridullah, S., Sherani, S., & Jahan, N. (2017). Preliminary Phytochemical Screening, Quantitative Analysis of Alkaloids, and Antioxidant

Activity of Crude Plant Extracts from Ephedra intermedia Indigenous to Balochistan. *The Scientific World Journal, 2017,* 5873648.

Gumisiriza, H., Birungi, G., Olet, E. A., & Sesaazi, C. D. (2019). Medicinal plant species used by local communities around Queen Elizabeth National Park, Maramagambo Central Forest Reserve and Ihimbo Central Forest Reserve, South western Uganda. *Journal of Ethnopharmacology, 239*, 111926.

Gutierrez-Lugo, M. T., Deschamps, J. D., Holman, T. R., Suarez, E., & Timmermann, B. N. (2004). Lipoxygenase Inhibition by Anadanthoflavone, a New Flavonoid from the Aerial Parts of *Anadenanthera colubrina Planta Medica, 70*, 263–265.

Habib, R., Mohyuddin, A., Khan, Z., & Mahmood, T. (2017). Analysis of Non-polar Chemical Profile of *Melia azedarach* L. *Scientific Inquiry and Review, 1*, 49-54.

Hashem, H. (2008). Principles of mass spectrometric analysis. Retrieved August 9th, 2021 from http://pharmgrads.blogspot.com/2008/04/lecture3.html.

Hayyan, M., Hashim, M. A., & AlNashef, I. M. (2016). Superoxide Ion: Generation and Chemical Implications. *Chemical Reviews, 116*, 3029–3085.

He, Y., Wang, Q., Ye, Y., Liu, Z., & Sun, H. (2020). The ethnopharmacology, phytochemistry, pharmacology and toxicology of genus *Albizia:* A review. *Journal of Ethnopharmacology, 257*, 112677.

Heinzen, H., de Vries, J. X., Moyna, P., Remberg, G., Martinez, R., & Tietze, L. F. (1996). Mass Spectrometry of Labelled Triterpenoids: Thermospray and Electron Impact Ionization Analysis. *Phytochemical Analysis, 7*, 237-244.

Holmes Jr, L., Rios, J., Berice, B., Benson, J., Bafford, N., Parson, K., & Halloran, D. (2021). Predictive Effect of *Helicobacter pylori* in Gastric Carcinoma Development: Systematic Review and Quantitative Evidence Synthesis. *Medicines, 8*, 1.

Holmes, K. K., Bertozzi, S., Bloom, B. R., Jha, P., Gelband, H., DeMaria, L. M., & Horton, S. (2017). Major Infectious Diseases: Key Messages from Disease Control Priorities. *Holmes KK, et al. The International Bank for Reconstruction and Development / The World Bank, Washington (DC), USA.*

Holopainen, J. K., Virjamo, V., Ghimire, R. P., Blande, J. D., Julkunen-Tiitto, R., & Kivimäenpää, M. (2018). Climate Change Effects on Secondary Compounds of Forest Trees in the Northern Hemisphere. *Frontiers in Plant Science, 9*, 1445.

Horiuchi, K., Shiota, S., Hatano, T., Yoshida, T., Kuroda, T., & Tsuchiya, T. (2007). Antimicrobial activity of oleanolic acid from *Salvia officinalis* and related compounds on vancomycin-resistant enterococci (VRE). *Biological & Pharmaceutical Bulletin, 30*, 1147–1149.

Hu, X., Shen, Y., Yang, S., Lei, W., Luo, C., Hou, Y., & Bai, G. (2018). Metabolite identification of ursolic acid in mouse plasma and urine after oral administration by ultrahigh performance liquid chromatography/quadrupole time-of-flight mass spectrometry. *RSC Advances, 8*, 6532.

Hussein, A. O., Mohammed, G. J., Hadi, M. Y., & Hameed, I. H. (2016a). Phytochemical screening of methanolic dried galls extract of *Quercus infectoria* using gas chromatography-mass spectrometry (GC-MS) and Fourier transform-infrared (FT-IR). *Journal of Pharmacognosy and Phytotherapy, 8*, 49-59.

Hussein, H. J., Hadi, M. Y., & Hameed, I. H. (2016b). Study of chemical composition of *Foeniculum vulgare* using Fourier transform infrared spectrophotometer and gas chromatography - mass spectrometry. *Journal of Pharmacognosy and Phytotherapy, 8*, 60-89.

Hyde, M. A., Wursten, B. T., Ballings, P., & Coates Palgrave, M. (2021). Flora of Zimbabwe: Cultivated Plants: Species information: *Albizia coriaria*. Retrieved June 11th, 2021 from https://www.zimbabweflora.co.zw/cult/species.php?species_id=184660.

ICRAF. (1992). A Selection of Useful Trees and Shrubs for Kenya. International Centre for Research in Agroforestry. Nairobi: Kenya.

India, J. (2015). Efficacy of some medicinal plants used in various parts of Kenya in treating selected bacterial and fungal pathogens. MSc Thesis. Kenyatta University, Nairobi, Kenya.

International Union of Pure and Applied Chemistry. (2019). Compendium of Chemical Terminology, 2nd ed. (the "Gold Book"). Compiled by A. D. McNaught and A. Wilkinson. Blackwell Scientific Publications, Oxford (1997). https://doi.org/10.1351/goldbook.

Invernizzi, C., Rovetta, T., Licchelli, M., & Malagodi, M. (2018). Mid and Near-Infrared Reflection Spectral Database of Natural Organic Materials in the Cultural Heritage Field. *International Journal of Analytical Chemistry, 2018*, 7823248.

Isah, T. (2019). Stress and defense responses in plant secondary metabolites production. *Biological Research, 52*, 39

Jain, P. K., Soni, A., Jain, P., & Jeetendra, B. (2016). Phytochemical analysis of *Mentha spicata* plant extract using UV-VIS, FTIR and GC/MS technique. *Journal of Chemical and Pharmaceutical Research, 8*, 1-6.

Janakiraman, N., & Johnson, M. (2015). Functional Groups of Tree Ferns (Cyathea) Using FT-IR: Chemotaxonomic Implications. *Romanian Journal of Biophysics, 25*, 131-141.

Janani, L., Lukyambuzi, H., & Kodi, P. (2014). Mordanting Methods for Dyeing Cotton Fabrics with Dye from *Albizia coriaria* Plant Species. *International Journal of Scientific and Research Publications, 4*, 1-5.

Jeong, S. Y., Zhao, B. T., Kim, Y. H., Min, B. S., & Woo, M. H. (2013). Cytotoxic and antioxidant compounds isolated from the cork of *Euonymus alatus* Sieb. *Natural Product Sciences, 19,* 366 - 371.

Jeruto, P., Mutai, C., Ouma, G., Lukhoba, C., Nyamaka, R. L., & Manani, S. D. (2010). Ethnobotanical survey and propagation of some endangered medicinal plants from south Nandi district of Kenya. *Journal of Animal & Plant Sciences, 8,* 1016-1043.

Jesus, J. A., Lago, J. H., Laurenti, M. D., Yamamoto, E. S., & Passero, L. F. (2015). Antimicrobial activity of oleanolic and ursolic acids: an update. *Evidence-Based Complementary and Alternative Medicine, 2015,* 620472.

Johns, T., Faubert, G. M., Kokwaro, J. O., Mahunnah, R. L. A., & Kimanani, E. K. (1995). Anti-giardial activity of gastrointestinal remedies of the Luo of East Africa. *Journal of Ethnopharmacology, 46*, 17-23.

Johns, T., Kokwaro, J. O., & Kimanani, E. K. (1990). Herbal Remedies of the Luo of Siaya District, Kenya: Establishing Quantitative Criteria for Consensus. *Economie Botany, 44*, 369-381.

Joshi, H., Saxena, G. K., Singh, V., Arya, E., & Singh, R. P. (2013). Phytochemical Investigation, Isolation and Characterization of Betulin from Bark of *Betula utilis*. *Journal of Pharmacognosy and Phytochemistry*, *8192*, 266–285.

Jurić, S., Sopko Stracenski, K., Król-Kilińska, Ż., Žutić, I., Uher, S. F., Đermić, E., . . . Vinceković, M. (2020). The enhancement of plant secondary metabolites content in Lactuca sativa L. by encapsulated bioactive agents. *Scientific Reports, 10*, 3737.

Kaba, J. S., Otu-Nyanteh, A., & Abunyewa, A. A. (2020). The role of shade trees in influencing farmers' adoption of cocoa agroforestry systems: Insight from semi-deciduous rain forest agroecological zone of Ghana. *NJAS-Wageningen Journal of Life Sciences, 92*, 100332.

Kaczmarek, B. (2020). Tannic Acid with Antiviral and Antibacterial Activity as A Promising Component of Biomaterials—A Minireview. *Materials, 13*, 3224.

Kafkas, N. E., Kosar, M., Öz, A. T., & Mitchell, A. E. (2018). Advanced Analytical Methods for Phenolics in Fruits. *Journal of Food Quality, 2018*, 3836064.

Kajobe, R., Kato, E. K., Otim, S. A., Kasangaki, P., & Abila, P. P. (2016). The status of honeybee pests in Uganda. *Bulletin of Animal Health and Production in Africa*, 105-117.

Kalaichelvi, K., & Dhivya, S. M. (2017). Screening of phytoconstituents, UV-VIS Spectrum and FTIR analysis of *Micrococca mercurialis* (L.) Benth. *International Journal of Herbal Medicine, 5*, 40-44.

Kama-Kama, F., Midiwo, J., Nganga, J., Maina, N., Schiek, E., Omosa, L. K., . . . Naessens, J. (2016). Selected ethno-medicinal plants from Kenya with *in vitro* activity against major African livestock pathogens belonging to the "*Mycoplasma mycoides* cluster". *Journal of Ethnopharmacology, 192*, 524-534.

Karan, B. N., Maity, T. K., Pal, B. C., Singha, T., & Jana, S. (2019). Betulinic Acid, the first lupane-type triterpenoid isolated via bioactivity-guided fractionation, and identified by spectroscopic analysis from leaves of *Nyctanthes arbortristis*: its potential biological activities *in vitro* assays. *Natural Product Research, 33*, 3287-3292.

Karpagasundari, C., & Kulothungan, S. (2014). Analysis of bioactive compounds in *Physalis minima* leaves using GCMS, HPLC, UV-Vis and FTIR techniques. *Journal of Pharmacognosy and Phytochemistry 3*, 196-201.

Katende, A. B., Birnie, A., & Tengnas, B. (1995). Useful Trees and Shrubs for Uganda: Identification, Propagation and Management for Agricultural and Pastoral Communities. *Technical Handbook No. 10. Regional Soil Conservation Unit, Nairobi, Kenya*.

Katuura, E., Omujal, F., Tumusiime, R. H., Nabukalu, D., & Byamukama, R. (2016). Documentation of indigenous knowledge on medicinal plants used to manage common influenza and related symptoms in Luwero district, central Uganda. *Journal of Medicinal Plants Research, 10*, 705-716.

Kavitha, A., Mary Kensa, V., Neelamegam, R., & Salom, G. T. V. (2019). Fourier Transform Infrared Spectroscopy (FT-IR) Analysis Of Ethanolic Extract Of *Rivina humilis* L. (Mixture). *Pramana Research Journal, 9*, 585-591.

Kenkel, J. (2003). Analytical Chemistry for Technicians. Third Edition, Boca Raton, Florida. Corporate Ltd. pp. 310-367.

Khiya, Z., Oualcadi, Y., Gamar, A., Berrekhis, F., Zair, T., & EL Hilali, F. (2021). Correlation of Total Polyphenolic Content with Antioxidant Activity of Hydromethanolic Extract and Their Fractions of the *Salvia officinalis* Leaves from Different Regions of Morocco. *Journal of Chemistry, 2021*, 8585313.

Khobragade, P. S., Hansora, D. P., Naik, J. B., Njuguna, J., & Mishra, S. (2017). Physico-mechanical properties of nano-polystyrene-decorated graphene oxide–epoxy composites. *Polymer International, 66*, 1402-1409.

Kigenyi, J. (2016). Coping with resource extinction: the case of medicinal plants in Kawete village, Iganga district, Uganda. *Culture and Environment in Africa Series* .Vol. 9, The Cologne African Studies Centre, Cologne, Germany.

Kigondu, E. V., Rukunga, G. M., Kerik, J. M., Tonui, W. K., Gathirwa, J. M., Kirira, P. G., . . . Ndiege, I. O. (2009). Anti-parasitic activity and cytotoxicity of selected Medicinal Plants from Kenya. *Journal of Ethnopharmacology, 123*, 505-509

Kim, J. S., & Lee, J. H. (2020). Correlation between Solid Content and Antioxidant Activities in Umbelliferae Salad Plants. *Preventive Nutrition and Food Science, 25*, 84–92.

Kiran, G. S., Jackson, S. A., Priyadharsini, S., Dobson, A., & Selvin, J. (2017). Synthesis of Nm-PHB (nanomelanin-polyhydroxy butyrate) nanocomposite film and its protective effect against biofilm-forming multi drug resistant *Staphylococcus aureus*. *Scientific Reports, 7*, 9167.

Kiria, M. J. (2018). Antimicrobial Activity and Constituents of the Root Bark of *Loncocarpus eriocalyx*. *MSc Thesis, University of Nairobi, Nairobi, Kenya*.

Kokwaro, J. O. (2009). Medicinal plants of East Africa, East African Literature Bureau, Nairobi, Kenya

Kokwaro, J. O. (1993). Medicinal plants of East Africa, 2nd Edn. East African Literature Bureau, Nairobi, Kenya. 416 pages.

Koma, O. S., & Sani, I. M. (2014). Betulinic Acid from Antimicrobial Root Wood Extract of *Dalbergia saxatilis* Hook f.(Fabaceae). *European Journal of Medicinal Plants, 4*, 686-694.

Krige, A. (2007). *Albizia adianthifolia* (Schumach.) W.Wight, National Herbarium, Pretoria. Retrieved June 4th, 2021 from http://www.plantzafrica.com/plantab/albizadian.html.

Król, A., Amarowicz, R., & Weidner, S. (2015). The effects of cold stress on the phenolic compounds and antioxidant capacity of grapevine (*Vitis vinifera* L.) leaves. *Journal of plant physiology, 189*, 97–104.

Kurek, J. (2019). Introductory Chapter: Alkaloids-Their Importance in Nature and for Human Life. *IntechOpen*. pp. 1-7.

Kuse, M., Yanagi, M., Tanaka, E., Tani, N., & Nishikawa, T. (2010). Identification of a Fluorescent Compound in the Cuticle of the Train Millipede *Parafontaria laminata armigera*. *Bioscience, Biotechnology, and Biochemistry, 74*, 2307-2309.

Kyazike, E. (2021). Exploring the preference for indigenous medicinal plant medicine in Buliisa District, Western Uganda. *Inkanyiso, Journal of Humanities & Social Sciences, 13*, 77-104.

Lacroix, D., Prado, S., Kamoga, D., Kasenene, J., Namukobe, J., Krief, S., & Brunois, F. (2011). Antiplasmodial and cytotoxic activities of medicinal plants

traditionally used in the village of Kiohima, Uganda. *Journal of Ethnopharmacology, 133*, 850–855.

Lamorde, M., Tabuti, J. R. S., Obua, C., Kukunda-Byobona, C., Lanyero, H., Byakika-Kibwika, P., . . . Merry, C. (2010). Medicinal plants used by traditional medicine practitioners for the treatment of HIV/AIDS and related conditions in Uganda. *Journal of Ethnopharmacology, 130*, 43-53.

Langat, P. K. (2013). Ethnobotanical study, toxicity and phytochemical screening of selected medicinal plants of Tinderet district, Nandi county, Kenya. *MSc Thesis, University of Nairobi, Nairobi, Kenya*.

Lawan, S. A., Saleh, A., Sani, B. G., Fa'iza, A. M., & Sadiya, A. Z. (2017). Preliminary Phytochemical Constituents and Phytotoxic Effect of *Albizia lebbeck* (L.) Benth on *Sorghum bicolor*. *Bayero Journal of Pure and Applied Sciences, 10*, 405-408.

Lee, D. Y., Jung, L., Lyu, H. N., Jeong, T. S., Lee, Y. H., & Baek, N. I. (2009). Triterpenoids from the fruits of *Cornus kousa* burg. As human acylcoa: Cholesterol acyltransferase inhibitors. *Food Science and Biotechnology, 18*, 223-227.

Leiderer. (1982). La médécine traditionnelle chez les Bekpak (Bafia) du Cameroun. *St. Augustin, Deutschland: Haus Volker und kulturen*.

Leite, S. P., Adami, T. B., Bjerk, T. R., dos Reis Souza, M. R., Cardoso, C. A. L., Krause, L. C., & Caramão, E. B. (2020). Ultrasonic assisted extraction of bioactive compounds from different parts of *Hancornia speciosa* Gomes. *Journal of Medicinal Plants Research, 14*, 300-308.

Leung, Y. L. (2014). *Staphylococcus aureus.* Reference Module in Biomedical Sciences. In: *Encyclopedia of Toxicology*, (3rd Edn), pp. 379-380. 379-380.

Lewis, G. P., & Arce, R. L. (2005). Tribe Ingeae. In: Legumes of the World. Lewis G., Schrire B., Macinker, B. & Lock M. Eds. *Royal Botanic Gardens, Kew, UK*. pp. 1-577.

Liguori, I., Russo, G., Curcio, F., Bulli, G., Aran, L., Della-Morte, D., . . . Abete, P. (2018). Oxidative stress, aging, and diseases. *Clinical Interventions in Aging, 13*, 757–772.

Lim, J. Y., Yoon, J., & Hovde, C. J. (2010). A brief overview of *Escherichia coli* O157:H7 and its plasmid O157. *Journal of Microbiology and Biotechnology, 20*, 5-14.

Lindblom, T. (2014). Reactions in the System Nitro-cellulose/ Diphenylamine with Special Reference to the Formation of a Stabilizing Product Bonded to Nitro-cellulose. Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology

Linus Pauling Institute. (2020). Flavonoids. Retrieved September 10th, 2020 from https://lpi.oregonstate.edu/mic/dietary-factors/phytochemicals/flavonoids.

Louppe, D., Oteng-Amoako, A. A., & Brink, M. (2008). Plant Resources of Tropical Africa 7: Timbers 1, PROTA Foundation, Backhuys Publishers, Leiden, the Netherlands.

Lucchini, J. J., Corre, J., & Cremieux, A. (1990). Antibacterial activity of phenolic compounds and aromatic alcohols. *Research in microbiology, 141*, 499–510.

Luvonga, M. W. (2007). Evaluation of antimicrobial activity of some plants used by traditional healers for treatment of microbial infections in Kakamega district: Kenya. MSc Thesis, Kenyatta University, Nairobi, Kenya.

Machumi, F., Innocent, E., & Yand, P. (2021). Seasonal impacts on antifungal activity and chemical composition of extracts from medicinal plants *Turraea holstii* and *Clausena anisata*. *Journal of Medicinal Plants Research, 15*, 133-139.

Makoye, P. M., John Daniel, I., Mbunde, M. N. M., Nelson E. , Sempombe, J., & Mugoyela, V. (2020). Phytochemical Screening, Antibacterial Activity and Bioautography of *Sorindeia madagascariensis*, *Mucuna stans*, and *Albizia harveyi*. *Journal of Diseases and Medicinal Plants, 6*, 65-71.

Malik, S. K., Ahmed, M., & Khan, F. (2018). Identification of novel anticancer terpenoids from *Prosopis juliflora* (Sw) DC (Leguminosae) pods. *Tropical Journal of Pharmaceutical Research, 17,* 661-668.

Manner, S., Skogman, M., Goeres, D., Vuorela, P., & Fallarero, A. (2013). Systematic exploration of natural and synthetic flavonoids for the inhibition of *Staphylococcus aureus* biofilms. *International Journal of Molecular Sciences, 14*, 19434-19451.

María, R., Shirley, M., Xavier, C., Jaime, S., Villalta, D., Siguencia, R., & Duche, J. (2018). Preliminary phytochemical screening, total phenolic content and antibacterial activity of thirteen native species from Guayas province Ecuador. *Journal of King Saud University – Science, 30*, 500–505.

Mbarara District Local Government. (2021). Mbarara district. Facts & figures. Retrieved May 10th, 2021 from https://www.mbarara.go.ug/district/facts-and-figures#:~:text=Climate%20and%20landscape,range%20of%2080%2D90%25.

Md Salim, R., Asik, J., & Sarjadi, M. S. (2021). Chemical functional groups of extractives, cellulose and lignin extracted from native *Leucaena leucocephala* bark. *Wood Science and Technology, 55*, 295–313

Mengesha, H., Birrie, H., & Gundersen, G. (1997). The molluscicidal effect of the bark of "Bitza" tree: a local discovery towards the control of schistosomiasis in the Abay river valley of Western Ethiopia. *Ethiopian Journal of Health Development, 11*, 89-92.

Minai, J. O. (2015). Assessing the spatial variability of soils in Uganda, MSc Dissertation, Purdue University, West Lafayette, Indiana.

Mohammed, G. J., Hameed, I. H., & Kamal, S. A. (2021). Study of secondary metabolites produced by *Aspergillus flavus* and evaluation of the antibacterial and antifungal activity. *African Journal of Biotechnology*. in press. Retrieved Septemeber 25th, 2021 from https://academicjournals.org/journal/AJB/article-in-press-abstract/study_of_secondary_metabolites_produced_by_aspergillus_flavus_and_evaluation_of_the_antibacterial_and_antifungal_activity.

Mohan, V. R., Tresina, P. S., & Daffodil, E. D. (2016). Antinutritional Factors in Legume Seeds: Characteristics and Determination. In: Reference Module in Food Science. *Encyclopedia of Food and Health*, 211-220.

Mugisha, M. K., Asiimwe, S., Namutebi, A., Borg-Karlson, A.-K., & Kakudidi, E. K. (2014). Ethnobotanical study of indigenous knowledge on medicinal and nutritious plants used to manage opportunistic infections associated with HIV/AIDS in western Uganda. *Journal of Ethnopharmacology, 155*, 194–202.

Müller, M., & Volmer, D. (2017). Interpretation of mass spectra. Institute for Bioanalytical Chemistry, Saarland University, 66123 Saarbrücken, Germany. Retrieved on July 20th, 2021 from https://www.uni-saarland.de/fileadmin/upload/lehrstuhl/jauch/An04_Massenspektroskopie_Skript_Volmer.pdf.

Muraina, I. A., Adaudi, A. O., Mamman, M., Kazeem, H. M., & Eloff, J. N. (2008). Effects of geographical location on the yield and bioactivity of *Anoigeissus leiocarpus*. *Journal of Pharmacy & Bioresources, 5*, 68-72.

Musinguzi, M., Tumushabe, A., Sekabira, K., Basamba, T. A., & Byarugaba, D. (2017). Medicinal plants use in and around Kalinzu Central forest reserve, Western Uganda. *Journal of Medicinal Plants Studies 5*, 44-49.

Muthaura, C. N., Keriko, J. M., Mutai, C., Yenesew, A., Gathirwa, J. W., Irungu, B. N., . . . Derese, S. (2015). Antiplasmodial potential of traditional phytotherapy of some remedies used in treatment of malaria in Meru-Tharaka Nithi County of Kenya. *Journal of Ethnopharmacology*, *175*, 315-323.

Nalubega, R. (2010). Antibacterial and phytochemical properties of selected poultry ethnomedicinal plants in Masaka district. MSc Thesis, Makerere University, Kampala, Uganda.

Nalubega, R., Kabasa, J. D., Olila, D., & Kateregga, J. (2011). Antibacterial activity and phytochemical screening of eleven plants used as poultry ethnomedicines in Southern Uganda. *Agriculture Journal, 6*, 303-309.

Nambejja, C., Tugume, P., Nyakoojo, C., & Kamatenesi-Mugisha, M. (2019). Medicinal plant species used in the treatment of skin diseases in Katabi Sub-

County, Wakiso District, Uganda. *Ethnobotany Research & Applications, 18*, 20.

Nambooze, J. (2019). Isolation and structural elucidation of antibacterial triterpeniods compounds from stem bark of *Psorospermum febrifugum* (Spach var. febrifugum). *MSc Thesis, Makerere University, Kampala, Uganda*.

Nampanzira, D. K., Kabasa, J. D., Nalule, S. A., Nakalembe, I., & Tabuti, J. R. S. (2015). Characterization of the goat feeding system among rural small holder farmers in the semi-arid regions of Uganda. *SpringerPlus, 4*, 188.

Namuhani, N., & Kimumwe, C. (2015). Soil Contamination with Heavy Metals around Jinja Steel Rolling Mills in Jinja Municipality, Uganda. *Journal of Health and Pollution, 5*, 61–67.

Namukobe, J., Kasenene, J. M., Kiremire, B. T., Byamukama, R., Kamatenesi-Mugisha, M., Krief, S., . . . Kabasa, J. D. (2011). Traditional plants used for medicinal purposes by local communities around the northern sector of Kibale National Park, Uganda. *Journal of Ethnopharmacology, 136*, 236–245.

Namukobe, J., Lutaaya, A., Asiimwe, S., & Byamukama, R. (2021). An Ethnobotanical Study of Medicinal Plants used in the Management of Dermatological Disorders in Buyende and Kayunga Districts, Uganda. *European Journal of Medicinal Plants, 32*, 15-40.

Nandiyanto, A. B. D., Oktiani, R., & Ragadhita, R. (2019). How to Read and Interpret FTIR Spectroscope of Organic Material. *Journal of Science & Technology, 4*, 97-118.

Nanyunja, K. R. (2003). Indigenous knowledge of the abundance of medicinal and food plants in Mount Moroto forest reserve. *Proceedings of the 11th World Forestry Congress*.

Nawaz, H., Shad, M. A., Rehman, N., Andaleeb, H., & Ullah, N. (2020). Effect of solvent polarity on extraction yield and antioxidant properties of phytochemicals from bean (*Phaseolus vulgaris*) seeds. *Brazilian Journal of Pharmaceutical Sciences, 56*, e17129.

Netala, V. R., Kotakadi, V. S., Nagam, V., Bobbu, P., Ghosh, S. B., & Tartte, V. (2015). First report of biomimetic synthesis of silver nanoparticles using aqueous callus extract of *Centella asiatica* and their antimicrobial activity. *Applied Nanoscience, 5*, 801–807

New Vision. (2019). ‘‘*Omugavu*’’ the multipurpose tree. Retrieved April 3rd, 2020 from http://greenwatch.or.ug/sites/default/files/2019-10/%27Omugavu%27%20the%20multipurpose%20%20tree.pdf.

Nicolescu, T. O. (2017). Interpretation of Mass Spectra. In: Mass Spectrometry, IntechOpen, pp. 23-78.

Nielsen, I. C. (1979). Notes on the genus *Albizia* Durazz. (Leguminosae-Mimosoideae) in mainland SE Asia. *Adansonia, 19*, 199-229.

Note, O. P., Chabert, P., Pegnyem, D. E., Weniger, B., Lacaille-Dubois, M., & Lobstein, A. (2010). Structure elucidation of new acacic acid-type saponins from *Albizia coriaria*. *Magnetic Resonance in Chemistry, 48*, 829–836.

Note, O. P., Mitaine-Offer, A. C., Miyamoto, T., Paululat, T., Mirjolet, J. F., Duchamp, O., . . . Lacaille-Dubois, M. (2009). Cytotoxic acacic acid glycosides from the roots of *Albizia coriaria*. *Journal of Natural Products, 72*, 1725-1730.

Nyamukuru, A., Tabuti, J. R. S., Lamorde, M., Kato, B., Sekagya, Y., & Aduma, P. R. (2017). Medicinal plants and traditional treatment practices used in the management of HIV/AIDS clients in Mpigi District, Uganda. *Journal of Herbal Medicine, 7*, 51–58.

Obakiro, S. B., Kiprop, A., Kowino, I., Kigondu, E., Odero, M. P., Omara, T., & Bunalema, L. (2020). Ethnobotany, ethnopharmacology, and phytochemistry of traditional medicinal plants used in the management of symptoms of tuberculosis in East Africa: a systematic review. *Tropical Medicine and Health, 46*, 68.

Ochwang'i, D. O., Kimwele, C. N., Oduma, J. A., Gathumbi, P. K., Mbaria, J. M., & Kiama, S. G. (2014). Medicinal plants used in treatment and management of cancer in Kakamega County, Kenya. *Journal of Ethnopharmacology, 151*, 1040–1055.

Ogukwe, C. E., Idika, I. D., & Awosu, E. A. (2018). Gas Chromatography-Mass Spectrophotometric (GC-MS) studies on therapeutic potentials of *Costus afer* Ker Gawl leaves. *World Journal of Pharmaceutical Research, 7*, 79-88.

Ogunkoya, L. (1981). Application of mass spectrometry in structural problems in triterpenes. *Phytochemistry, 20*, 121-126.

Ogunmoye, A., Oladosu, I., Onocha, P., & Choudhary, I. (2018). Isolation of Betulinic acid from the stem bark and root of *Uapaca guineensis*. *Journal of Research and Reviews in Science, 4*, 92-96.

Oh, H. M., Lee, S. W., Yun, B. R., Hwang, B. S., Kim, S. N., Park, C. S., . . . Rho, M. C. (2014). *Vigna angularis* inhibits IL-6-induced cellular signalling and ameliorates collagen-induced arthritis. *Rheumatology, 53*, 56–64.

Okello, J., Okullo, J. B. L., Eilu, G., Nyeko, P., & Obua, J. (2018). Physicochemical composition of *Tamarindus indica* L. (Tamarind) Fruits in the agro-ecological zones of Uganda. *Food Science and Nutrition, 6*, 1179–1189.

Olala, C. N. (2014). Identification of plants used for treatment of malaria and factors influencing their use in Boro division, Siaya county, Kenya. *MSc Thesis, Kenyatta University, Nairobi, Kenya*.

Olila, D., Ziraba, B. R., & Kamoga, D. (2007). Bio-prospective studies on medicinal plants used to manage poultry diseases in the Mount Elgon region of Uganda. *Research Journal of Pharmacology, 1*, 56-60.

Oliveira, R. N., Mancini, M. C., de Oliveira, F. C. S., Passos, T. M., Quilty, B., Thiré, R. M. d. S. M., & McGuinness, G. B. (2016). FTIR analysis and quantification of phenols and flavonoids of five commercially available plants extracts used in wound healing. *Revista Matéria, 21,* 767– 779.

Oloyede, G. K., Ali, M., & Lateef, M. (2019). Enzyme inhibition, Antioxidant and Insecticidal activities of flavonoids and fixed oil from *Albizia zygia* (J. F.Macbr). *Life Science Journal, 16*, 33-40.

Omara, T. (2020). Antimalarial Plants Used Across Kenyan Communities. *Evidence-Based Complementary and Alternative Medicine, 2020*, 4538602.

Omara, T., Kagoya, S., Openy, A., Omute, T., Ssebulime, S., Kiplagat, K. M., & Bongomin, O. (2020a). Antivenin plants used for treatment of snakebites in Uganda: Ethnobotanical reports and pharmacological evidences. *Tropical Medicine and Health, 48,* 6.

Omara, T., Kiprop, A. K., Ramkat, R. C., Cherutoi, J., Kagoya, S., Nyangena, D. M., . . . Koske, M. C. (2020b). Medicinal Plants Used in Traditional Management of Cancer in Uganda: Ethnobotanical Surveys, Phytochemistry and Anticancer Studies. *Evidence-Based Complementary and Alternative Medicine, 2020*, 3529081.

Omeli, M. R. W. (2011). Tree Attribute Ranking And Phenology Study: Farmers' Knowledge of Trees Commonly Found on Coffee Farms Bordering Mabira Forest Reserve in Mukono District, Uganda. *MSc Thesis, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya*.

Opio, D. R., Andama, E., & Kureh, G. T. (2017). Ethnobotanical Survey of Antimalarial Plants in Areas of: Abukamola, Angeta, Oculokori and Omarari of Alebtong District in Northern Uganda. *European Journal of Medicinal Plants, 21*, 1-14.

Orodho, J. A., Kirimuhuzya, C., Otieno, J. N., Magadula, J. J., & Okemo, P. (2011). Local management of tuberculosis by traditional medicine practitioners in Lake Victoria region. *The Open Complementary Medicine Journal, 3*, 1-9.

Orwa, J. A., Mwitari, P. G. M., Mtu, E., & Rukunga, G. M. (2007). Traditional healers and the management of malaria in Kisumu District, Kenya. *East African Medical Journal, 84*, 51-55.

Oryema, C., Bukenya-Ziraba, R., Omagor, N., & Opio, A. (2010). Medicinal plants of Erute county, Lira district, Uganda with particular reference to their conservation. *African Journal of Ecology, 48,* 285–298.

Othman, L., Sleiman, A., & Abdel-Massih, R. M. (2019). Antimicrobial Activity of Polyphenols and Alkaloids in Middle Eastern Plants. *Frontiers in Microbiology, 10*, 911.

Owuor, B. O., Ochanda, J. O., Kokwaro, J. O., Cheruiyot, A. C., Yeda, R., Okudo, C. A., & Akala, H. M. (2012). *In vitro* antiplasmodial activity of selected Luo and Kuria medicinal plants. *Journal of Ethnopharmacology, 144*, 779–781.

Panda, P., & Tripathy, G. (2009). Wound healing activity of aqueous and methanolic bark extract of *Vernonia arborea* in Wistar rats. *Natural Products Radiance, 8*, 6–11.

Parsonnet, J. (1995). Bacterial infection as a cause of cancer. *Environmental health perspectives, 103*, 263–268.

Pękal, A., & Pyrzynska, K. (2014). Evaluation of Aluminium Complexation Reaction for Flavonoid Content Assay. *Food Analysis Methods, 7*, 1776-1782.

Peng, G., Guan, H., Wang, X., & Shi, Y. (2017). Simultaneous determination of 14 active constituents of Shengjiang Xiexin decoction using ultrafast liquid chromatography coupled with electrospray ionization tandem mass spectrometry. *Acta pharmaceutica Sinica B, 7*, 193–201.

Pereira, F. B. M., Domingues, F. M. J., & Silva, A. M. S. (1996). Triterpenes from *Acacia dealbata*. *Natural Products Letters, 8*, 97-103.

Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., . . . Bitto, A. (2017). Oxidative Stress: Harms and Benefits for Human Health. *Oxidative Medicine and Cellular Longevity, 2017*, 8416763.

Pleńkowska, J., Gabig-Cimińska, M., & Mozolewski, P. (2020). Oxidative Stress as an Important Contributor to the Pathogenesis of Psoriasis. *International Journal of Molecular Sciences, 21*, 6206.

Pongpiachan, S. (2014). FTIR Spectra of Organic Functional Group Compositions in PM2.5 Collected at Chiang-Mai City, Thailand during the Haze Episode in March 2012. *Journal of Applied Sciences Research, 14*, 2967-2977.

Poole, K. (2004). Efflux-mediated multiresistance in Gram-negative bacteria. *Clinical microbiology and infection, 10*, 12-26.

Prachayasittikul, S., Saraban, P., Cherdtrakulkiat, R., Ruchirawat, S., & Prachayasittikul, V. (2010). New bioactive triterpenoids and antimalarial activity of *Diospyros rubra* Lec. *EXCLI Journal, 9*, 1–10.

Prashant, A., & Krupadanam, G. L. D. (1993). Dehydro-6-hydroxyrotenoid and lupenone from *Tephrosia villosa*. *Phytochemistry, 32*, 484-486.

Rahul, C., Pankaj, P., Sarwan, S. K., & Mahesh, J. K. (2010). Phytochemical screening and antimicrobial activity of *Albizzia lebbeck*. *Journal of Chemical and Pharmaceutical Research, 2*, 476-484.

Rambo, D. F., Biegelmeyer, R., Toson, N. S. B., Dresch, R. R., Moreno, P. R. H., & Henriques, A. T. (2019). The genus *Erythrina* L.: A review on its alkaloids, preclinical, and clinical studies. *Phytotherapy Research, 5*, 1258-1276.

Rani, N., Sharma, S., & Sharma, M. (2016). Phytochemical analysis of *Meizotropis pellita* by FTIR and UV-VIS spectrophotometer. *Indian Journal of Science and Technology, 9*, 1–4.

Räsänen, R.-M., Hieta, J.-P., Immanen, J., Nieminen, K., Haavikko, R., Yli-Kauhaluoma, J., & Kauppila, T. J. (2019). Chemical profiles of birch and alder bark by ambient mass spectrometry. *Analytical and Bioanalytical Chemistry, 411*, 7573–7583.

Rasigade, J. P., & Vandenesch, F. (2014). *Staphylococcus aureus*: a pathogen with still unresolved issues. Infection, genetics and evolution *Journal of Molecular Epidemiology and Evolutionary Genetics in Infectious Diseases, 21*, 510-514.

Rath, S., Rud, T., Pieper, D. H., & Vital, M. (2020). Potential TMA-Producing Bacteria Are Ubiquitously Found in Mammalia. *Frontiers in Microbiology*, *10*, 2966.

Reddy, S. B., Nagaraja, M. S., Kadalli, G. G., & Champa, B. V. (2018). Fourier Transform Infrared (FTIR) Spectroscopy of Soil Humic and Fulvic Acids Extracted from Paddy Land Use System. *International Journal of Current Microbiology and Applied Sciences, 7*, 834-837.

Ronzio, R. A. (2020). Naturally Occurring Antioxidants. *Textbook of Natural Medicine (Fifth Edition), e12*, 731-751.

Rukunga, G. M., & Waterman, P. G. (2001a). A new oleanane glycoside from the stem bark of *Albizia gummifera*. *Fitoterapia, 72*, 140–145.

Rukunga, G. M., & Waterman, P. G. (2001b). Triterpenes of *Albizia versicolor* and *Albizia schimperana* stem barks. *Fitoterapia, 72*, 188–190.

Saldaña, M., Valenzuela, S. A., Moor, S. R., Metola, P., & Anslyn, E. V. (2020). K-5 Thin-Layer Chromatography: Three-Dimensional Analysis of Pigments from Plant Materials Using an Interlocking Building-Block Photography Box. *Journal of Chemical Education, 97*, 4414–4419.

Sallau, M. S., Uttu, A. J., Ibrahim, H., Idris, Y. A., & Dama, J. H. (2016). Isolation of a Major Antimicrobial Compound from Stem Bark of *Glossonema boveanum* (Decne). *Biotechnology Journal International, 16*, 1-10.

Sandeep, K., Yadav, A., Yadav, M., & Yadav, Y. P. (2017). Effect of climate change on phytochemical diversity, total phenolic content and in vitro antioxidant activity of *Aloe vera* (L.) Burm.f. *BMC Research Notes, 10*, 60.

Sangster, A. W., & Stuart, K. L. (1965). Ultraviolet Spectra of Alkaloids. *Chemical Reviews, 65*, 69–130.

Santiago, L. A., & Mayor, A. B. R. (2014). Lupeol: An antioxidant triterpene in *Ficus pseudopalma* Blanco (Moraceae). *Asian Pacific Journal of Tropical Biomedicine, 4*, 109-118.

Schmidt, J., & Huneck, S. (1979). Mass spectroscopy of natural products. V—Mass spectroscopic studies of ring a substituted allobetulane derivatives. *Organic Mass Spectrometry, 14*, 646–655.

Schultz, F., Anywar, G., Quave, C. L., & Garbe, L. A. (2021a). A Bibliographic Assessment Using the Degrees of Publication Method: Medicinal Plants from the Rural Greater Mpigi Region (Uganda). *Evidence-Based Complementary and Alternative Medicine, 2021*, 6661565.

Schultz, F., Anywar, G., Tang, H., Chassagne, F., Lyles, J. T., Garbe, L., & Quave, C. L. (2020a). Targeting ESKAPE pathogens with anti-infective medicinal plants from the Greater Mpigi region in Uganda. *Scientific Reports, 10*, 11935.

Schultz, F., Anywar, G., Wack, B., Quave, C. L., & Garbe, L. (2020b). Ethnobotanical study of selected medicinal plants traditionally used in the rural Greater Mpigi region of Uganda. *Journal of Ethnopharmacology, 256*, 112742.

Schultz, F., Osuji, O., Nguyen, A., Anywar, G., Scheel, J., Caljon, G., . . . Garbe, L.-A. (2021c). Pharmacological Assessment of the Antiprotozoal Activity, Cytotoxicity and Genotoxicity of Medicinal Plants Used in the Treatment of Malaria in the Greater Mpigi Region in Uganda. *Frontiers in Pharmacology, 12*, 678535.

Schultz, F., Osuji, O. F., Wack, B., Anywar, G., & Garbe, L. A. (2021b). Antiinflammatory Medicinal Plants from the Ugandan Greater Mpigi Region Act as Potent Inhibitors in the COX-2 / PGH2 Pathway. *Plants, 10*, 351.

Seung-Joo, L., Umano, K., Shibamoto, T., & Lee, K.-G. (2005). Identification of volatile components in basil (*Ocimum basilicum* L.) and thyme leaves (*Thymus vulgaris* L.) and their antioxidant properties. *Food Chemistry, 91*, 131–137.

Shaheen, A., Hanif, M. A., Rehman, R., Jilani, M. I., & Shikov, A. (2020). Alkanet. In: Muhammad A.H., Haq N., Muhammad M.K., Hugh J.B. (Eds). Medicinal Plants of South Asia. Novel sources for drug discovery. *Amsterdam; Oxford: Cambridge, MA Elsevier*, 1-12.

Shai, L. J., McGaw, L. J., Aderogba, M. A., Mdee, L. K., & Eloff, J. N. (2008). Four pentacyclic triterpenoids with antifungal and antibacterial activity from *Curtisia dentata* (Burm.f) C.A. Sm. leaves. *Journal of Ethnopharmacology, 119*, 238–244.

Shehu, M. W., Bello, I., Abdulkadir, N., Shehu, A., Jamil, S. E., & Waziri, S. A. (2018). Utilization of medicinal plants used in the management of HIV/AIDS opportunistic infections in njeru sub-county, Buikwe district, Uganda. *MOJ Bioequivalence & Bioavailability, 5*, 66–72.

Shenta, A. A., & Al-Maliki, A. D. M. (2013). Isolation and Identification of Three Alkaloids Compounds from *Albizia lebbeck* L. Leaves and study of Their Antimicrobial Activity Against Pathogenic Bacteria of Urinary Tracts Inflammatory *in vitro*. *University of Thi-Qar Journal of Science, 3*, 99-111.

Shin, Y. G., Cho, K. H., Chung, S. M., Graham, J., Das Gupta, T. K., & Pezzuto, J. M. (1999). Determination of betulinic acid in mouse blood, tumor and tissue homogenates by liquid chromatography–electrospray mass spectrometry. *Journal of Chromatography B, 732*, 331–336.

Shiota, M. (2021). Oxidative stress and prostate cancer. In: Oxidative Stress and Dietary Antioxidants, Cancer (Second Edition). pp. 15-26.

Shiracko, N., Owuor, B. O., Gakuubi, M. M., & Wanzala, W. (2016). A survey of ethnobotany of the AbaWanga people in Kakamega county, Western province of Kenya. *Indian Journal of Traditional Knowledge, 15*, 93-102.

Shrivastava, S. R., Shrivastava, P. S., & Ramasamy, J. (2018). World health organization releases global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. *Journal of Medical Society, 32*, 76.

Siddique, H. R., & Saleem, H. (2011). Beneficial health effects of lupeol triterpene: A review of preclinical studies. *Life Sciences, 88*, 285–293.

Šiman, P., Filipová, A., Tichá, A., Niang, M., Bezrouk, A., & Havelek, R. (2016). Effective Method of Purification of Betulin from Birch Bark: The Importance of Its Purity for Scientific and Medicinal Use. *PLoS ONE, 11*, e0154933.

Singab, A. N., Bahgat, D., Al-Sayed, E., & Eldahshan, O. (2015). Saponins from Genus *Albizia*: Phytochemical and Biological Review. *Medicinal & Aromatic Plants, S3*, 001.

Sirama, V. O. (2014). Ethnobotanical, Bioactivity and Phytochemical Evaluation of Anthelmintic Herbal Remedies of Migori County, Kenya. *MSc Thesis, University of Nairobi, Nairobi, Kenya*.

Song, M., Hang, T. J., Wang, Y., Jiang, L., Wu, X. L., Zhang, Z., . . . Zhang, Y. (2006). Determination of oleanolic acid in human plasma and study of its pharmacokinetics in Chinese healthy male volunteers by HPLC tandem mass spectrometry. *Journal of Pharmaceutical and Biomedical Analysis,, 40*, 190–196.

Sripad, G., Prakash, V., & Rao, M. S. N. (1982). Extrability of polyphenols of sunflower seed in various solvents. *Journal of Biosciences, 4*, 145-152.

Srivastava, P., & Chaturvedi, R. (2010). Simultaneous determination and quantification of three pentacyclic triterpenoids—betulinic acid, oleanolic acid, and ursolic acid—in cell cultures of *Lantana camara* L. *In vitro Cellular & Developmental Biology - Plant, 46*, 549–557.

Ssegawa, P., & Kasenene, J. M. (2007). Medicinal plant diversity and uses in the Sango bay area, Southern Uganda. *Journal of Ethnopharmacology, 113,* 521-540.

Sulaiman, C. T., Ramesh, P. R., Mahesh, K., Madhu, K. M., Anandan, E. M., Praveen, M., & Indira, B. (2020). Chemical profiling of a polyherbal formulation by tandem mass spectroscopic analysis with multiple ionization techniques. *Future Journal of Pharmaceutical Sciences, 6*, 40.

Suleiman, M. H. A., & Ateeg, A. A. (2020). Antimicrobial and Antioxidant Activities of Different Extracts from Different Parts of *Zilla spinosa* (L.) Prantl. *Evidence-Based Complementary and Alternative Medicine, 2020*, 6690433.

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA: a cancer journal for clinicians, 71*, 209–249.

Sut, S., Poloniato, G., Malagoli, M., & Dall'Acqua, S. (2018). Fragmentation of the main triterpene acids of apple by LCAPCI-MSn. *Journal of Mass Spectrometry, 53*, 882–892.

Suttiarporn, P., Chumpolsri, W., Mahatheeranont, S., Luangkamin, S., Teepsawang, S., & Leardkamolkarn, V. (2015). Structures of Phytosterols and Triterpenoids with Potential Anti-Cancer Activity in Bran of Black Non-Glutinous Rice. *Nutrients, 7*, 1672-1687.

Szczurek, A. (2021). Perspectives on Tannins. *Biomolecules, 11*, 442.

Tabuti, J. R., Dhillion, S. S., & Lye, K. A. (2003). Ethnoveterinary medicines for cattle (Bos indicus) in Bulamogi county, Uganda: plant species and mode of use. *Journal of Ethnopharmacology, 88*, 279–286.

Tabuti, J. R., Lye, L. A., & Dhillion, S. S. (2003). Traditional herbal drugs of Bulamogi, Uganda: plants, use and administration. *Journal of Ethnopharmacology, 88*, 19-44.

Tabuti, J. R. S., & Magadula, B. B. (2007). The ethnobotany and ecological status of *Albizia coriaria* Welw. ex Oliv. in Budondo Sub-county, Eastern Uganda. *African Journal of Ecology, 45*, 126–129.

Taiz, L., & Zeiger, E. (2003). Plant physiology. 3rd edn. *Annals of Botany, 91*, 750-751.

Tamokou, J. D., Mpetga, D. S., Lunga, P. K., Tene, M., Tane, P., & Kuiate, J. (2012). Antioxidant and antimicrobial activities of ethyl acetate extract, fractions and compounds from stem bark of *Albizia adianthifolia* (Mimosoideae). *BMC Complementary and Alternative Medicine, 12,* 99.

Tchimene, M. K., Nwaehujor, C. O., Ezenwali, M., Okoli, C. C., & Iwu, M. M. (2016). Free Radical Scavenging Activity of Lupeol Isolated from the Methanol Leaf

Extract of *Crateva adansonii* Oliv. (Capparidaceae). *International Journal of Pharmacognosy and Phytochemical Research, 8*, 419-426.

Tedankara. (2021). Mass Spectroscopy (MS). Retrieved on August 5th, 2021 from https://library.tedankara.k12.tr/carey/ch13-ms.html.

Thammavong, S. (2012). Chemical Constituents of *Albizia myriophylla* Wood and Biological Activities. *MSc Thesis, Prince of Songkla University, Hat Yai, Thailand*.

Thawabteh, A., Juma, S., Bader, M., Karaman, D., Scrano, L., Bufo, S. A., & Karaman, R. (2019). The Biological Activity of Natural Alkaloids against Herbivores, Cancerous Cells and Pathogens. *Toxins, 11*, 656.

The New Vision. (2010). Museveni hailed for new district. Retrieved on May 20th, 2021 from https://web.archive.org/web/20140416043723/http://www.newvision.co.ug/D/8/16/724804.

The Plant List. (2019). *Albizia coriaria.* Retrieved on April 10th, 2021 from http://www.theplantlist.org/.

Thube, S., Katekar, S., & Kureshi, T. (2014). Screening of *Albizia lebbeck* bark extract as a potential antivenom agent against Cobra *Naja naja*. In: Annual International Conference on Pharmacology and Pharmaceutical Sciences. Global Science & Technology Forum (GSTF).

Tijjani, A., Ndukwe, I., & Ayo, R. (2012). Isolation and Characterization of Lup-20(29)-ene-3, 28-diol (Betulin) from the Stem-Bark of *Adenium obesum* (Apocynaceae). *Tropical Journal of Pharmaceutical Research, 11*, 259-262.

Tincho, M. B., Morris, T., Meyer, M., & Pretorius, A. (2020). Antibacterial Activity of Rationally Designed Antimicrobial Peptides. *International Journal of Microbiology, 2020*, 1-9.

Tocher, D. R. (2003). Thin-layer Chromatography. In: Chromatography. *Encyclopedia of Food Sciences and Nutrition (2nd Edn)*, 1267-1274.

Todar, K. (2014). Todar's Online Textbook of Bacteriology. 4 pages. Retrieved September 15th, 2020 from http://textbookofbacteriology.net/pseudomonas.html.

Togashi, N., Shiraishi, A., Nishizaka, M., Matsuoka, K., Endo, K., Hamashima, H., & Inoue, Y. (2007). Antibacterial activity of long-chain fatty alcohols against *Staphylococcus aureus*. *Molecules, 12*, 139–148.

Tong, S. Y., Davis, J. S., Eichenberger, E., Holland, T. L., & Fowler, V. G. (2015). *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. *Clinical Microbiology Reviews, 28*, 603-661.

Trease, G. E., & Evans, W. C. (1989). Phenols and phenolic glycosides. In: Textbook of Pharmacognosy, vol. 12, pp. 343–383, Balliese, Tindall and Co Publishers, London, UK.

TROFACO. (2019). A list of tree species that communities with whom TROFACO work have planted so far. Retrieved May 10th, 2020 from https://trofaco.org/wp-content/uploads/2019/12/TROFACO-trees.pdf.

Tropical Plants Database. (2020). *Albizia coriaria* Welw. ex Oliv. (Fabaceae). Retrieved July 23rd, 2020 from http://tropical.theferns.info/viewtropical.php?id=Albizia%20coriaria.

Trubetskaya, A., Johnson, R., Monaghan, R. F. D., Ramos, A. S., Brunsvik, A., Wittgens, B., . . . Budarin, V. (2021). Combined analytical strategies for chemical and physical characterization of tar from torrefaction of olive stone. *Fuel, 291*, 120086.

Tsabang, N., Yedjou, C. G., & Tchounwou, P. B. (2017). Phytotherapy of High Blood Pressure in Three Phytogeographic Regions of Cameroon. *Pharmaceutica Analytica Acta, 7*, 530.

Tugume, P., Kakudidi, E. K., Buyinza, M., Namaalwa, J., Kamatenesi, M., Mucunguzi, P., & Kalema, J. (2016). Ethnobotanical survey of medicinal plant species used by communities around Mabira central forest reserve, Uganda. *Journal of Ethnobiology and Ethnomedicine, 12*, 28.

Turrens, J. F. (2003). Mitochondrial formation of reactive oxygen species. *The Journal of physiology, 552*, 335–344.

USDA. (2021). Agricultural Research Service, National Plant Germplasm System. Germplasm Resources Information Network (GRIN-Taxonomy). National Germplasm Resources Laboratory, Beltsville, Maryland.

Uwaya, J. O., Okunrobo, L. O., & Igbinaduwa, P. O. (2017). *Albizia zygia* (D.C.) Macbr (Fabaceae): A Comparative Investigation of Phytochemical Composition, Proximate Analysis and Anti-Seizure Properties of Methanol Extracts of Its Leaves and Stem-Bark. *Nigerian Journal of Pharmaceutical and Applied Science Research, 6*, 76-80.

Uzor, P. F. (2020). Alkaloids from Plants with Antimalarial Activity: A Review of Recent Studies. *Evidence-Based Complementary and Alternative Medicine, 2020*, 8749083.

Velioglu, Y. S., Mazza, G., Gao, L., & Oomah, B. D. (1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *Journal of Agricultural and Food Chemistry, 46*, 4113–4117.

Wahyuono, S. (1985). Phytochemical Investigation of *Amsonia randiflora* Family Apocynaceae. MSc Thesis, University of Arizona, Tucson, Arizona.

Wain, J., Hendriksen, R. S., Mikoleit, M. L., Keddy, K. H., & Ochiai, R. L. (2015). Typhoid fever. *Lancet, 385*, 1136-1145.

Wanyama, P. A. G., Kiremire, B. T., Murumu, J. E. S., & Kamoga, O. (2011). Textile dyeing and phytochemical characterization of crude plant extracts derived from selected dye-yielding plants in Uganda. *International Journal of Natural Products Research, 1*, 26-31.

Weather atlas. (2021). Weather forecast Kole, Uganda. Retrieved May 20th, 2021 from https://www.weather-atlas.com/en/uganda/kole.

WHO. (2017). Global Priority List of Antibiotic-Resistant Bacteria to Guide Research, Discovery, and Development of New Antibiotics. Geneva: WHO Press, pp.1-7. Retrieved Februray 23rd, 2021 from https://www.who.int/medicines/publications/WHO-PPL-Short_Summary_25Feb-ET_NM_WHO.pdf?ua=1.

WHO. (2019). WHO Global Report on Traditional and Complementary Medicine. Retrieved March 4th, 2020 from https://www.who.int/traditional-complementary-integrative-medicine/WhoGlobalReportOnTraditionalAndComplementaryMedicine2019.pdf?ua=1.

WHO. (2020a). Infectious diseases kill over 17 million people a year: WHO warns on global crisis. Retrieved February, 8th 2020 from https://www.who.int/whr/1996/media_centre/press_release/en/.

WHO. (2020b). Infectious diseases kill over 17 million people a year: WHO warns on global crisis. Retrieved Februray 20th, 2021 from https://www.who.int/whr/1996/media_centre/press_release/en/.

WHO. (2020c). The top 10 causes of death. Retrieved 10th November 2020 from https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death.

WHO. (2020d). World Health Report. Infectious diseases and cancer. Retrieved on November 10th, 2020 from https://www.who.int/whr/1996/media_centre/press_release/en/index7.html.

Widyawati, P. S., Dwi, T., Budianta, W., & Kusuma, F. A. (2014). Difference of solvent polarity to phytochemical content and antioxidant activity of *Pluchea indicia* Less leaves extracts. *International Journal of Pharmacognosy and Phytochemistry Research, 6*, 850-855.

Wilson, K., & Walker, J. (2018). Principles and Techniques of Biochemistry and Molecular Biology (7 Edn). Cambridge University Press, New York. pp. 434-442.

World Agroforestry. (2019). *Albizia coriaria*. Retrieved November 8th, 2019 from http://old.worldagroforestry.org/usefultrees/pdflib/Albizia_coriaria_UGA.pdf.

Worldometer. (2021). Uganda Population. Retrieved January 15th, 2021 from https://www.worldometers.info/world-population/uganda-population/.

Wu, M., & Li, F. (2015). *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. *Molecular Medical Microbiology, 3,* 1547-1564.

Xu, F., Huang, X., Wu, H., & Wang, X. (2018). Beneficial health effects of lupenone triterpene: A review. *Biomedicine & Pharmacotherapy, 103* 198–203.

Yam-Puc, M. A., Santana-Hernández, A. A., Yah-Nahuat, P. N., Ramón-Sierra, J. M., Cáceres-Farfán, M. R., Borges-Argáez, R. L., & Ortiz-Vázquez, E. (2019). Pentacyclic triterpenes and other constituents in propolis extract from *Melipona beecheii* collected in Yucatan, México. *Revista Brasileira de Farmacognosia, 29,* 358-363.

Yang, W., Chen, X., Li, Y., Guo, S., Wang, Z., & Yu, X. (2020). Advances in Pharmacological Activities of Terpenoids. *Natural Product Communications, 15,* 1-13.

Yang, W., Liu, J., Blažeković, B., Sun, Y., Ma, S., Ren, C., . . . Wang, Y. (2018). *In vitro* antibacterial effects of Tanreqing injection combined with vancomycin or linezolid against methicillin-resistant *Staphylococcus aureus*. *BMC Complementary and Alternative Medicine, 18,* 169.

Yetayih, M. M., & Ravichandran, Y. D. (2020). Extraction and GC-MS Analysis of the Essential Oil from the Peel of *Solanum incanum* and its Antibacterial Activity Studies. *Asian Journal of Chemistry, 32,* 2001-2006.

Yi, Z. B., Yu, Y., Liang, Y. Z., & Zeng, B. (2007). Evaluation of the antimicrobial mode of berberine by LC/ESI-MS combined with principal component analysis. *Journal of pharmaceutical and biomedical analysis, 44*, 301–304.

Zahari, A., Ablat, A., Omer, N., Nafiah, M. A., Sivasothy, Y., Mohamad, J., . . . Awang, K. (2016). Ultraviolet-visible study on acid-base equilibria of aporphine alkaloids with antiplasmodial and antioxidant activities from *Alseodaphne corneri* and *Dehaasia longipedicellata*. *Scientific Reports, 6*, 21517.

Zargoosh, Z., Ghavam, M., Bacchetta, G., & Tavili, A. (2019). Effects of ecological factors on the antioxidant potential and total phenol content of *Scrophularia striata* Boiss. *Scientific Reports, 9*, 16021.

Zengin, G., Mahomoodally, M. F., Paksoy, M. Y., Picot-Allain, C., Glamocilja, J., Sokovic, M., . . . Custodio, L. (2019). Phytochemical characterization and bioactivities of five Apiaceae species: natural sources for novel ingredients. *Industrial Crops and Products, 135*, 107–121.

Zhang, W., Jiang, H., Yang, J., Jin, M., Du, Y., Sun, Q., . . . Xu, H. (2019). Safety assessment and antioxidant evaluation of betulin by LC-MS combined with free radical assay. *Analytical Biochemistry, 587*, 113460.

APPENDICES

Appendix I: Agroecological zones of Uganda

No.	Agro-ecological zone	General vegetation	Major districts
1	Lake Victoria Crescent	Forest/savanna mosaic characterized by patches of dense forest in the south and scattered trees in shrubs and grassland of the North	Kampala, Mukono, Wakiso, Mpigi**,** Masaka, Luwero, Kayunga, Kiboga, Nakasongola, Kalangala
2	Mid Northern	Flat terrain covered by thick savannah grassland	Lira, Apac, **Kole**, Kitgum, Gulu, Pader, Oyam, Amolatar, Alebtong, Otuke, Dokolo, Kwania
3	South East	Vegetation predominantly forest /savanna mosaics	**Jinja**, Iganga, Kamuli, Bugiri, Buyende, Kaliro, Mayuge, Luuka, Namutumba, Namayingo
4	Karamoja Drylands	Vegetation is characterized by thorny bushes, woodlands, occasional small trees and patches of grassland	Moroto, Kotido, Nakapiripirit
5	West Nile	Savanna vegetation with open mixtures of trees and shrubs standing within tall grass	Arua, Moyo, Adjumani, Yumbe
6	Lake Albert Crescent	Vegetation ranges from rainforest to savanna grasses	Masindi, Hoima, Kibale, Kiboga
7	Southern Highlands	High altitude forest/Savannah mosaic at high altitudes and swamp Forest	Kisoro, Kabale, Rukungiri, Kanungu
8	Southern Dry lands	Vegetation contains forests, savanna mosaic and grass savanna	Rakai, Sembabule, **Mbarara**, Ntungamo, Rukungiri
9	Eastern	Vegetation ranges from montane forest to high open moorland	Pallisa, Tororo, Kumi, Kaberamaido, Katakwi, Soroti, Mbale, Sironko, Kapchorwa
10	Western Highlands	Natural equatorial forest vegetation and rich natural savannah grasslands in the relatively drier areas	Bushenyi, Kasese, Bundibugyo, Kamwenge, Kyenjojo, Kabarole

Adapted from Kajobe *et al.* (2016). Districts in **bold** are those where *A. coriaria* leaves were sampled.

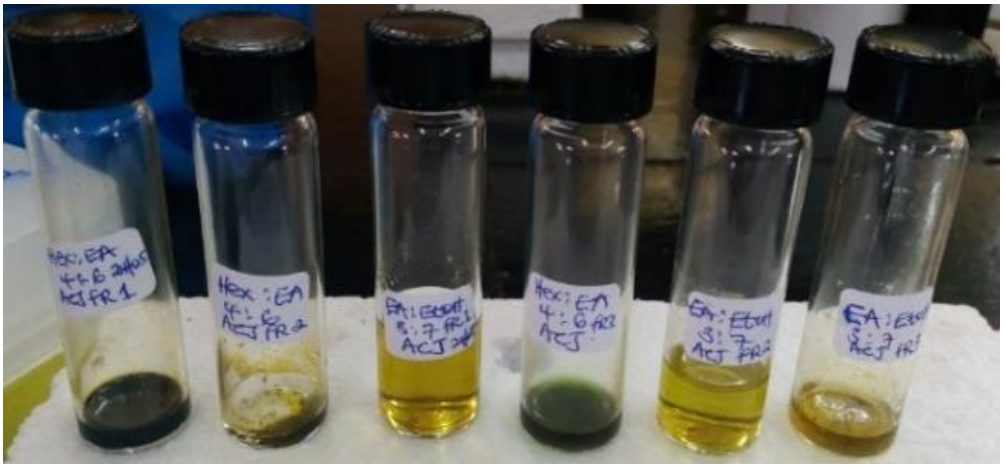
Appendix II: Fresh leaves, leaf extracts, column chromatography elution and fractions of *A. coriaria* extracts

(a) (b) (c)

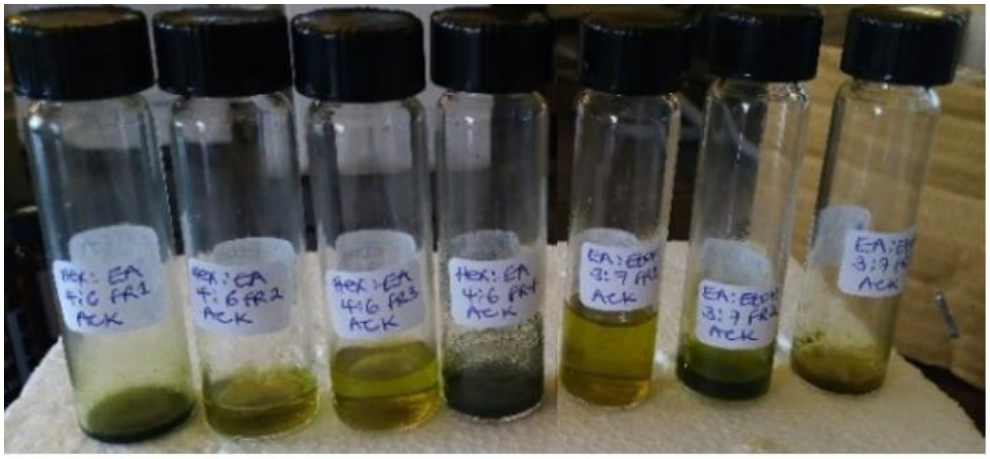
Portions of sampled *A. coriaria* leaves from (a) Jinja, (b) Kole, and (c) Mbarara district.

Portions of organic solvent extracts of *A. coriaria* leaves.

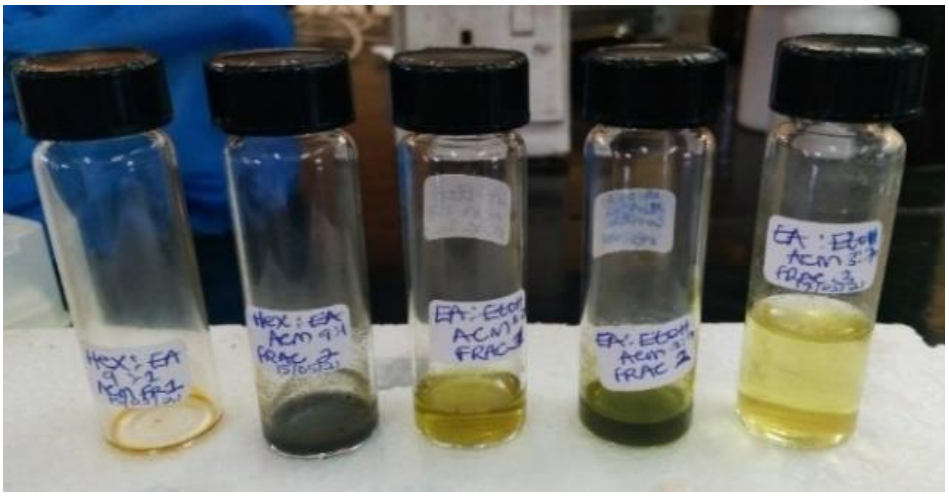
Chromatographic isolation of compounds in ethanolic extracts of *A. coriaria* leaves from Mbarara using hexane: ethyl acetate (9:1) solvent system.



(a)



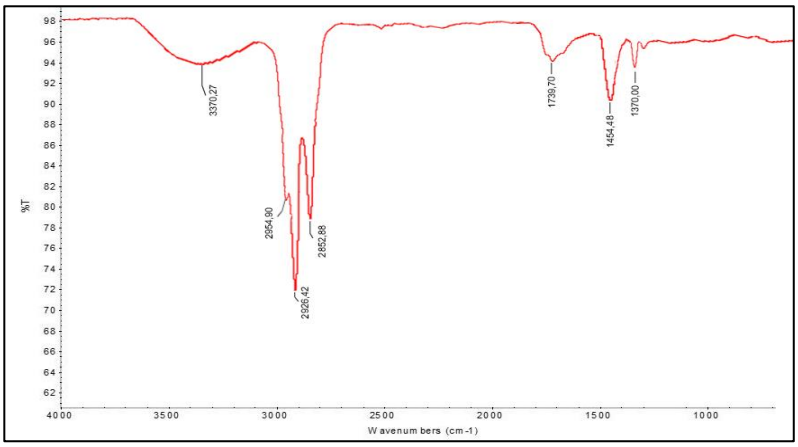
(b)



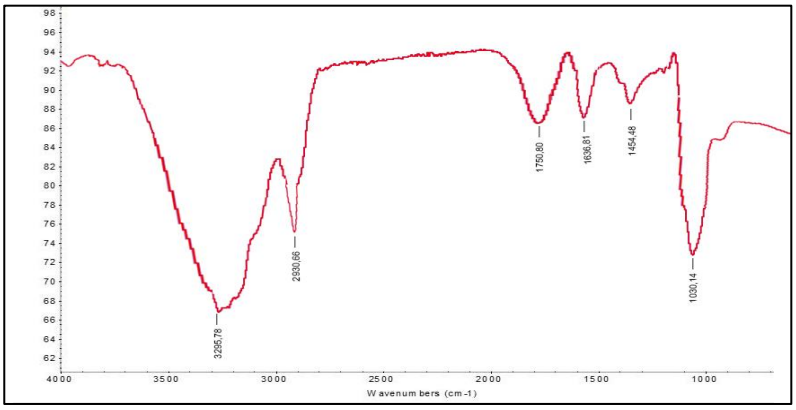
(c)

Fractions of ethanolic extracts of *A. coriaria* leaves from (a) Jinja, (b) Kole, and (c) Mbarara districts.

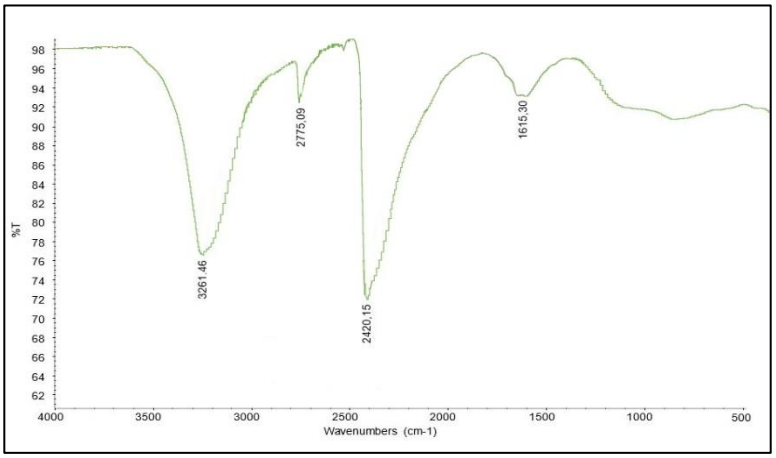
Appendix III: FTIR spectra of *A. coriaria* leaf extracts



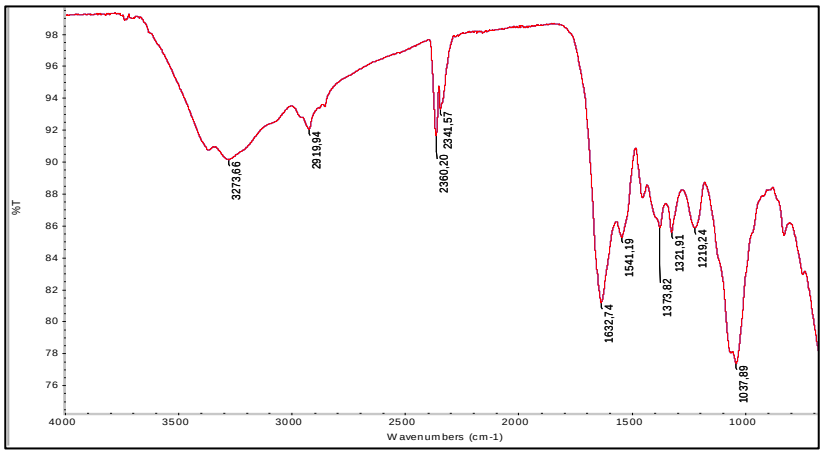
Ethyl acetate extract of *A. coriaria* leaves from Jinja



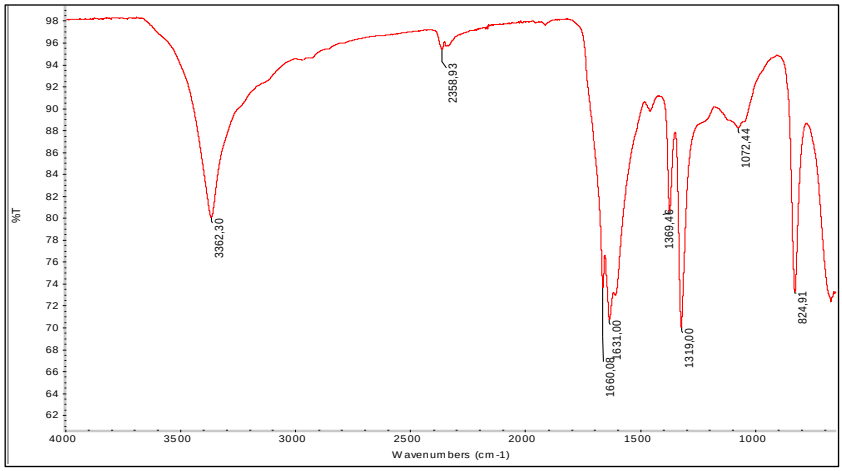
Ethyl acetate extract of *A. coriaria* leaves from Kole



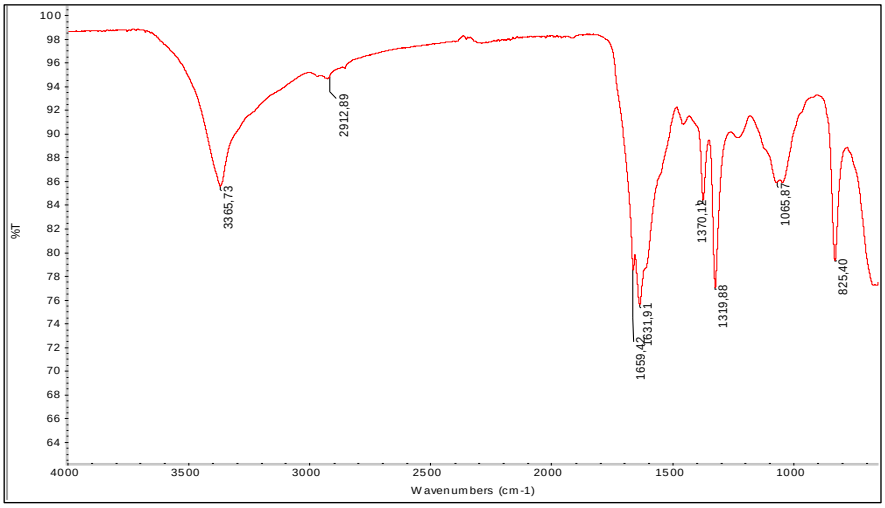
Ethyl acetate extract of *A. coriaria* leaves from Mbarara



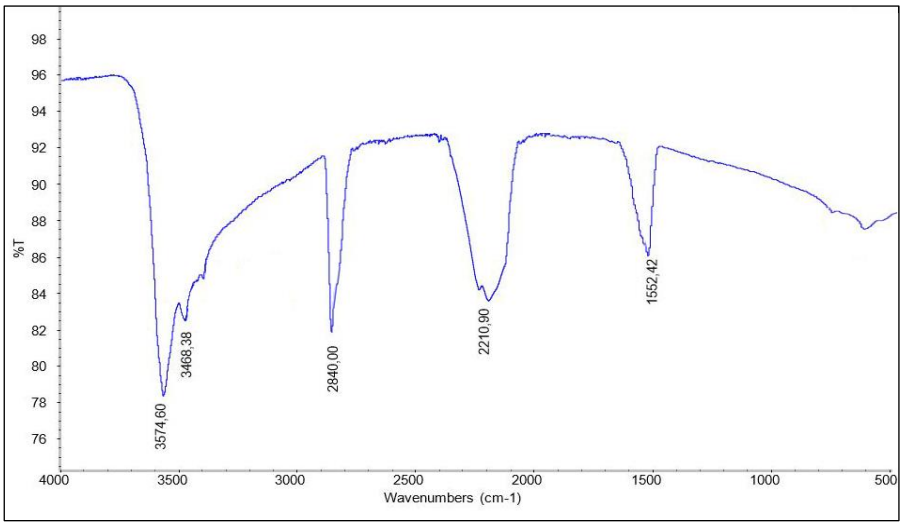
Ethanolic extract of *A. coriaria* leaves from Jinja



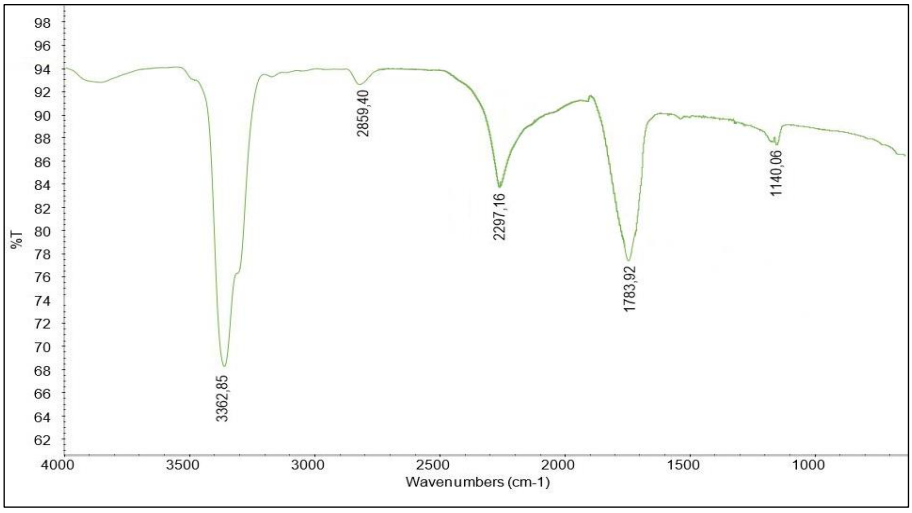
Ethanolic extract of *A. coriaria* leaves from Kole



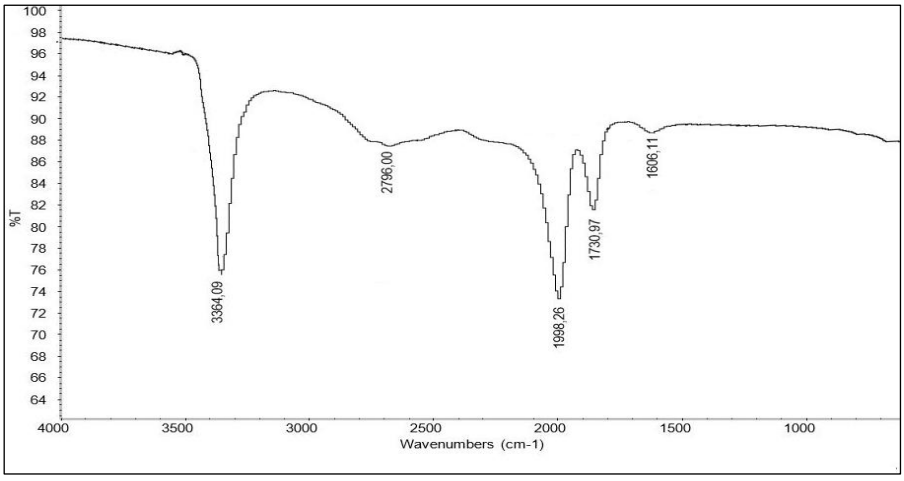
Ethanolic extract of *A. coriaria* leaves from Mbarara



Aqueous extract of *A. coriaria* leaves from Jinja

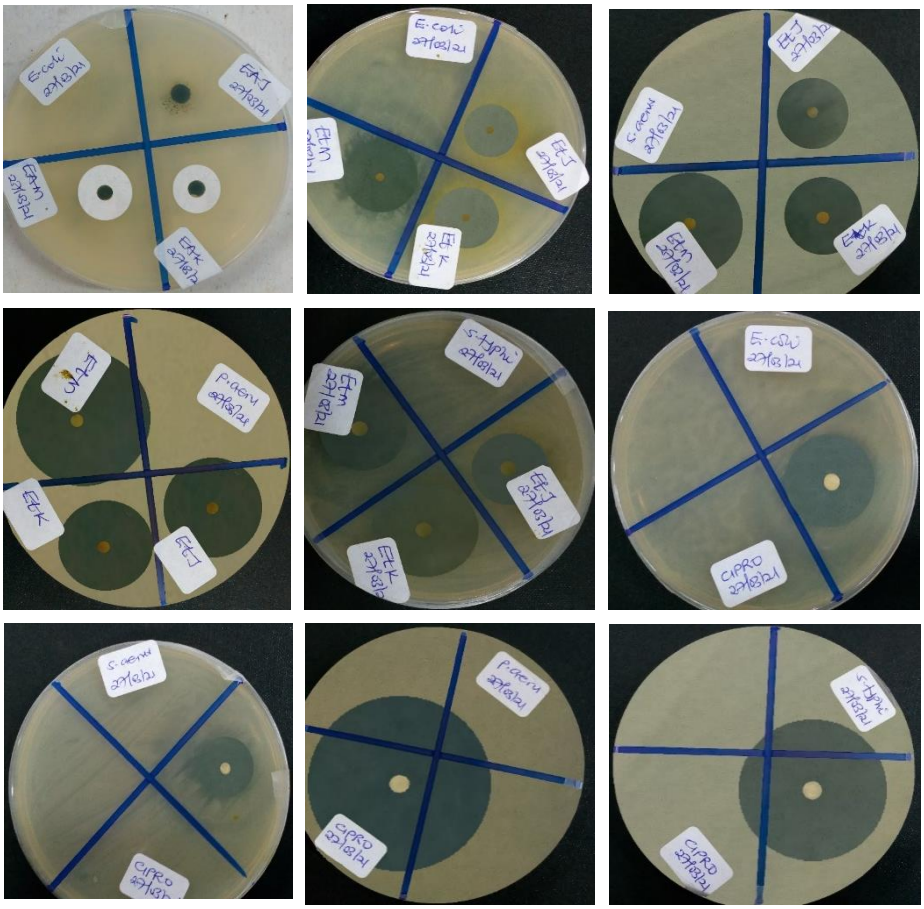


Aqueous extract of *A. coriaria* leaves from Kole



Aqueous extract of *A. coriaria* leaves from Mbarara

Appendix IV: Inhibition zones of *A. coriaria* leaf extracts



Legend: E. coli = *Escherichia coli*, S. aureus = *Staphylococcus aureus*, P. aeru = *Pseudomonas aeruginosa*, S. typhi = *Salmonella typhi*, CIPRO = Ciprofloxacin, EAJ, EAK and EAM = ethyl acetate extract of leaves from Jinja, Kole, and Mbarara respectively; EtJ, EtK and EtM = Ethanolic extract of *A. coriaria* leaves from Jinja, Kole and Mbarara districts, respectively.

Appendix V: Statistical summaries (ANOVA and Pearson's correlation)

1. One-Way ANOVA summary for the different solvent extract yields

Extract yield					
Source	DF	Adj SS	Adj MS	*F*-value	**P**
Factor	2	433.1	216.5	1.89	**0.230907**
Error	6	686.6	114.4		
Total	8	1119.7			

2. One-Way ANOVA summary for total phenolic content of the extracts

Total phenolic content					
Source	DF	SS	MS	*F*-value	**P**
Factor	2	1360.5	680.2	40.55	**0.002208**
Error	6	915.5	152.6		
Total	8	2276.0			

3. One-Way ANOVA summary for total flavonoid content of the extracts

Total flavonoid content					
Source	DF	Adj SS	Adj MS	*F*-value	**P**
Factor	2	119.1534	59.5767	**6.23**	**0.023560**
Error	6	21.6093	5.4023		
Total	8	176.554			

4. One-Way ANOVA summary for antioxidant activity (IC_{50}) of the extracts

Antioxidant activity					
Source	SS	DF	MS	*F*-value	**P**
Factor	65.1215	2	32.5607	**21.02**	**0.007548**
Error	30.4266	4	1.5494		
Ss/Bl	24.229	2			
Total	95.5481	8			

5. Pearson's correlation coefficients for TPC, TFC and antioxidant activity (IC_{50})

Parameter	Total flavonoid content	Antioxidant activity
Total phenolic content	0.898 (p = 0.001)**	-0.831 (p = 0.006)**
Total flavonoid content	1.000	-0.755 (p = 0.019)*

*Correlation is significant at $p < 0.05$, **Correlation is also significant at $p < 0.01$.

6. One-Way ANOVA summary for antibacterial activity (zone of inhibition diameter) of the extracts

Antibacterial activity (zone of inhibition)					
Source	SS	DF	MS	***F*-value**	**P**
Treatment (between columns)	85.52	3	28.51	**0.6512**	**0.5881**
Residual (within columns)	1401	32	43.78		
Total	1486	35			

Appendix VI: Publications from this thesis

1. **Omara, T.**, Kiprop, A. K., & Kosgei, V. J. (**2021**). Intraspecific variation of phytochemicals, antioxidant and antibacterial activities of different solvent extracts of *Albizia coriaria* leaves from some agroecological zones of Uganda. *Evidence-Based Complementary and Alternative Medicine, 2021*, Article ID 2335454, 14 pages. https://doi.org/10.1155/2021/2335454

2. **Omara, T.**, Kiprop, A. K., & Kosgei, V. J. (**2021**) *Albizia coriaria* Welw ex Oliver: A review of its ethnobotany, phytochemistry and ethnopharmacology. *Advances in Traditional Medicine.* https://doi.org/10.1007/s13596-021-00600-8