

**ANTIMICROBIAL ACTIVITY OF ALBIZIA GRANDIBRACTEATA AGAINST
NEISSERIA GONORRHEAE.**

MUSASIA IVYN MUNYULI

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**A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN
TROPICAL AND INFECTIOUS DISEASES OF THE UNIVERSITY OF NAIROBI.**

**UNIVERSITY OF NAIROBI INSTITUTE FOR TROPICAL AND INFECTIOUS
DISEASES (UNITID).**

2019.

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.....

SIGNATURE

Ivyn Munyuli Musasia (BSN)
W64/88432/2016
University of Nairobi

.....

DATE

I confirm that this dissertation was written by the above-named student and has been submitted with our approval as university supervisors:

Prof. KENNEDY ABUGA, PhD
Associate Professor, School of Pharmacy.
University of Nairobi.

SIGNATURE

DATE

Prof. JULIUS OYUGI, PhD
Deputy Director, UNITID.
Associate Professor, Department of Medical Microbiology
University of Nairobi.

SIGNATURE

DATE

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NAME: MUSASIA IVYN MUNYULI

REGISTRATION NUMBER: W64/ 88432 / 2016

UNIVERSITY OF NAIROBI

COLLEGE OF HEALTH SCIENCES

INSTITUTE OF TROPICAL AND INFECTIOUS DISEASES

COURSE: MASTER OF SCIENCE IN TROPICAL AND INFECTIOUS DISEASES.

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DEDICATION

This dissertation is dedicated to my parents Mr. Phaniel Isayangwa Musasia and Mrs. Caroline Siavuka Musasia.

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LIST OF ABBREVIATIONS AND SYMBOLS

BSAC	British Society of Antimicrobial Chemotherapy
DMSO	Dimethyl Sulfoxide
ESC	Extended Spectrum Cephalosporin
MBC	Minimum Bactericidal Concentration
MIC	Minimum Inhibitory Concentration
MTM	Modified Thayer Martin
PPV	Positive Predictive Value
STI	Sexually Transmitted Infection
WHO	World Health Organization
WHO-GASP	World Health Organization Gonococcal Surveillance Program

ABSTRACT

BACKGROUND

Gonorrhoea is a common sexually transmitted infection affecting millions of people worldwide in any given year. In Africa alone, it affects approximately 11.4 million people annually. In Kenya, very few studies have been conducted with regards to the prevalence of the infection. It adversely affects sexual and reproductive health and is implicated in complications such as pelvic inflammatory disease in women and infertility in both men and women. Studies conducted in the recent years have also shown that gonococcal infection, increases Human Immunodeficiency Virus shedding hence increasing its transmission.

Available medication to treat the infection is often expensive and has numerous adverse effects. Furthermore, the emergence of drug resistant strains of the bacteria especially to the Extended Spectrum Cephalosporins, which are a last resort treatment further complicates gonococcal management as there are limited treatment options. There is therefore need to find alternative, easily accessible, inexpensive treatment with high efficacy.

Plants have diverse chemical properties and provide numerous potent antimicrobial agents that can be utilized in the treatment of various ailments. *Albizia grandibracteata* has gained ethno-medicinal application due to its anti-gonococcal properties, among the Luhya community in Kenya. This study therefore seeks to assess the antimicrobial potency of *Albizia grandibracteata* against *Neisseria gonorrhoeae*.

OBJECTIVE

The main aim of this study was to evaluate the antimicrobial activity of *Albizia grandibracteata* whole roots extract against *Neisseria gonorrhoeae*.

METHODOLOGY

Albizia grandibracteata whole roots were conservatively harvested in Kakamega forest, Kakamega County in September 2018. They were air dried in the laboratory and milled into powder before use. Solvent extraction was done by cold maceration whereof the resulting crude extracts were evaporated to dryness *in vacuo*. The crude extracts were then tested for antigonococcal activity using the agar well diffusion method. The MIC and MBC values were determined using the broth dilution technique.

RESULTS

The findings of this study showed that whole roots extracts of *Albizia grandibracteata* have significant activity against *Neisseria gonorrhoea* isolates. Results from the agar well diffusion exhibited that from the solvents used at different concentrations. Dichloromethane, Dichloromethane: Methanol mixture, Methanol, Chloroform, cold water, Ethyl acetate and water

decoction extracts showed significant antigonococcal activity with mean diameter zones of inhibition ranging from 53.1 % to 3.8 % that of ceftriaxone.

Tube dilutions done for the solvents showed MIC values that ranged from 0.6 mg/ml to 5.0 mg/ml and MBC values that ranged from 0.3 mg/ml to 10 mg/ml.

CONCLUSION

From this study, *Albizia grandibracteata* extracts showed concentration dependent antigonococcal activity, thus confirming its scientific basis in traditional treatment for gonococcal infections.

CHAPTER ONE: INTRODUCTION

1.1 BACKGROUND

Gonorrhoea is a bacterial infection and is the second most common sexually transmitted infection worldwide. The prevalence and incidence rates have been hard to make out because resources are mostly lacking in areas where the disease is dormant. Incidence that is available by WHO suggests that about one million STI's are acquired daily and of the 357 million new infections, 78 million infections worldwide are caused by *Neisseria gonorrhoeae* of which 11.4 million infections were from Africa (1). Few studies have been conducted in Kenya on the prevalence of gonorrhoea. Among these studies was one conducted among circumcised young men in Kisumu County in 2009, showed the incidence of gonorrhoea to be at 5.6% (2), while another study indicates a 1.1% prevalence of gonorrhoea among female sex workers in Nairobi (3). This therefore makes gonorrhoea a disease of public concern.

Gonorrhoeal infection greatly affects sexual and reproductive health worldwide as it is implicated in complications for example pelvic inflammatory disease and infertility in women (4). Studies have also shown, infection with gonorrhoea greatly increases the risk of a person acquiring Human Immunodeficiency Virus (5). The Global Burden of Disease estimates that gonorrhoea is responsible for 225,400 years lived with disability and 313,900 disability adjusted life years (6), thus having serious financial implications on the economic growth of a country.

Antibiotic resistance has increased rapidly and with the emergence of multi-drug resistant strains of *Neisseria gonorrhoeae*, the bacterial infection has become of public health concern with data from 77 countries, according to WHO- GASP showing wide spread resistance to antibiotics used to treat the infection; ciprofloxacin at 97%, azithromycin at 81% and extended spectrum cephalosporin's (ESC's) which are a last resort treatment at 66% resistance (7). This is a worrying trend that makes gonorrhoea a difficult disease to treat as therapeutic options are limited.

The current management guidelines for gonorrhoea advocate for prevention, through safe sexual behavior, which involve consistent and correct condom use; proper treatment; early diagnosis; conclusive tracking and review of new infections and rational antibiotic utilization. As much as all these measures have been put in place, there are some factors that pose a challenge to the efforts. They include: lack of awareness, stigma associated with the infection and poor capacity building of health workers. All these are stumbling blocks towards all the goals put in place to control the disease.

1.2 PROBLEM STATEMENT

Persons infected with gonorrhoea pose a huge health risk especially the ones who are asymptomatic. It is possible to control the infection through safe sex practices, early treatment and diagnosis, complete tracking and reporting of new infections and proper use of antibiotics.

Despite all the efforts being put in place, to curb the disease, challenges arise as the infection is prevalent in most parts of the world, Kenya included. This possess a great challenge on the individual economically, physically, socially, emotionally and mentally as it affects their daily life activities.

Ethno- medicine is an established concept, which has been used for very many years by different communities for primary healthcare as opposed to conventional drugs. This has been exhibited by the large proportion of the population in Sub Saharan Africa who meet their primary health care needs from traditional healers who use herbal medicine. This is because they are inexpensive, easily accessible, cater for individual preferences and most recently due to the emergence of the 'green movement' that advocates for inherent safety and desirability of natural products.

Owing to their diverse chemical properties, plants provide numerous potent antimicrobial agents that have been used in the successful treatment of various ailments including venereal diseases. There is little information regarding their efficacy and safety levels. Also, few studies have been conducted with regards to the use of traditional medicinal plants in the treatment of various strains of *Neisseria gonorrhoeae* in Kenya, therefore this study will try to evaluate the antimicrobial potency of *Albizia grandibracteata*, against *Neisseria gonorrhoeae* strains.

CHAPTER TWO: LITERATURE REVIEW

2.0 INTRODUCTION

Gonorrhoea is caused by a bacterium *Neisseria gonorrhoeae* a Gram negative cocci that affects the mucous membranes. It is associated with muco-purulent discharge. It mainly affects the oropharynx, anorectum, genital areas and ocular membrane in children. It is transmitted mostly through oral, anal or vaginal sex (8). It is estimated that men have a 20% risk of acquiring the infection from an act of vaginal sex with an infected woman, while for women the risk of acquiring the infection from an act of intercourse with an infected man lies between 60%-80%. It can also be transmitted vertically through spontaneous vertical delivery.

It is often asymptomatic but clinically manifests in men, women and children. In men it presents with urethritis, urethral discharge, and painful urination. In women it primarily affects the endocervix, extends to the urethra and vagina and presents with suppuration, lower abdominal pain and pain during sexual intercourse due to the inflammation of the uterine cervix.

Oropharyngeal gonorrhoea is also a common manifestation that can be acquired through oral sex with an infected partner. It is estimated that in 90% of the patients always present as asymptomatic, while the remaining 10% present with a sore throat (9). The infection may spread- Disseminated gonococcal Infection and may manifest as skin lesions on the upper and lower limbs; tenosynovitis and arthritis – on the knees, wrists and ankles; endocarditis; meningitis and conjunctivitis. In children it causes ophthalmia neonatorum.

Complications may arise if left untreated. They include infertility in women due to pelvic inflammatory disease that results to obliteration and fibrosis of the fallopian tubes. Ectopic pregnancies may also occur. In children, the infection may lead to blindness (10). In men it may lead to infertility due to prostatitis and epididymitis (11); urethral strictures may form due to the pathological process of the infection. Studies have also shown an increased risk of developing prostate cancer by 20% due to a previous infection with gonorrhoea (12). Infection with gonorrhoea has been shown to increase the risk of acquiring Human Immunodeficiency Virus (13).

2.1 DIAGNOSIS AND ANTIBIOTIC RESISTANCE

Gonorrhoea 'superbug' is spreading globally, with reported cases of fluoroquinolone resistance, cephalosporin resistance and tetracycline resistance in Sub Saharan Africa (16). Penicillin were successfully used for decades, for the effective treatment of gonorrhoea after sulfonamide failure (20). Penicillin resistance was acquired through mutation of genes associated with cell wall biosynthesis thus affecting periplasmic drug concentration (21), hence the drugs in this category were rendered no longer effective for the treatment of gonococcal infection.

Tetracycline was also used for the treatment of gonorrhoea infection, but discontinued due to resistance acquired through mutation in specific genes and due to plasmid encoded Tet M protein

blocks at the 30s ribosomal subunit which is the target site for this particular antibiotic, hence inactivity.

In the United States of America, ciprofloxacin a fluoroquinolone, is no longer used in the treatment of gonorrhoea, due to wide spread resistance reported in 17 cities between 1990 and 2006 thus leading to increased incidence of the infection (14). In Kenya a study was conducted in 2012, four clinics showed 53.2% prevalence of fluoroquinolone resistant gonorrhoea (15). Fluoroquinolone resistance is conferred through amino acid substitutions in DNA gyrase and DNA topoisomerase (16), making it ineffective against the treatment of gonorrhoea.

There has been an increase in *Neisseria gonorrhoea* isolates that are resistant to oral cephalosporin and an emanation of intermediate level resistance to injectable cephalosporin (17). Resistance to oral cephalosporin was initially reported in Japan from two male patients with gonococcal urethritis (18). This was then subsequently followed by resistance to third generation cephalosporin that was reported in many regions in Japan (19). Reduced susceptibility of cephalosporin was also reported worldwide in countries such as India (20), United States of America (21) and Greece (22). In South Africa a study conducted on *Neisseria gonorrhoea* isolates from two gay men, showed resistance to cefixime an extended spectrum cephalosporin (23).

In 2016, United Nations world health assembly supported the WHO global health sector strategy on sexually transmitted infections 2016-2021. Among its strategies was to reduce the incidence of gonorrhoea by 90% through addressing the problem that of its antimicrobial resistance. Dual drug therapy for anorectal, genital and oropharyngeal gonococcal infection was then recommended (24). The use of 250mg of intramuscular ceftriaxone and 1g of oral Azithromycin as a single dose or 400mg of oral Cefixime and 1g of oral Azithromycin as a single dose in the absence of ceftriaxone. A single dose of either intramuscular 250mg ceftriaxone, 400mg oral cefixime or 2g intramuscular spectinomycin can be used in singular therapy of gonococcal infection. For the treatment guidelines to be effective, local and global resistance patterns are to be put into consideration.

In the event of treatment failure, retreatment of the infection is recommended. Intramuscular ceftriaxone 500mg and 2g oral azithromycin as single dose or 800mg oral cefixime and 2g of oral azithromycin as a single dose or intramuscular gentamicin 240mg and 2g of oral azithromycin as a single dose and in the absence of oropharyngeal gonococcal infection Spectinomycin 2g intramuscular and 2g oral azithromycin as a single dose is used.

For neonatal gonococcal conjunctivitis, topical ocular prophylaxis is done with either 1% tetracycline hydrochloride, 0.5% erythromycin eye ointment, water-based solution of 2.5% povidine iodine or 1% silver nitrate solution. If infected the recommended treatment is with 50mg/kg intramuscular ceftriaxone as a single dose or a single dose of intramuscular kanamycin 25mg/kg or a single dose of intramuscular spectinomycin 25mg/kg. Table 2.1 summarizes the different classes of antibiotics and their mechanical action used in the treatment of gonococcal infection.

Table 2. 1 Antibiotics used in the treatment of gonococcal infection.

CLASS	ANTIBIOTIC	MECHANISM OF ACTION
Sulfonamides	Trimethoprim/ sulfamethoxazole	Folic acid synthesis inhibitors
Penicillin	Amoxicillin Ampicillin Ampicillin + sulbactam	Cell wall synthesis inhibitors
Aminoglycoside	Gentamicin Kanamycin Spectinomycin	Protein synthesis inhibitor at the 30s ribosomal subunit
Macrolide	Erythromycin Azithromycin	Protein synthesis inhibitor at the 50s ribosomal subunit
Tetracycline	Doxycycline Oxytetracycline	Protein synthesis inhibitors at the 30s ribosomal subunit
Fluoroquinolone	Ciprofloxacin Grepafloxacin	DNA synthesis inhibitors
Cephalosporin	Ceftriaxone Cefixime	Cell wall synthesis inhibitors
Monobactam	Aztreonam	Cell wall synthesis inhibitors

Low and middle income countries rely on syndromic management of infections which involves identification of congruous, easy to recognize clinical manifestations, which subsequently manage treatment without the use of laboratory assays. Management of symptoms lacks antibiotic susceptibility testing, poor positive predictive value (PPV) resulting in the overuse of antibiotics hence antimicrobial resistance (25).

Diagnosis of gonorrhoea is often done in the laboratory using Gram stain and culture, which provides presumptive diagnosis of gonorrhoea especially among symptomatic men with urethritis. It is a good option in low income settings but often takes a long time to get the test results, the procedure is labor intensive and is less reliable for cervical, rectal and pharyngeal gonococcal infections (26). Other newer, more efficient and effective techniques such as the nuclear amplification assays are now available but are often expensive and are unable to provide data on antimicrobial susceptibility (27). Despite this, they are highly sensitive and specific and can be used to generate results from a wide array of samples. For example, urine, vulvovaginal, cervical and urethral swabs. Accurate diagnostic tests used to diagnose asymptomatic infections are scarce in low and middle income countries where the burden of infection is high. In countries where they are available they are often very expensive and geographically inaccessible hence getting results become a challenge. This therefore impedes on appropriate treatment and follow up for those who are infected (28).

2.2 ALBIZIA SPECIES

2.2.1 ETHNOMEDICINAL VALUE

In various parts of the world, medicinal plants have been used to treat many infectious diseases, with the World Health Organization (WHO) estimating that more than 70% of the world's population residing in developing countries make use these plants for primary health care (29). Plants are possible sources of antimicrobial agents (30) and through the discovery of conventional drugs such as vincristine, digoxin, atermisinin, quinine from medicinal plants show their potential in provision of novel and potent pharmaceutical agents (31). As a result of this discoveries, there is increased research in ethno pharmacology of medicinal plants.

Albizia species are endemic in India and are widely used worldwide for their medicinal properties(32). In traditional Chinese medicine, the flowers of these species are implicated with the treatment of anxiety, depression and insomnia. *Albizia lebeck* is used as a constricting agent by some cultures and is used to treat oils, cough, eye infections, flu, gingivitis, lung associated ailments and it is purported to have psychoactive properties. *Albizia amara* is also used as a blistering agent and is used in the treatment of piles, diarrhea and gonorrhoea. *Albizia grandibracteata* is used in Western Kenya traditionally for the treatment of malaria, gonorrhoea, flu, fever and headache. This information was retrieved from ethno botanical records which show how indigenous communities use different plants and has not been verified via scientific evaluation of their effectiveness claims.

2.2.2 PHARMACOLOGICAL IMPORTANCE

The *Albizia* species contain bioactive compounds some of which have been isolated and identified and have been evaluated scientifically found to contain specific pharmacological properties. Some isolated phytoconstituents include: saponins, alkaloids, terpenes and flavonoids. They have been proven to possess anti diabetic properties and sedation properties as in *Albizia julibrissin* (33); antioxidant properties has been seen in *Albizia amara* (34); anti-inflammatory properties in *Albizia lebeck* (35); for the treatment of asthma, arthritis, burns and cytotoxic activity against human aqueous cell carcinoma; antiplasmodial activity has been seen in *Albizia saman* (36), *Albizia adinocephala* stem and bark extracts have been found to inhibit malarial enzyme plasmepsin and *Albizia lebeck* has been proven to have hepatoprotective properties (37) and its bark extract shows good prognosis when bruises, boils and hemorrhoids. Lipophilic extracts of *Albizia gummifera* used in a study in Uganda illustrated significant antitrypanosomal activity (38). Antifungal activity especially on *candida albicans* and cytotoxic activity has been seen in *Albizia inundata* against human head and neck squamous cell and melanoma cells (39). Table 2.2 summarizes various medicinal uses of different *Albizia* species and the parts with medicinal value.

Table 2.2 Summary of the medicinal uses of different Albizia species and the parts used.

PLANT SPECIES	PART USED	MEDICINAL USE
<i>Albizia adinocephala</i>	Stem bark	Anti-plasmodial activity
<i>Albizia amara</i>	Leaves	Antioxidant properties
<i>Albizia gummifera</i>	Root bark	Anti-trypanosomal activity
<i>Albizia inundata</i>		Antifungal activity – <i>candida albicans</i> Cytotoxic activity against head and neck squamous cell and melanoma cells
<i>Albizia julibrissin</i>	Flowers	Anti-plasmodial activity
<i>Albizia lebbek</i>	Leaves Stem bark	Anti-inflammatory activity (asthma, arthritis, burns) Cytotoxic activity against human aqueous cell carcinoma Anti-diabetic activity and sedation properties
<i>Albizia saman</i>	Leaves	Antiplasmodial activity

2.2.3 ALBIZIA GRANDIBRACTEATA

This tree is commonly referred to as the ‘Red Nogo’ and is mainly located in tropical Africa. It is found in South Sudan, Eastern Democratic Republic of Congo, Uganda, Kenya, Rwanda, Burundi and Northern Tanzania along the rainforests, grasslands and riverine forests (40). In Kenya, It is found in Kakamega forest in Kakamega County and is locally referred to as ‘*mukhunzuli*’ among the luhya community (41). It is a mid-sized deciduous tree that belongs to the family Fabaceae. It has a straight trunk of 20 meters and a flattened crown. The bark is fairly smooth, pale grey-brown. It has compound leaves that appear pink–red when young. It has colorful flowers with hemispherical heads, mostly pink with dark red anthers, seen well beyond their petals (42).



Figure 2. 1 A picture of *Albizia grandibracteata* on site, in Kakamega forest (a photograph taken by the author in August 2018)

It has various traditional medicinal uses: Its leaf infusion is used in the Democratic Republic of Congo in vapor for the treatment of fever, in Uganda, its leaves are pounded and taken to treat diarrhea and in Kenya the root infusion is used for the treatment of tonsillitis (43), fever, malaria, headache and flu (44). In the treatment of gonorrhoea, the whole root is boiled and its resulting decoction administered in a cup, to be taken over a period of seven days (41). The prognosis of the treatment depends on early reporting of the symptoms. A study conducted in Uganda revealed that methanolic extracts of leaves and saponins isolated from have shown antitumor activity against KB and MCF7 cell lines (45).

2.2.4 PLANTS EFFECTIVE AGAINST *NEISSERIA GONORRHEA* ISOLATES

Other plants have been identified and their extracts have proven effective against the activity *Neisseria gonorrhoeae*. A study done on the root extracts of *jatropha curcas* was found to have significant antigonococcal activity (46). Ethanol extract from the leaves of *Eupatorium odoratum* used by Nepalese folk, was found to be effective against *Neisseria gonorrhoeae* with a maximum zone of inhibition of the ten medicinal plants used in the study (47). In rural South Africa, *aloe ferox* is commonly used for the treatment of sexually transmitted infections, upon *in vitro* testing, methanol extracts of the plant showed antimicrobial activity against six strains of *Neisseria gonorrhoeae* (48). A study conducted in central Uganda, revealed ether root extract from *cassia alata* showed highest potency against clinically resistant *Neisseria gonorrhoeae*, while crude extracts of the same showed concentration dependent *Neisseria gonorrhoeae* inhibition (49). *In vitro* activity of *eugenol* an active compound of *Ocimum sanctum* was found to have anti gonococcal activity against multi drug resistant isolates of *Neisseria gonorrhoeae*

(50). Lastly, a study on the activity of topical microbicides – polyherbal cream showed bacteriostatic activity on WHO strains and clinical isolates of *Neisseria gonorrhoea*. This was inclusive of the strains that were resistant to ciprofloxacin, penicillin and tetracycline (51).

2.3 STUDY JUSTIFICATION

The burden of sexually transmitted infections varies by sex and geographical area with the greatest infection occurring in resource poor countries. The second most sexually transmitted bacterial infection in the world is gonorrhoea. World Health Organization gives an estimate 78 million cases of gonococcal infections every year. Global incidence of gonococcal infections is estimated to be at 19 per 1,000 cases in women while in men it is estimated to be at 24 per 1,000. The global prevalence of gonorrhoea as at 2012 was estimated to be at 0.8 % among women and 0.6% among men aged 15-49 years of age. The highest prevalence was reported in Africa and Western Pacific Region.

Often times, gonorrhoea presents as a co-infection with chlamydia. When left untreated or undiagnosed it results to a sequel of complications: - pelvic inflammatory disease, infertility, ectopic pregnancies, miscarriages; adverse pregnancy outcomes such as congenital infections, still births and preterm delivery. It also affects an individual psychologically and increases the transmission of HIV.

Gonorrhoea has become a challenge globally as the current treatment regimens are not effective, due to the emergence of the drug resistant strains. The last resort treatment, which are the Expanded Spectrum Cephalosporin are often expensive, cause discomfort as they are administered through injection and are often associated with adverse drug reactions such as blood anomalies and disulfiram like reaction with ethanol. Tests for the asymptomatic detection of the gonorrhoea are inaccessible, often very expensive impeding greatly on early and prompt treatment and follow up. There is therefore a need to find alternative, easily accessible, inexpensive treatment with high efficacy – greater than 95% that can be used to safely treat the infection. Studies conducted on various medicinal plants against gonococcal activity have shown potent bioactive compounds that inhibit growth and activity of different isolates of *Neisseria gonorrhoea*. Therefore, this study proposes to assess the antimicrobial potency of *Albizia grandibracteata* against strains of *Neisseria gonorrhoeae*.

2.4 RESEARCH QUESTION

What is the antimicrobial potency of *Albizia grandibracteata* against *Neisseria gonorrhoeae*?

2.5 OBJECTIVES

2.5.1 MAIN OBJECTIVE

This study was conducted to evaluate the antimicrobial activity of *Albizia grandibracteata* extracts against *Neisseria gonorrhoeae*.

2.5.2 SPECIFIC OBJECTIVES

The specific objectives of this study were to:

1. Determine the antimicrobial susceptibility pattern of *Neisseria gonorrhoeae*.
2. Determine the Minimum Inhibition Concentration (MIC) of extracts of *Albizia grandibracteata* against *Neisseria gonorrhoeae*.
3. Determine the Minimum Bactericidal Concentration (MBC) of extracts of *Albizia grandibracteata* against *Neisseria gonorrhoeae*

CHAPTER THREE: METHODOLOGY

3.0 INTRODUCTION

This was an experimental study that employed solvent extraction using different solvents facilitating the release of different bioactive compounds from the plant material. The extraction process involved pre-washing, drying and milling of the plant material to obtain a fine powder that improved the dynamics of analytical extraction and also increased the surface area of the material with the solvent system (52) .

Antimicrobial screening assays were then used to test crude extracts of *Albizia grandibracteata* against *Neisseria gonorrhoea*. Agar well diffusion and broth dilution techniques were then used to obtain the MIC and MBC of the plant extracts as per a study conducted by (53) and (54) .

3.1 PLANT COLLECTION AREA

The roots of the plant were collected in Kakamega forest in Kakamega County. The forest is located Northwest of Nairobi County and near the Uganda border. It is the only tropical forest in Kenya with the last residuum of the early Guineo- Congolian rainforest and is found between 00^o 08' 30.5" to 00^o 23' 12.5" North and 34^o 18' 08" to 34^o 57' 26.5" East from an altitude of 1520 to 1680 meters above sea level (55). It covers an area of 238 km² which is inclusive of Kakamega National Reserve and Kisere Forest Reserve which are gazetted and are under government protection.

It experiences long period of rains in the months of April and May and short duration of rains in the months of September and October. It receives a mean yearly rainfall of 2000 mm with a mean annual temperature of 20.5°C. It consists of at least 380 plant species, 330 bird species, mammals especially primates such as the potto, white and lack Columbus monkeys and the vervet monkey, amphibians, reptiles and invertebrates. It is a common tourism attraction site for bird watching (56) . Conservative harvesting of the roots was done in the Isecheno and Buyangu blocks where the *Albizia grandibracteata* species are found.

The forest neighbors the Kenyan Bantu luhya tribe, who have a population density of 618.20 persons per square kilometer as of 2016 according to data collected by kneoma (57). Their main economic activity is farming (58) ,as per 2015 report by the Kenya National Bureau of Statistics hence the forest faces the threat of extinction due to increased human activity such as agriculture and encroachment as a result of increased population.

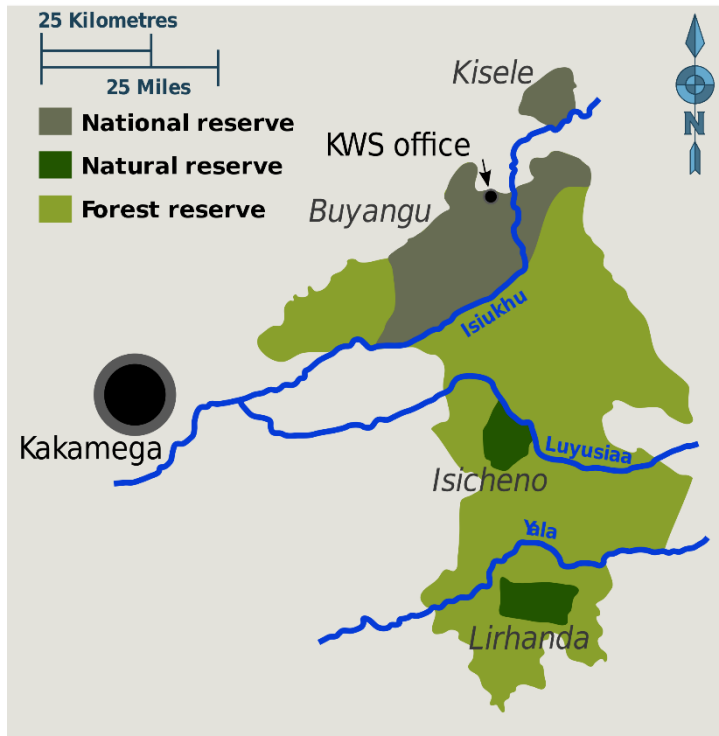


Figure 3. 1 A Map of Kakamega forest
courtesy of Wikimedia Commons (59)

3.2 PLANT MATERIAL

Whole roots of *Albizia grandibracteata* were collected from Kakamega forest, Kakamega County in September 2018. In order to ensure proper botanical placement, a plant herbarium specimen was prepared and deposited at the University herbarium, Department of Botany, School of Biological Sciences, University of Nairobi for authentication. The voucher specimen was then documented and given a voucher specimen number – IMS 2018/01. The plant material was then air dried under ambient condition, ground to powder and kept in a plastic container for use.



Figure 3. 2 Fresh voucher specimen number – IMS 2018/01 (a photograph taken by the author in August 2018)

3.3 EXTRACTION OF THE PLANT MATERIAL

3.3.1 SOLVENTS, REAGENTS, MATERIALS AND EQUIPMENT

General purpose reagents: - ethyl acetate, chloroform, dichloromethane, methanol (Kobian Kenya Ltd, Kenya) and water were distilled and used for extraction. Dimethyl Sulfoxide (Fischer Scientific, Loughborough, United Kingdom) was used to prepare aqueous suspensions for antimicrobial activity.

Filtrations to remove particulate matter from crude extracts was done using Whatmann filter paper No.1 (Whatmann International Ltd, Maidstone, England).

A Heidolph VV2000® rotary vacuum evaporator (Heidolph Electro GmbH &Co. KG, Kelheim, Germany) connected to a Laborota4000 cooler (Polyscience, Niles, USA) a WB2000® water bath (Heidolph Electro GmbH &Co. KG, Kelheim, Germany) and a diaphragm vacuum pump (KNF Neuberger GmbH, Freiburg, Germany) was used to reduce to dryness extracted plant material for antimicrobial activity testing.

All glassware used in the antimicrobial activity studies were sterilized in a Memmert® universal oven (Memmert GmbH &Co. KG, Schwabach, Germany) using dry heat at 150°C for an hour. A portable autoclave (Dixon's Surgical Instruments Ltd, Essex, UK) was used to sterilize the nutrient media at 121°C for fifteen minutes. The microorganisms- *Neisseria gonorrhoeae* were incubated in a CO₂ incubator.

The bench work involving the use of the microorganisms was carried out in a Bioflow® laminar flow cabinet (Vermeulin L. J BVBA, Westmalle, Belgium) while the loops were sterilized by a Bunsen burner flame.

3.3.2 CRUDE EXTRACTS OF ORGANIC SOLVENTS

The dried whole roots of *Albizia grandibracteata* were milled into powder. The extraction technique employed was maceration. This study used, cold extraction technique which involved, placing 100g of the powdered plant material in a stoppered conical flask and soaking it with 800 mls of different solvents: - methanol, ethyl acetate, dichloromethane, chloroform and dichloromethane-methanol (50:50). They then underwent maceration for 24 hours with continuous magnetic stirring.

The extracts of each solvent were filtered using Whatman filter paper and filtrates collected in stoppered conical flasks.

3.3.3 PREPARATION OF AQUEOUS EXTRACT

For the maceration one hundred grams of the plant powder was soaked in 800 milliliters of distilled water and stirred continuously by magnetic stirring for 24 hours.

Decoction was applied to 100g of the powdered plant material soaked in 800mls of distilled water, boiled and magnetically stirred for an hour, allowed to cool and was strained using Whatman filter paper and filtrate collected in a conical flask.

3.3.4 CONCENTRATION OF THE EXTRACT

Organic extracts were dried *in vacuo* by a rotary evaporator to dry excess solvent hence concentrating the extract. The apparatus contained a vacuum chamber which created a reducing pressure that subsequently reduced the boiling point of a solvent thus concentrating the extract.

Aqueous extracts were separately freeze dried using dry ice which left behind a dry residue that was the concentrated extract.

All extracts were tightly sealed and refrigerated at 4°C until their use.

The solvent extraction process is illustrated in figure 3.3.

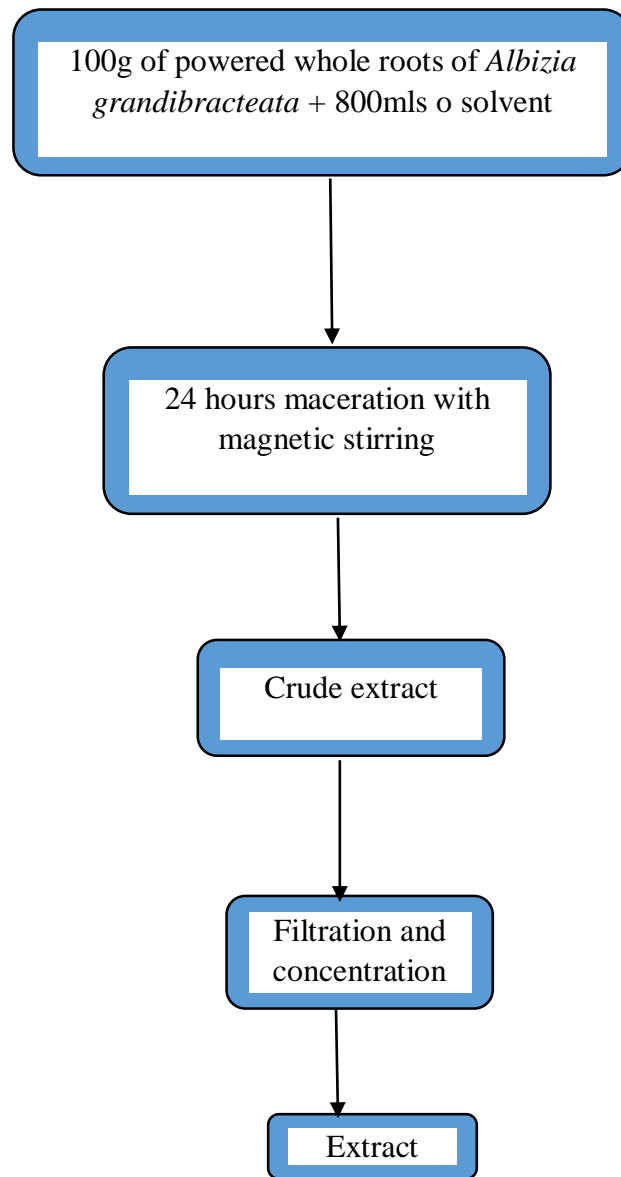


Figure 3. 3 Solvent extraction scheme of *Albizia grandibracteata* whole root.

3.3.5 DETERMINATION OF EXTRACT YIELDS

The percentage yield of the dried plant extract as calculated using the formula below:

$$(\%) \text{YIELD} = (W_2 - W_1) \div W_0 \times 100$$

Where, W_2 is the weight of the extract and the container, W_1 is the weight of the empty container alone and W_0 is the weight of the powder of the plant material (60) which was 100 grams.

3.4 ANTIGONOCOCCAL ACTIVITY TESTS

There are different bio assays that are used to determine *in vitro* antimicrobial activity of different antimicrobial agents. They include: disc diffusion, well diffusion, agar and broth dilution. In this era of antibiotic resistance, research and development of new antimicrobial agents has been on the forefront with a lot of emphasis being put on antimicrobial activity screening and evaluation of methods.

The agar well diffusion method and the Macro dilution technique were used to determine antigonococcal activity of *Albizia grandibracteata* against *Neisseria gonorrhoeae* isolate (61).

3.4.1. TEST MICROORGANISMS

The microorganisms *Neisseria gonorrhoeae* strain was obtained from the stock culture of University of Nairobi, Department of Medical Microbiology Laboratory.

3.4.2. ANTIMICROBIAL SUSCEPTIBILITY TESTS

3.4.2.1 ANTIBIOTIC SENSITIVITY TEST

Antibiotic susceptibility testing of *Neisseria gonorrhoeae* isolates was examined using the Kirby Bauer disc diffusion method. The method employed antibiotic discs with the following concentrations: penicillin (10 μ g), ciprofloxacin (30 μ g), tetracycline (30 μ g), ceftriaxone (30 μ g), and azithromycin (15 μ g) as per CLSI guidelines and protocol (2012).

3.4.2.2 AGAR WELL DIFFUSION METHOD

The antimicrobial screening of the aqueous and solvent extracts was determined by Agar well diffusion method recommended by the Clinical and Laboratory Standards Institute and Okeke et al. (62)

3.4.2.3 PREPARATION OF PLANT EXTRACTS.

The plant extracts were dissolved in DMSO, to prepare stock solutions. A stock solution of 40mg/ml was obtained from each extract and two fold serial dilutions were made using DMSO

to obtain the following concentrations: 40 mg/ml, 20 mg/ml, 10 mg/ml, 5 mg/ml and lastly 2.5 mg/ml. This is because DMSO can make solutions with both polar and non – polar compounds and is capable of forming a homogenous mixture with many of organic solvents and water. Fifty microliters of each concentration was filled in each well.

Ceftriaxone 150 mg working standard with a potency of 84.2%, weighed at 29.4 mg was dissolved in 10 mls of DMSO to achieve a final concentration of 0.294 mg/ml. It later underwent dilution using distilled water to a final concentration of 0.1 mg/ml. This acted as the positive control.

3.4.2.4 INOCULUM AND INOCULATION PROCEDURE.

The inoculation density was attained by achieving a standardized concentration of 1.5×10^8 colony forming units/ml. This was done using the growth method, where two to five single colonies from a GC agar plate culture were suspended in 4 mls of Mueller Hinton broth with a sterile loop and incubated at 37°C, in 5% CO₂ until visibly turbid. This is known as the 0.5 McFarland standard (63). The stock solution formed was used within 10 minutes of standardization to prevent loss of viability and change of inoculum size.

A sterile swab was then dipped into the broth and used to transfer the standardized suspension onto plates with already prepared GC agar base (GC agar base medium, HIMEDIA, India) with Bovine haem powder (OXIOD, UK), Vitox and VCNT enrichment supplements. The sterile cotton swab was then used to make streaks on the dried media. The plate was then rotated at 60 degrees to ensure the inoculum was distributed evenly on the entire agar plate.

To ensure that excess moisture is removed, the lid were left aside for a few minutes, but not more than 10 minutes.

3.4.2.5 PREPARATION OF THE AGAR WELLS

The wells were made by punching the agar using sterile cork borers (6 mm in diameter) into agar plates containing inoculums.

The Modified Thayer Martin agar contained: GC agar medium (HIMEDIA, India) with bovine haem powder (OXOID, UK) and Vitox and VCNT enrichment supplement were prepared as per the manufacturer's instructions and autoclaved at 121°C for 15 minutes for sterility.

Each plate comprised of only five wells equidistant to each other to avoid overlapping of the different zones of inhibition. The five wells were treated with different concentrations of the plant extracts; another plate was designated for the controls only- where for the positive control, a standard antibiotic solution- ceftriaxone 0.1mg/ml was used and the other two wells that were punched onto the agar medium and were designated for the negative control and the extract displaying the highest zone of inhibition. Fifty microliters each of DMSO, the most active extract

and ceftriaxone solution were then introduced into the wells. These plates were left to dry in a sterile conditions for an hour, inverted and incubated at 37°C for 24 hours in 5% CO₂, after which the diameter of the zones of inhibition were calculated if present, to the nearest millimeter with the use of a calibrated ruler. The same was applied to the plate with the controls to measure antibacterial activity assessment.

To ensure reliability, each test was done in triplicates and the average measurements of the extracts and antibiotic calculated. After the incubation, all the plates were examined for the presence of inhibition per concentration as a property of antimicrobial activity. The results were compared to the activity of the standard.

3.5 DETERMINATION OF INHIBITORY CONCENTRATION (MIC) AND MINIMUM BACTERICIDAL ACTIVITY (MBC)

3.5.1 DETERMINATION OF MIC

This study defined MIC as the highest dilution of the plant extract that inhibited growth of *Neisseria gonorrhoeae*. Plant extracts which showed zones of inhibition in the agar well diffusion method, were further tested to determine their MIC values by broth dilution as per the Clinical and Laboratory Standard Institute guidelines(64).

Macro dilution was used as a quantitative measurement to determine in vitro antimicrobial property of *Albizia grandibracteata* against *Neisseria gonorrhoeae* isolate.

3.5.2 METHOD OF DILUTION

The dilution susceptibility method was achieved by serially diluting (two fold) the plant extracts in sterile Mueller Hinton broth thus obtaining different dilutions, which were used to determine the MIC and MBC.

Growth technique: Four to six properly isolated colonies of the microorganism with the identical morphologic characteristics were sort out from the agar plates and used in different ways. A few of them were used to make the cell suspension by being aseptically transferred into the test tubes containing 5 mls of freshly prepared MH broth medium and incubated at 37°C for 18- 24 hours in 5% CO₂ until visibly turbid in order to achieve the 0.5 McFarland turbidity standard. The rest were utilized in making Gram stain smears to unveil the characteristic gram negative diplococci, as a confirmatory test for the actual growth of *Neisseria gonorrhoeae*.

Dilution technique: Ten test tubes were allocated for each plant extract and a stock solution of 10mg/ml for each extract was then prepared. The first test tube contained 2 mls of the stock

solution (10 mg/ml). Serial dilutions were done in two fold for the other eight test tubes to obtain the following concentrations: 5 mg/ml, 2.5mg/ml, 1.3 mg/ml, 0.6 mg/ml, 0.3 mg/ml, 0.2 mg/ml, 0.1 mg/ml and lastly 0.04 mg/ml. A suspension of the microorganism of fifty microliters was then added to each of the broth dilutions.

A test tube containing two milliliters of sterile MH broth and fifty microliters of the suspended test organism was used as the positive control while a test tube containing two milliliters of sterile MH broth and fifty microliters of sterile water was used as the negative control.

The test tubes were then incubated for 18 -24 hours at 37°C in 5% CO₂.

3.5.3 MINIMUM INHIBITORY CONCENTRATION (MIC).

After overnight incubation, turbidity of the solutions was assessed, to determine whether there was bacterial growth. To ensure growth or lack of it in each tube, a sterile loop of the suspensions in each tube were sub cultured onto plates with GC agar incubated for 18-24 hours, in 5% CO₂ at 37°C.

After overnight incubation, the plates were examined for presence of growth of *Neisseria gonorrhoeae*. The lowest concentration of extract dilution showing no visible growth was then recorded as the MIC.

3.5.4 MINIMUM BACTERICIDAL ACTIVITY (MBC).

MBC was described as the least concentration of antimicrobials that will kill the microorganism after an overnight incubation. Using the MIC tubes, the tube that comes after the MIC tube, contains the MBC.

After MIC determination, the test tube without growth of the bacteria was sub cultured onto GC agar plates and incubated for 24 hours, in 5% CO₂ at 37°C.

MBC was defined the lowest concentration of the sub cultured test tube which does not show any visible growth after macroscopic evaluation (65). All the readings were taken in triplicate.

CHAPTER FOUR: RESULTS

4.0 INTRODUCTION

This chapter described the study results on percentage yield acquired from the plant using cold maceration as a solvent extraction technique, antibiotic susceptibility pattern of the organism, mean diameter of zones of inhibition from the crude extract of the different solvents, Minimum inhibitory concentration and minimum bactericidal concentration from the crude extracts.

4.1 PERCENTAGE YIELD

During solvent extraction, cold maceration technique was used to retrieve possible bioactive components from the plant material that were responsible for its antigonococcal activity. This involved soaking the ground plant material in 800 mls of solvent with continuous stirring for 24 hours after which the mixture was filtered and the filtrate then went through rotary evaporation to dry the excess solvent.

The results are displayed in the table 4.1.

Table 4. 1 Percentage yield acquired from different solvents during solvent extraction

SOLVENT	W ₁ (grams)	W ₂ (grams)	W ₃ (grams)	% YIELD
METHANOL	42.9	46.7	3.8	3.8
DCM	56.6	57.1	0.5	0.5
ETHYL ACETATE	50.6	51.4	0.8	0.8
CHLOROFORM	46.8	48.1	1.2	1.2
DCM:METHANOL (50:50)	50.6	52.7	2.2	2.2
WATER DECOCTION	58.3	65.2	6.9	6.9
COLD WATER EXTRACT	42.5	45.0	2.5	2.5

The percentage yield of the dried whole root of *Albizia grandibracteata* ranged from 6.9% to 0.5%. The highest yield was obtained from the aqueous extract that underwent decoction at 6.9%, which was followed by methanol at 3.8%, then the cold water extract at 2.5%, then the dichloromethane- methanol 50:50 at 2.5% and the lowest yield was extracted from dichloromethane at 0.5%.

4.2 ANTIBIOTIC SUSCEPTIBILITY PATTERN

Five antibiotic discs were employed to evaluate the antibiotic sensitivity of *Neisseria gonorrhoea* to each drug with the use of the Kirby Bauer disc diffusion method. The test was done in triplicates and the mean of the zones of inhibition calculated.

Ceftriaxone 30 μ g displayed the biggest zone of inhibition at 38.0 mm, followed by azithromycin 15 μ g at 36.0mm, then ciprofloxacin at 34.0mm, then tetracycline 30 μ g at 18.0mm and lastly penicillin 10 μ g at 12.0mm.

The controls used for the purpose this study were ceftriaxone working standard at a concentration of 0.1 mg/ml as the positive control which exhibited a mean one of inhibition of 38.0mm, hence showing susceptibility of the organism towards it. Fifty microliters of pure analytic standard DMSO was used as a negative control and displayed no activity against the organism. See table 4.2 for more details on its antibiotic susceptibility pattern.

Table 4. 2. Antibiotic susceptibility pattern of various antibiotics

ANTIBIOTIC	CONCENTRATION (μ g/ml)	ZONE OF INHIBITION (mm)
Ceftriaxone	30	38.0
Penicillin	10	12.0
Ciprofloxacin	30	34.0
Azithromycin	15	36.0
Tetracycline	30	18.0

4.3 ANTIMICROBIAL ACTIVITY OF THE CRUDE EXTRACTS

4.3.1 ETHYL ACETATE EXTRACT

The crude extract from ethyl acetate was diluted in different concentrations ranging from 40 g/ml to 2.5 mg/ml and were used for antimicrobial susceptibility testing.

At 40 mg/ml the mean diameter of zone of inhibition was 21.0 mm, at 20 mg/ml it was at 18.0 mm, at 10 mg/ml it was at 16.0 mm, 5 mg/ml it was at 15.0 mm and at 2.5 mg/ml zone of inhibition was at 13.0 mm.

Figure 4.1 below shows zones of inhibition displayed against the gonococci on a culture plate.

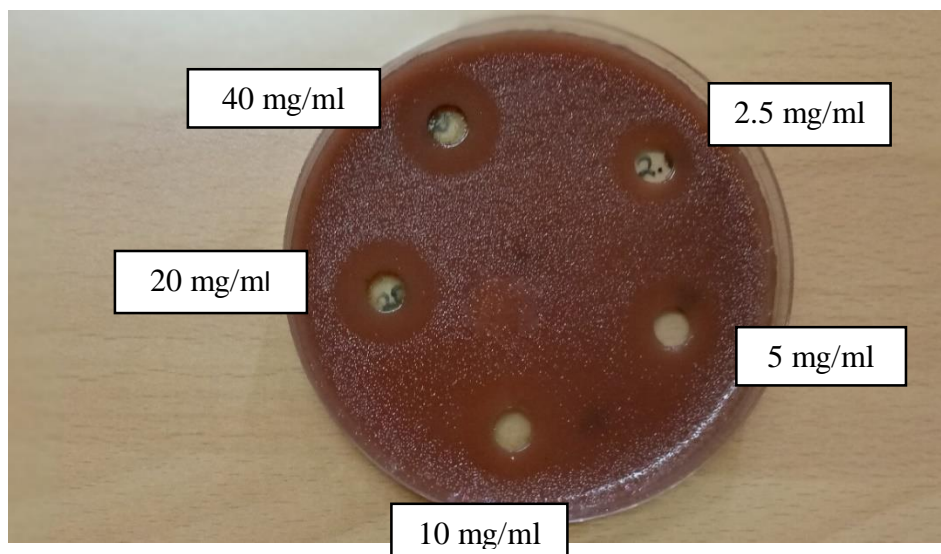


Figure 4. 1. Culture plate showing zones of inhibition from Ethyl acetate crude extract.

4.3.2 DICHLOROMETHANE EXTRACT

The crude extract from dichloromethane had different concentrations that ranged from 20mg/ml to 2.5 mg/ml. At a concentration of 20 mg/ml the mean diameter of the zone o inhibition was at 22.0 mm, at 10 mg/ml it as at 21.0 mm, at 5mg/ml it was at 20.0 mm and at 2.5 mg/ml it was at 18.0 mm.

These results are seen in figure 4.2 below.

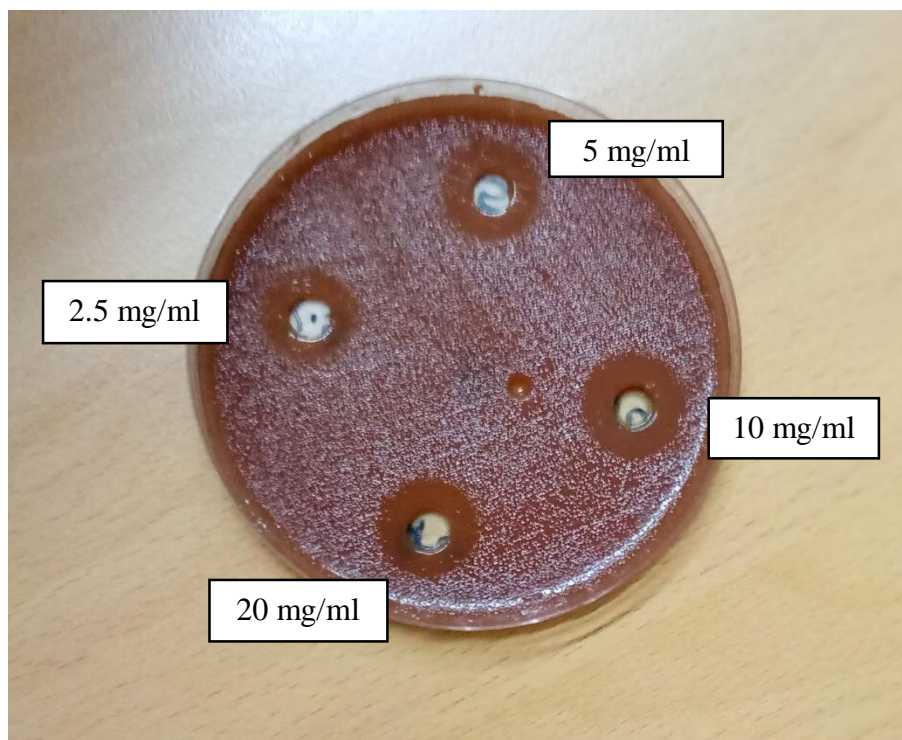


Figure 4. 2 Culture plate showing zones of inhibition from Dichloromethane crude extract.

4.3.3 DICHLOROMETHANE-METHANOL (50:50) EXTRACT

The crude extract from the above mixture was obtained and various concentrations were prepared to test for antigonococcal properties.

At 40 mg/ml a mean diameter zone of inhibition of 20.0 mm was obtained, at 20 mg/ml it was at 19.0 mm, at 10 mg/ml it was at 17.0 mm, while at 5 mg/ml and 2.5 mg/ml the mean diameter zones were 16.0 mm and 14.0 mm respectively.

Figure 4.3 below, displays the results below.

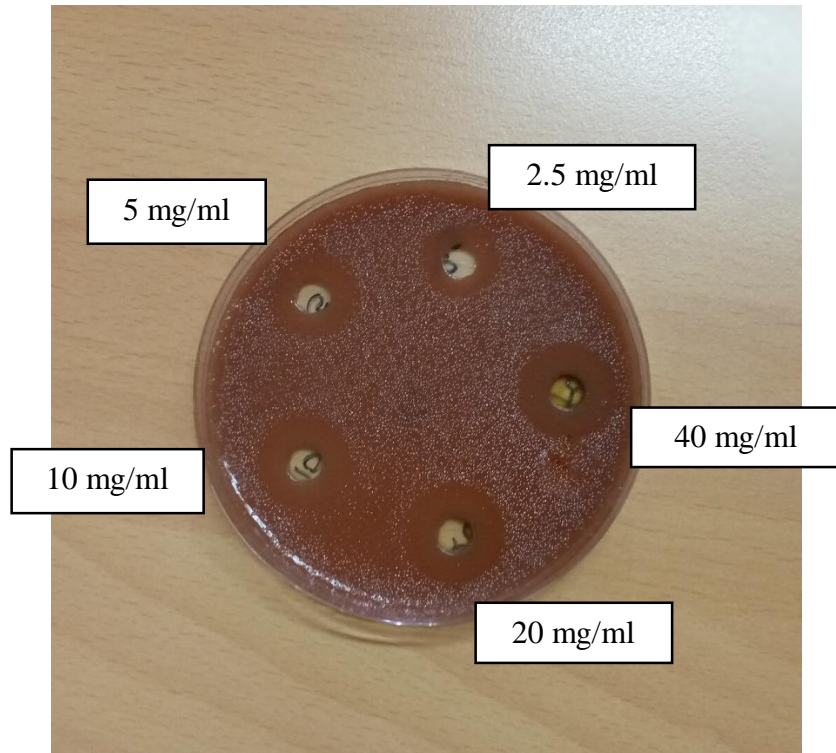


Figure 4. 3 Culture plate showing zones of inhibition from Dichloromethane-Methanol (50:50) mixture crude extract.

4.3.4 CHLOROFORM EXTRACT

At a concentration of 2.5 mg/ml the mean diameter zone of inhibition was at 16.0 mm, at 5mg/ml it was at 19.0 mm, at 10 mg/ml it was at 20.0 mm at 20mg/ml it was at 21.0 mm and lastly at 40 mg/ml the zone of inhibition was at 23.0 mm

Figure 4.4 displays results from the culture of chloroform crude extract against *Neisseria gonorrhoeae*.

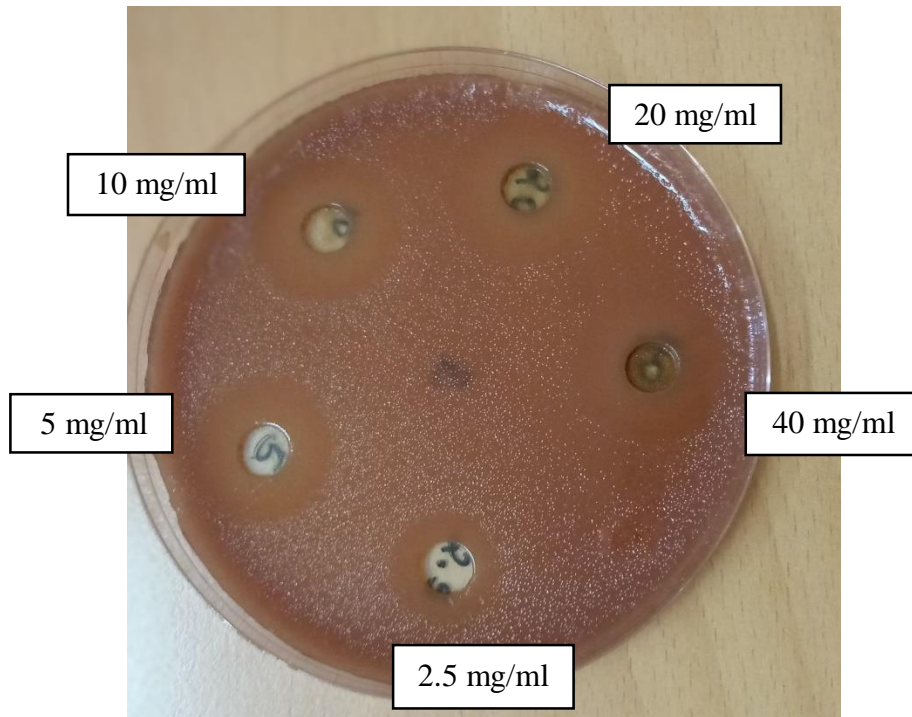


Figure 4. 4 Culture plate showing zones of inhibition from Chloroform crude extract.

4.3.5 COLD WATER EXTRACT

Five concentrations were made from the crude extract of cold water using and were used to test for antigonococcal properties that may be present in it.

The mean diameter zone of inhibition remained constant at 6.0 mm for three concentrations that ranged from 10 mg/ml to 2.5 mg/ml. At 40 mg/ml the mean diameter zone of inhibition was at 14.0 mm at 10.0 mm at 20 mg/ml.

The results are captured in figure 4.5 as seen below.

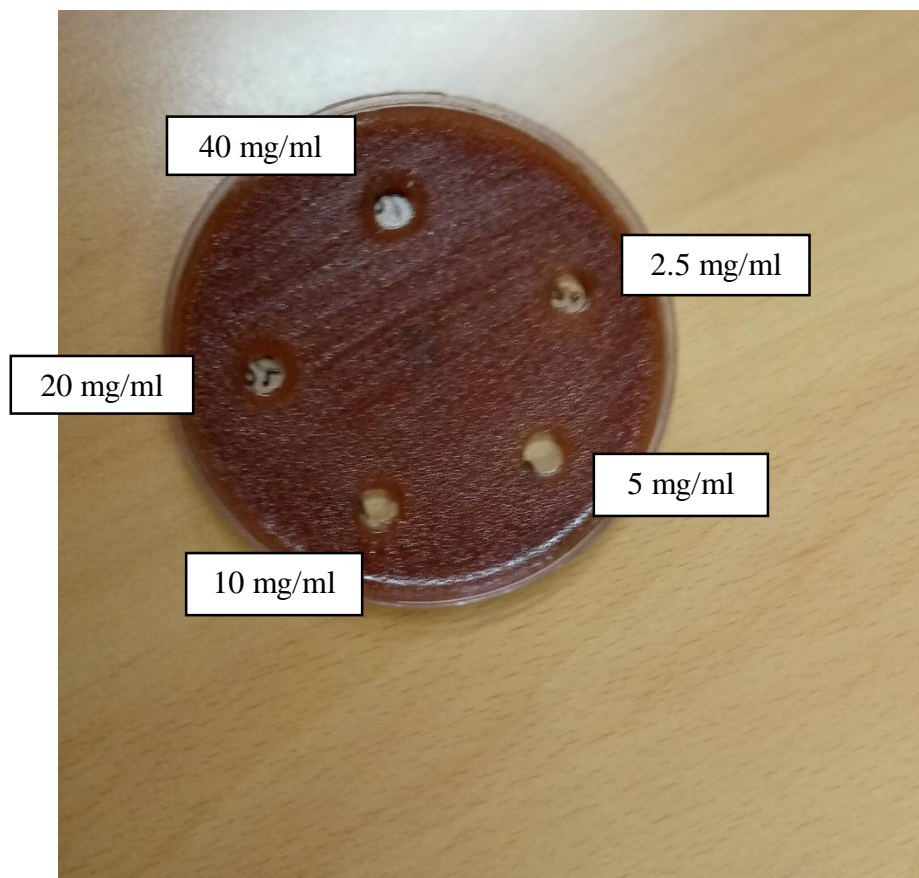


Figure 4. 5 Culture plate showing zones of inhibition from Cold water crude extract.

4.3.6 WATER DECOCTION EXTRACT

Antigonococcal properties present in water decoction crude extract were tested using five concentrations that ranged from 2.5 mg/ml to 40 mg/ml.

At a concentration of 2.5 mg/ml the mean zone of inhibition was at 7.2 mm, at 5 mg/ml, it was at 9.0 mm, at 10 mg/ml it was at 10.0 mm at 20 mg/ml it was at 11.0 mm and at 40 mg/ml it was at 13.0 mm.

The results above are seen in figure 4.6 below.

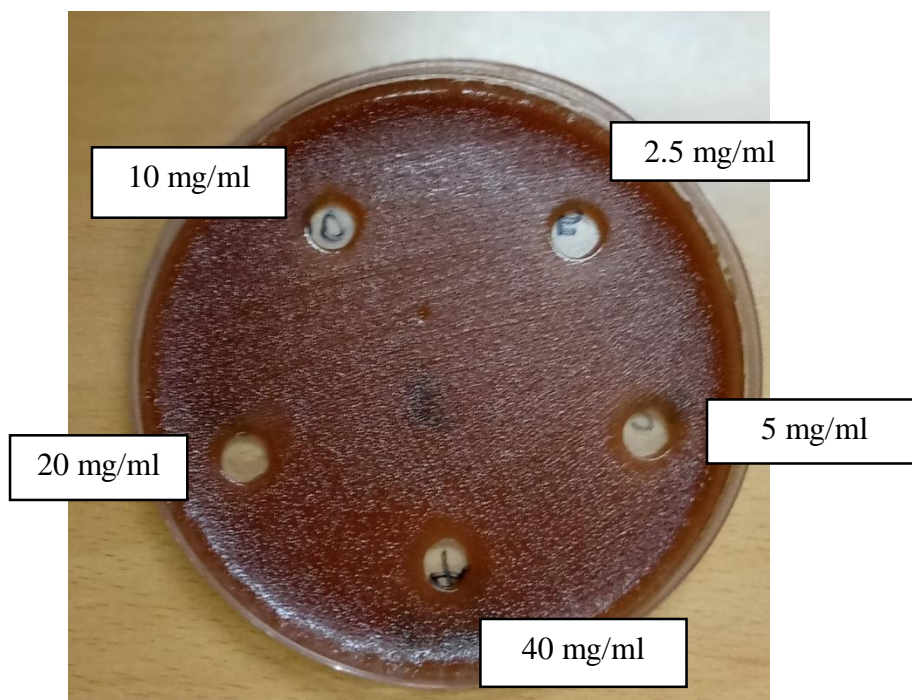


Figure 4. 6 Culture plate showing zones of inhibition from Water decoction crude extract.

4.3.7 METHANOL EXTRACT

Methanol crude extract was diluted in different concentrations and the following results were obtained from its antimicrobial activity property

At 40 mg/ml the zone o inhibition displayed was at 21.0 mm, at 20 mg/ml it was at 20.0 mm, at 10 mg/ml it was at 18.0 mm, at 5 mg /ml it was at 15.0 mm and lastly at 2.5 mg/ml it was at 14.0 mm.

The above results can be seen in figure 4.7 below.

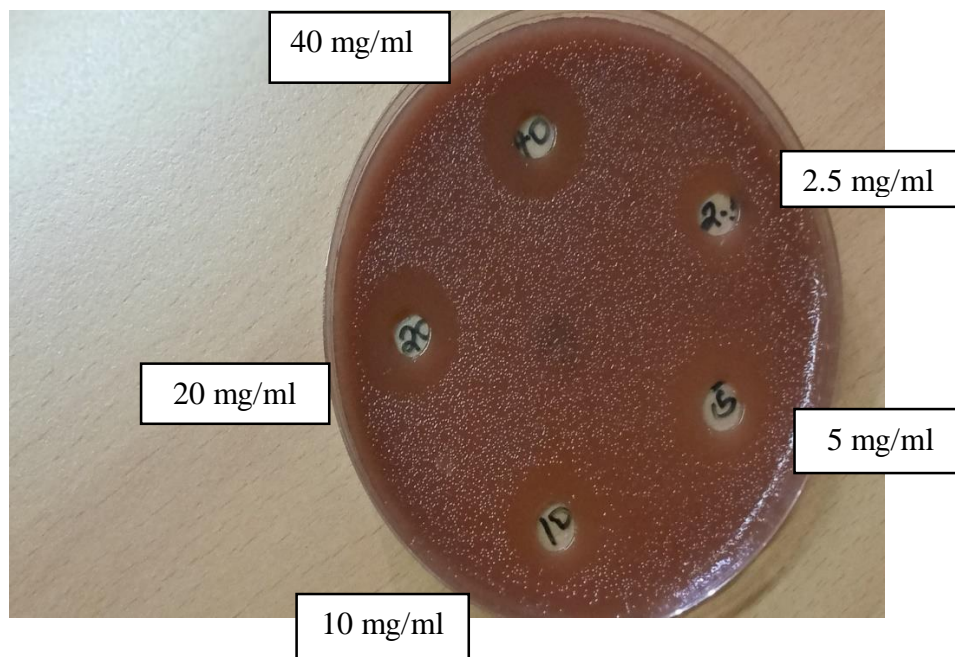


Figure 4. 7 Culture plate showing zones of inhibition from Methanol crude extract.

4.3.8 CONTROLS

The controls for this study were performed on a separate plate. The controls included: DMSO which acted as negative control, working standard ceftriaxone 0.1mg/ml and chloroform 40mg/ml which were used as the positive controls.

DMSO showed no zone of inhibition against *Neisseria gonorrhoeae*, while Ceftriaxone working standard and chloroform displayed a zone of inhibition of 38.0 mm and 23.0 mm respectively. These results are seen in figure 4.8 below.

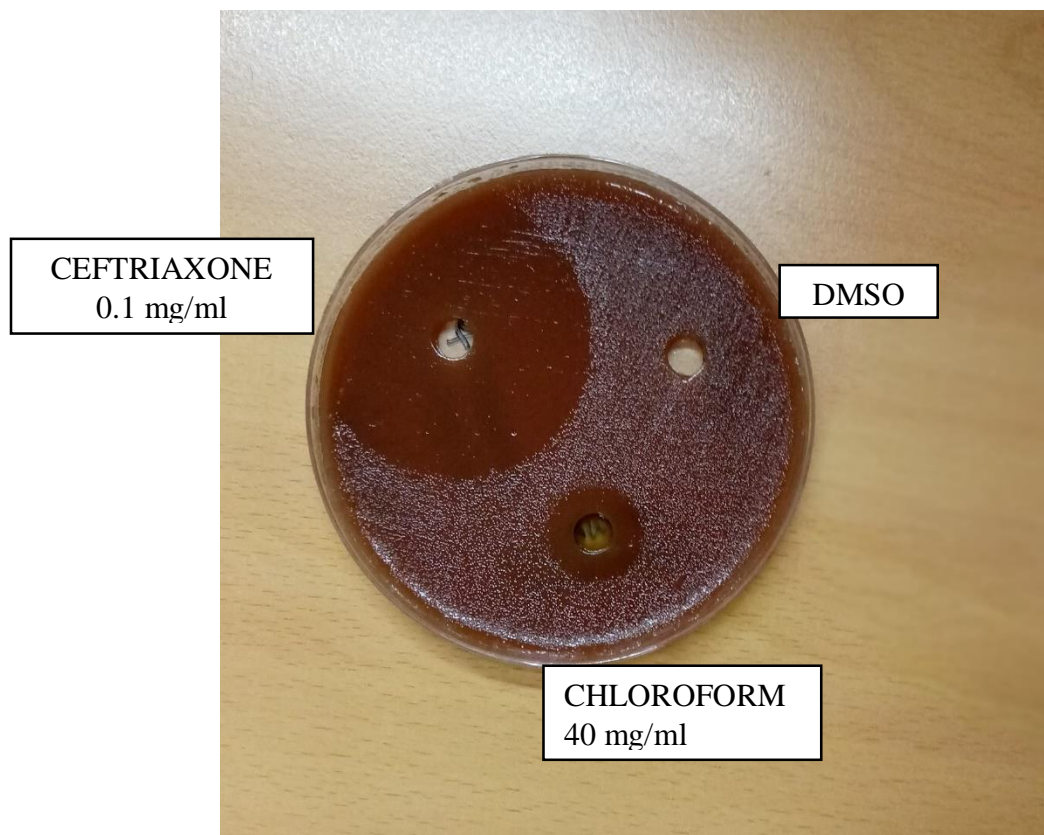


Figure 4. 8 Culture plate showing zones of inhibition from the positive and negative controls.

4.3.9. PERCENTAGE OF STANDARD

This was calculated by comparing the zone of inhibition of each test solution with that of the standard used.

$$\% \text{ of Standard} = \frac{\text{Inhibition diameter of sample}}{\text{Inhibition diameter of standard}} * 100$$

Table 4. 3 Zones of Inhibition of the extracts

TEST SUBSTANCES	40 mg/ml	20 mg/ml	10 mg/ml	5 mg/ml	2.5 mg/ml
	Diameters of Zones of Inhibition (mm)				
	D (% of std)	D (% of std)	D (% of std)	D (% of std)	D (% of std)
DICHLOROMETHANE	-	16 (50.0)	15 (46.9)	14 (43.8)	12 (37.5)
DCM- METHANOL (50:50)	14 (43.8)	13 (40.6)	11 (34.4)	10 (31.3)	8 (25.0)
ETHYL ACETATE	15 (46.9)	12 (37.5)	10 (31.3)	9 (28.1)	7 (21.9)
COLD WATER	8 (25.0)	4 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)
DECOCTION	6 (18.8)	5 (15.6)	4 (12.5)	3 (9.4)	1.2 (3.8)
METHANOL	15 (46.9)	14 (43.8)	12 (37.5)	9 (28.1)	8 (25.0)
CHLOROFORM	17 (53.1)	15 (46.9)	14 (43.8)	13 (40.6)	10 (31.3)

D = diameter of the zone of inhibition less the 6 mm diameter of the well; % of std = D of test substance / D of the standard * 100; a zone of inhibition of 0.0 mm implies no activity; - = Test not done.

Table 4. 4 Zones of Inhibition of the Controls

TEST SUBSTANCES	CEFTRIAXONE (0.1 mg/ml)	DMSO (PURE)	CHLOROFORM (40 mg/ml)
D	32.0	0.0	17.0
D (% of std)	100.0	0.0	53.1

4.4 MINIMUM INHIBITORY CONCENTRATION AND MINIMUM BACTERICIDAL CONCENTRATION

A stock solution of 10 mg/ml was prepared and serially diluted to obtain various concentrations ranging from 10mg/ml to 0.000391 mg/ml for each solvent except for cold water crude extract in order to determine the MIC.

To determine the MBC, the lowest concentration from the MIC tube dilutions were sub cultured onto plates with MTM media.

The results are seen in table 4.5 below.

Table 4. 5 Results from MIC and MBC tube dilutions.

SOLVENT	MIC (mg/ml)	MBC (mg/ml)
DICHLOROMETHNE	2.5	5.0
DICHLOROETHANE: METHANOL (50:50)	1.3	2.5
ETHYL ACETATE	1.3	2.5
WATER DECOTION	5.0	10.0
CHLOROFORM	5.0	10.0
METHANOL	0.6	0.3

This study defined MIC as the highest dilution of plant extract that inhibited growth of the organism after overnight incubation.

The MIC values ranged from 0.6 mg/ml to 5.0 mg/ml. Methanol extract had the highest MIC of 0.6 mg/ml followed by DCM-Methanol and ethyl acetate (50:50) at 1.3 mg/ml, then by dichloromethane extract at 2.5 mg/ml and lastly water decoction and chloroform extract with the lowest MIC values of 5.0 mg/ml.

4.4.1. MINIMUM BACTERICIDAL ACTIVITY

The lowest concentration of the sub cultured MIC test tubes that did not show any visible growth after overnight incubation upon macroscopic examination was defined as the MBC.

MBC values obtained for *Albizia grandibracteata* crude extracts ranged from 0.3 mg/ml to 10.0 mg/ml. The methanol crude extract exhibited the highest MBC of 0.3 mg/ml while water decoction and chloroform extract exhibited the lowest MBC values of 10.0 mg/ml.

The cold water extract was excluded from determination of MIC and MBC as it did not show active zones of inhibition at 10mg/ml.

CHAPTER FIVE: DISCUSSION

The search for potential antimicrobial agents has led to an increase in drug discovery studies over the years due to a rise in antibiotic resistant bacterial strains. The WHO recommends that countries formulate standard procedures to validate medicinal plant products for incorporation into mainstream healthcare (66).

There is limited data published on the antimicrobial activity of *Albizia grandibracteata*. This is the first time *Albizia grandibracteata* whole roots have been shown to have antimicrobial activity against *Neisseria gonorrhoeae*. Other studies that have been done have shown that it has anticytotoxic properties present in its methanolic extract (45).

The study used cold maceration technique for the extraction process. It played an important role when it came to the determination of the plant material yield extracted, as it facilitated obtaining yield from a given extract without altering the functional properties required. The technique used led to good extraction yields from all the solvents except Dichloromethane which had the lowest yield that further affected the amount needed for it to be dissolved to make a stock solution of 40 mg/ml of DMSO for the antimicrobial assays.

Different antibiotics were used to test for susceptibility and resistance pattern of *Neisseria gonorrhoeae*. These results can be interpreted to mean that: *Neisseria gonorrhoeae* was susceptible to ceftriaxone and ciprofloxacin as it presented with zones of inhibition of 38.0 mm and 31.0 mm respectively. It showed resistance to tetracycline and penicillin as it displayed zones of inhibition less than 30.0 mm and 26.0 mm respectively according to the CLSI 2013 (67).

Neisseria gonorrhoeae showed susceptibility to azithromycin with a zone of inhibition greater than 28.0 mm. A study conducted by BSAC on standardized disc susceptibility method in 2009, gave these guidelines for azithromycin susceptibility breakpoints for Disc diffusion involving *Neisseria gonorrhoeae*. (68)

Seven solvents were used to evaluate the in vitro activity of *Albizia grandibracteata* whole roots against *Neisseria gonorrhoeae*. Agar well diffusion method was used. Their activity was then compared to that of the standard as a percentage.

At a concentration of 40 mg/ml chloroform showed significant activity against *Neisseria gonorrhoeae* with a zone of inhibition of 53.1% that of ceftriaxone. This was followed by ethyl acetate and methanol at 46.9 %, then dichloromethane- methanol (50:50) at 43.8 % and lastly the decoction at 18.8 %. Due to poor yield obtained during solvent extraction dichloromethane was unable to produce the yield to prepare a concentration of 40 mg/ml.

At a concentration of 20 mg/ml the zones of inhibition ranged from 50.0 % to 12.5 % that of ceftriaxone. All solvents were active against *Neisseria gonorrhoeae* but Dichloromethane was significantly active against gonococci with a zone of 50.0% that of ceftriaxone.

At a concentration of 10 mg/ml methanol, chloroform, dichloromethane, ethyl acetate and DCM-Methanol (50:50) extracts and the decoction were active as they had zones of inhibition ranging between 12.5 % - 46.9 % that of ceftriaxone . Water cold extract showed no activity.

At a concentration of 5 mg/ml water cold extract showed no activity. The water decoction extract remained active at zone of inhibition of 9.4% that of ceftriaxone. The rest of the solvents displayed very high activity with zones ranging from 43.8 % to 28.1 % that of ceftriaxone.

Lastly at a concentration of 2.5 mg/ml cold water extract showed no activity. At this concentration dichloromethane and chloroform displayed the highest activity with mean zones of inhibition of greater than 30.0% that of ceftriaxone, followed by methanol , ethyl acetate and DCM-Methanol (50:50) with zones greater than 20.0 % that of ceftriaxone and lastly water decoction extract with a mean zone of 3.8 % that of ceftriaxone.

All crude extracts showed concentration dependent inhibition for *Neisseria gonorrhoeae*. Chloroform extract showed the highest antigonococcal activity, while cold water displayed the lowest activity against *Neisseria gonorrhoeae*. Ceftriaxone a synthetic drug was used as the standard. These extracts are in crude forms yet gave significant antigonococcal activity to that of ceftriaxone. Chloroform crude extract and dichloromethane at 40 mg/ml and 20 mg/ml respectively gave zones greater than 50.0 % that of the ceftriaxone. This can be interpreted to mean that, if the crude extracts undergo purification, they could yield similar or even better activity compared to ceftriaxone.

Traditionally, the plants whole roots are prepared by boiling in water and the decoction is drunk over a period of seven days, by patients who have symptomatic gonococcal infection (41) . Results from the water decoction show significant antigonococcal activity, while the use of other solvents greatly increases its antigonococcal activity. This therefore validates its use in traditional medicine.

Minimum inhibition Concentration was used to quantify the activity of *Albizia grandibracteata* extracts. Methanol crude extract showed the highest MIC value of 0.6 mg/ml, followed by ethyl acetate and dichloromethane-methanol (50:50) at 1.3 mg/ml, then dichloromethane at 2.5 mg/ml and lastly water decoction and chloroform at 5.0 mg/ml. this can be interpreted to mean that at higher concentrations the crude extracts have bactericidal activity against *Neisseria gonorrhoea*.

Minimum bactericidal Concentration was the lowest concentration of the sub cultured MIC test tubes where growth was absent. The MBC values ranged from 0.3 mg/ml to 10.0 mg/ml. The highest value was obtained from the methanol crude extract at 0.3 mg/ ml while the lowest were obtained from water decoction and chloroform at 10.0 mg/ ml. This results can be interpreted to mean that at lower concentrations the crude extracts have bacteriostatic activity against *Neisseria gonorrhoea*.

This therefore suggests that *Albizia grandibracteata* crude extracts, when used traditionally as an antimicrobial, is bacteriostatic. Most of the preparations made traditionally don't have specific concentrations, hence accounting for the use of the extracts by traditional folk for the treatment of gonococcal infection.

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

This study made use of the crude extracts from *Albizia grandibracteata* whole roots for its preliminary antimicrobial screening. Non-polar solvents such as Chloroform, Ethyl acetate, Dichloromethane, Methanol and polar solvents such as aqueous extract in form of water decoction and cold water crude extract were used and found to have significant activity against gonococci.

This is proof that in their crude aqueous form the extracts of *Albizia grandibracteata* are effective against gonococci. Use of non-polar solvents further improved its activity with increasing concentration therefore confirming the validity of its use in traditional medicine for the treatment of gonococcal infections.

The extracts were tested against standard drug – Ceftriaxone and solvents such as chloroform and dichloromethane showed mean ones of inhibition of greater than 50.0% that of the standard. The extracts were in crude forms that contain impurities that may have hindered further antimicrobial activity. If purified, they may be able to produce a similar activity or even better activity compared to that of the standard.

The crude extracts of *Albizia grandibracteata* were found to have bacteriostatic activity against gonococci at lower concentrations and bactericidal activity at higher concentrations against gonococci, further proving the presence of antimicrobial properties present in the medicinal plant.

6.2 RECOMMENDATIONS

This study therefore recommends that more reagents, conditions and modes of extraction should be carried out on various parts of the plant and their corresponding extracts tested again on *Neisseria gonorrhoeae*. This would be important as it would help determine the best solvent for extraction and its most active part for maximum use of the plant crudely or for isolation of active components.

Phytochemistry profiling should also be conducted on the available plant extracts to determine the bioactive components of *Albizia grandibracteata*. This compounds should isolated and interpreted to determine whether or not it is a new antimicrobial agent.

Lastly, other strains and isolates of gonorrhoeae, especially resistant ones should be tested against the extract to see whether it has similar or even better activity against them. Other bacterial species such as enterobacteriaceae, staphylococcus and streptococcus should also be studied for antagonism by this plant, as *Albizia grandibracteata* has been implicated in the treatment of diarrhea and tonsillitis traditionally.

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APPENDICES

- I. Research Permit - Kenya Wildlife Service
- II. Material Transfer Agreement form – Kenya Wildlife Service and UNITID
- III. Prior Informed Consent form
- IV. NACOSTI Permit

APPENDIX I: Kenya Wildlife Service Research Permit



ISO 9001:2008 Certified

KWS/BRM/5001

17 July 2018

Ms Ilyn M. Musasia
P.O.Box 73306-00200
NAIROBI
e-mail: imusasia17@gmail.com
mobile: 0785420827

Dear *Ms. Musasia,*

PERMISSION TO CONDUCT RESEARCH IN KAKAMEGA FOREST NATIONAL RESERVE

We acknowledge receipt of your application requesting for permission to conduct research on a project titled: '**Anti-microbial activity of *Albiza grandibracteata* against *Neisseria gonorrhoeae***'. The study will generate data and information that will assist in the production of drugs for treatment of drug-resistant strains of *Neisseria gonorrhoeae*.

You have been granted permission to conduct the study from **July 2018 – July 2019** upon payment to KWS academic research fees of **ksh.6000** (Masters Study). You will seek for permission to access Kakamega Forest Reserve from Kenya Forest Service (KFS) administration officials. You will enter into a Materials Transfer Agreement (MTA) and Prior Informed Consent (PIC) with the resource providers and proceed for the fieldwork. You will also be required to work closely with our Senior Scientist in-charge of Western Conservation Area (WCA), whom you will give the progress report on the study.

You will submit a bound copy of your MSc thesis to the KWS Deputy Director, Biodiversity Research and Monitoring on completion of the study.

Yours *Sincerely,*

SAMUEL M. KASIKI, PhD, OGW
DEPUTY DIRECTOR
BIODIVERSITY RESEARCH AND MONITORING

Copy to:

- Senior Scientist, WCA
- Warden, Kakamega Forest N. Reserve
- Research Scientist, KFNR
- Forest Conservator, Buyangu/Isecheno Forest Stations

P.O Box 40241-00100, Nairobi, Kenya.

Tel: +254-020-2379407/8/9-15. Mobile: +254-735 663 421, +254-726 610 508/9.

Email: kws@kws.go.ke Website: www.kws.go.ke

APPENDIX II: MATERIAL TRANSFER AGREEMENT

MATERIAL TRANSFER AGREEMENT (MTA)

BETWEEN

KENYA WILDLIFE SERVICE (KWS)

AND

UNIVERSITY OF NAIROBI INSTITUTE OF TROPICAL AND INFECTIOUS DISEASES (UNITID)

FOR

The Transfer of Material:

Plant sample (whole roots of *Albizia grandibracteata*) and the extract arising from research on Antimicrobial activity of *Albizia grandibracteata* on *Neisseria gonorrhoeae*



ARTICLE 1 – PARTIES TO THE AGREEMENT

1.1 The present Material Transfer Agreement hereinafter referred to as “**this Agreement**” is the Standard Material Transfer Agreement

1.2 This Agreement is :

BETWEEN Kenya Wildlife Service, P.O. BOX 40241-00100, Nairobi, hereinafter referred to as “**the provider**”

AND The University of Nairobi, College of Health Sciences, Institute of Tropical and Infectious Diseases, P.O.BOX 19676-00202, Nairobi, hereinafter referred to as “**the recipient**”

1.3 The parties to **this Agreement** hereby agree as follows:

ARTICLE 2 – DEFINITION OF TERMS

In **this Agreement** the expressions set out below shall have the following meaning:

Material Transfer Agreement shall mean an agreement between the holder of an access permit and relevant lead agency or community on access to genetic resources and benefit sharing (Kenya law Legal Notice 160, Wildlife Act 2013/KALR Act 1 of 2013)

Material genetic material

Provider shall mean the person(s) providing the genetic resource and or associated knowledge – KWS.

Prior Informed Consent shall mean a consent obtained by the user from the provider as the case may be after fully disclosing all the required information that permits access to their genetic resources and associated traditional knowledge, under mutually agreed terms. This **MTA** is executed with prior informed consent and also indicated in the mutually agreed terms described in this MTA.

Modifications shall mean substances created by recipients that contains / incorporate/ are derived from research specimens, progeny and unmodified derivatives.

Recipient person(s) / party receiving the material under this agreement – University of Nairobi Institute of Infectious Diseases.

Progeny is the unmodified descendant of the accessed genetic resource.

Duplicates a referenced representative sample of the genetic material accessed by an Access Permit holder.

Research Specimen collection of genetic material under a designated depository institution

Benefit Sharing -shall mean the benefits arising from the use of the material, their progeny and derivatives and associated traditional knowledge, practices and innovations. It may include both monetary and non-monetary returns such as up-front payments, royalties, salaries, institutional

development and strengthening, technical and academic training, the transfers of technology and information exchange and sharing as specified in the MTA.

Derivatives – shall include but not be limited to modified or unmodified extracts and any compounds or chemical structure based on or derived from plant genetic resource and its progeny, inclusive of analogues.

Genetic Materials – shall mean any biological material of plant material origin containing functional units of heredity of actual or potential value.

Information – shall mean any traditional knowledge, maps, drainages, photographs, plans, manuscripts, records, reports, recommendations, estimates, documents and any other data arising out of the received by any of the provider, Collaborators or User in connection with the resource.

Third Party – shall mean any other party other than the parties (the recipient and the provider) to this MTA

Unauthorized Disclosure – shall mean the placement of confidential information including indigenous traditional knowledge into public domain by publication or disclosure to a third party without the written prior informed consent of the original holders of that knowledge.

Intellectual property Rights –rights to publication of scientific papers and potential unforeseen offshoots.

ARTICLE 3 – TERMS AND CONDITIONS

The materials that shall be transferred are strictly for academic purposes. Upon transfer of the material, the Recipient shall provide the provider with a list of documents and describe the source of the material and the geographical position system coordinates of the material sources before they are transferred to the depositories. Evidence (including sample reference codes such as assigned accession numbers) of receipt of the materials by the designated depositories shall be available to KWS as agreed. The evidence that shows that the Recipient has obtained Prior informed consent, Access permit and Research Clearance will be attached.

ARTICLE 4- RIGHTS AND OBLIGATIONS OF THE PROVIDER

- I. KWS on behalf of the state retains ownership of the biological materials including any material contained or incorporated in modifications, until transferal to designated depositories for long term curation and management.
- II. KWS also retains rights to any intellectual property it owns in the material.
- III. KWS retains the right to access, audit and monitor the use and application of the biological material provided under this MTA. No rights under intellectual property of Kenya or rights in any other material or confidential information provided by the state to the recipient under this agreement is granted or implied as a result of providing this material to the recipient, other than expressly set forth herein

ARTICLE 5- RIGHTS AND OBLIGATIONS OFF THE RECIPIENT

- I. The Recipient shall use the genetic material for the described and permitted use only.
- II. The Recipient shall be responsible for ensuring that all permits required for the movement of the material is obtained and that sufficient proof of such permits is provided by KWS.
- III. In no circumstance shall the Recipient collect a sample in such a way that will threaten or be detrimental to the supply of that material in the wild.
- IV. Intellectual property rights arising from the study of the materials will be jointly shared by KWS and UNITID.
- V. The Recipients shall acknowledge this agreement and contribution of KWS and where applicable, local communities and stakeholders in all and any publications, patents or presentations involving the use of the material.
- VI. The Recipient should notify the KWS in case of any discovery. This shall be covered under a different agreement.
- VII. To the extent permitted by law, the recipients will indemnify and keep KWS and the State harmless from any claim, action and damage or cost deriving from or in connection with the Recipients' transfer or use of material.
- VIII. No commercialization shall take place as result of this project.
- IX. The material obtained under this agreement shall only be transferred by the recipients to a third party with prior written authorization from the provider.
- X. The Recipient agrees to provide to the Provider a copy of any interim report, final report, publications and other materials resulting from the use of transferred material. The recipient also agrees to identify in each in each report or other material the Project's Access permit number that collected the original research specimen from which the transferred material is derived. In addition, the recipients agree to provide notice in writing to the Provider in not less than 60 days before the recipient files an application for a patent or intellectual property claim resulting from use of transferred material.

ARTICLE 6- DURATION OF THE AGREEMENT

- I. This agreement is binding throughout the existence of the biomaterial
- II. The Recipient may terminate this agreement by a written notice to KWS at least three months in advance of desired date of termination.
- III. KWS may without assigning any reason thereof, suspend or terminate this agreement any time with written notice to the recipients.
- IV. On termination of this agreement, the Recipient agrees that any remaining material (apart from samples transferred to designated depositories) upon verification will be destroyed (unless requested by KWS ATO RETURN THE REMAINING MATERIAL) and to provide proof thereof to KWS no later than 30 days from the date of expiry or termination , whichever comes first.

- V. The above sections on ownership of the material and intellectual property, confidentiality, publications, warranty disclaimer, limitation of liability and indemnification shall survive expiration or earlier termination of this agreement.

ARTICLE 7- APPLICABLE LAW

As defined by the Kenya Wildlife Act 2013, Kenyan ABS laws and relevant Kenyan Domestic legislation where applicable.

Where applicable as defined in relevant Biodiversity Multi-lateral Environmental Agreement where Kenya is a party to

ARTICLE 8- DISPUTE SETTLEMENT

Any Dispute arising from this agreement shall be settled in the following manner;

- i. Amicable dispute settlement. The parties shall attempt in good faith to resolve the dispute by negotiation.
- ii. Mediation: if the dispute is not resolved by negotiation as described in paragraph i), the parties may choose mediation through a neutral third party mediator to be mutually agreed.
- iii. Arbitration: if the dispute has not been settled by mediation as described in paragraph ii), a party may submit the dispute for arbitration under Arbitration Act, 1999 of the Laws of Kenya or any statutory modifications or enactments in replacement thereof, if mutually agreed upon by the parties at the time. If the dispute is not settled by arbitration under the Arbitration Act, 1995, and if the parties mutually agree, the dispute shall be finally settled under the rules of arbitration of the International Chamber of Commerce by one or more arbitrators appointed in accordance with said rules

ARTICLE 9- FORCE MAJEURE

- i. Neither party (ies) shall be liable to the other party (ies) for any delay or nonconformance of its obligations under this Agreement arising from any cause beyond its reasonable control, including, but not limited to, any of the following: government Act, war, fire, drought, explosion, civil commotion or industrial disputes of a third party or impossibility of obtaining gas or electricity or materials.
- ii. The affected party (ies) must promptly notify the other party (ies) in writing, but in no circumstances later than 14 days, of the cause and likely duration of the cause.
- iii. Such notice having been given, the performance of the affected party's obligations, to the extent affected by the cause, shall be suspended during the period the cause persists.
- iv. Without prejudice to the above, the affected party (ies) must take all reasonable measures to minimize the impact of any force majeure on the performance of its obligations under the **Agreement** and to ensure, as soon as practicable, the resumption of normal performance of the obligations affected by the force majeure.

ARTICLE 10- NOTICES

Any notice or other document to be served under this Agreement must be delivered by hand or sent by registered mail to the addresses below:

a. PROVIDER

Kenya Wildlife Service
P.O. BOX 40241-00100
Nairobi, KENYA.

b. RECIPIENT

University of Nairobi,
College of health sciences,
University of Nairobi Institute of Tropical and Infectious Diseases.
P.O.BOX 19676-00202,
Nairobi, KENYA.

All notices or documents shall be deemed to have been served at the date and time of delivery of the said notices or documents to the recipient party.

ANNEXES:

ANNEX I

Description of the Material and Purpose of transfer:

Plant samples from *Albizia grandibracteata* – whole roots will be used for the purpose of research on the antimicrobial activity of the plant root extract against *Neisseria gonorrhoeae*

Analysis:

Laboratory analysis will be carried out in University of Nairobi Laboratory.

Quantity of material required:

In order to maximize current and future use of the material, the plant sample will be preserved by drying in paper envelopes. Intended collection will include 10 kg (5 envelopes). The herbarium specimen will be deposited in University of Nairobi, College of Biological Sciences Herbarium.

ANNEX II

Labelling:

The collected material shall be coded and geo- referenced prior to use. All the site from which the material is collected shall have a GPS reference, and this information shall be included in the material coding record. This shall be verified by KWS before use. Project data shall be accessible to all partners. Data analysis, writing and publication will involve the PI and also co-authorship by partners including KWS as appropriate.

ARTICLE 11 – SIGNATURE

PROVIDING INSTITUTION

for

The Director General, KWS

NAME..... *Dr. Madhus Oban, PhD*

SIGNATURE..... *[Signature]*

DATE..... *24th August, 2018*

RECIPIENT INSTITUTION

The Director, UNITID

NAME..... *Prof James M.N. M'umunya*

SIGNATURE..... *[Signature]*

DATE..... *23/8/18*



APPENDIX III: PRIOR INFORMED CONSENT

PRIOR INFORMED CONSENT FOR THE RESEARCH ON ANTIMICROBIAL ACTIVITY OF *ALBIZIA GRANDIBRACTEATA* AGAINST *NEISSERIA GONORRHOEA*.

This mutually agreed terms MAT have been generated after various consultations with local collaborators and the Resource Provider. These are the Mutually Agreed Terms entered into on the DAY 33.. MONTH 08.....YEAR 2018. by and between Ms. Ivyn Munyuli Musasia (Principal Investigator) and Kenya Wildlife Service.

WITNESSETH:

Whereas Kenya Wildlife Service manages and conserves the country's Wildlife under the Wildlife (Conservation and Management) Act, 2013.

Whereas Kenya Wildlife Service grants PIC on Wildlife user rights and it also administers Material Transfer agreements on all wildlife specimens on behalf of the government.

WHEREAS, Ms. Ivyn Munyuli Musasia of University of Nairobi Institute of Tropical Infectious Diseases is the principal investigator on the study of Antimicrobial activity of *Albizia grandibracteata* against *Neisseria gonorrhoeae*, her role will be conducting research, collecting samples, analyzing sample extracts from the plant extract.

NOW THEREFORE, IT IS HEREY AGREED by parties as follows:

In connection with this research activities, the following conditions will be met:

- i. This will be a joint partnership between the Principal Investigator and KWS
- ii. The PI will provide KWS with a checklist of all specimens collected including photographs and coordinates
- iii. Both background and generated intellectual property will be respected and where jointly owned. The resource provider will be notified appropriately and no commercialization i.e. copyright materials, patents, technology transfer will take place without consultation and approval by KWS
- iv. All collected material will remain property of Kenya
- v. In the event of transfer off any accessed biological specimen the PI will enter into an MTA with KWS
- vi. Biological materials collected pursuant to this agreement shall not be transferred to third parties without prior written consent from KWS
- vii. That these mutually agreed terms will form the basis for a collaborative research agreement between the PI and KWS
- viii. The terms and conditions set forth herein may be revisited and / o amended at a future date, in the event that such revisions are required in connection it changes to the proposed research, or are justified by preliminary research findings. All such amendments or revisions shall be in writing and signed by all parties hereto.

IN WITNESS WHEREOF, the parties hereto are duly authorized representatives have hereunto subscribed their hands and seals on the date and year first mentioned below:

Read and acknowledged by Applicant: Principal Investigator (PI)

NAME: Ms. IVYN MUNYULI MUSASIA
ADDRESS: 73306 – 00200 NAIROBI
CELLPHONE NUMBER: 0728- 012- 274
EMAIL: imusasia17@gmail.com

SIGNATURE..... *[Signature]* DATE..... *11/11/18*

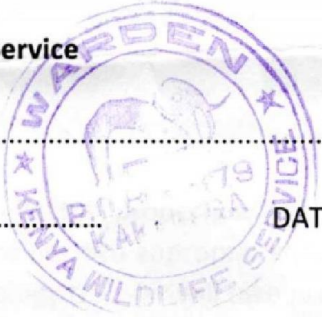
For the Kakamega Forest Community

NAME..... *Dennis SHITANDAYI*
DESIGNATION..... *[Signature]*
ADDRESS..... *P.O Box 18 KAKUNGA-KAKAMEGA*
CELLPHONE NUMBER..... *+254725991646*
EMAIL..... *shitad1@yahoo.com*

**SIGNED on behalf of the PROVIDER
Director –General, Kenya Wildlife Service**

NAME..... *Emmy Atai*
SIGNATURE..... *[Signature]* DATE..... *11/11/18*

For the warden



APPENDIX IV. NACOSTI RESEARCH PERMIT



**NATIONAL COMMISSION FOR SCIENCE,
TECHNOLOGY AND INNOVATION**

Telephone: +254-20-2213471,
2241349,3310571,2219420
Fax: +254-20-318245,318249
Email: dg@nacosti.go.ke
Website : www.nacosti.go.ke
When replying please quote

NACOSTI, Upper Kabete
Off Waiyaki Way
P.O. Box 30623-00100
NAIROBI-KENYA

Ref. No. **NACOSTI/P/18/04309/25692**

Date: **19th November, 2018**

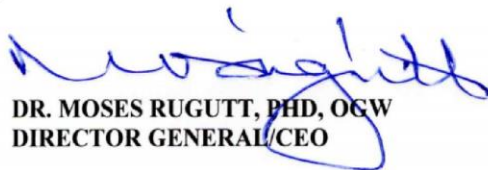
Ivyn Munyuli Musasia
University of Nairobi
P.O Box 30197-00100
NAIROBI.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on “*Antimicrobial activity of albizia grandibracteata against neisseria gonorrhoeae,*” I am pleased to inform you that you have been authorized to undertake research in **Kakamega County** for the period ending **16th November, 2019.**

You are advised to report to **the County Commissioner, the County Director of Education and the County Director of Health Services, Kakamega County** before embarking on the research project.

Kindly note that, as an applicant who has been licensed under the Science, Technology and Innovation Act, 2013 to conduct research in Kenya, you shall deposit **a copy** of the final research report to the Commission within **one year** of completion. The soft copy of the same should be submitted through the Online Research Information System.


DR. MOSES RUGUTT, PHD, OGW
DIRECTOR GENERAL/CEO

Copy to:

The County Commissioner
Kakamega County.

The County Director of Education
Kakamega County.