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FACULTY OF APPLIED FUNDAMENTAL SCIENCES

DEPARTMENT OF BIOTECHNOLOGIES

CONTRIBUTION TO THE STUDY OF
ANTIMICROBIAL ACTIVITY OF CRUDE
EXTRACTS FROM LEAVES AND BARK
OF *PHYTOLACCA DODECANDRA*
(UMUHOKO) AGAINST *NEISSERIA*
GONORRHOEA AND *CANDIDA ALBICANS*

Scientia et Lux

A dissertation submitted in partial

Fulfillment of the requirements for the award of

Bachelor's Degree in Biotechnologies.

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DECLARATION

I do hereby declare that this project submitted in partial fulfillment of the requirements of bachelor's degree in Biotechnologies, at INES/RUHENGERI, is my original work and has not previously been submitted elsewhere. Also, I do declare that a complete list of references is provided indicating all the sources of information quoted or cited.

Date

Signature.....

NGABONZIMA Gaspard

DEDICATION

To:

My brothers and sisters;

All my friends and collaborators.

ACKNOWLEDGEMENTS

First of all, my thanks are launched to Almighty GOD for the protection, care and guidance that enabled me to live in suitable conditions since my existence till now, as well as the completion of my studies.

I cannot find the suitable words to thank my supervisor Mr. KAGIMBURA Ervine, whose ideas, guidance and supervision have enlightened me a lot and offered me an appreciated experience.

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Let me take this opportunity to express my deepest thanks to Mr. BIRIHANZE Jean Bosco and his wife UWURUKUNDO Brigitte for their invaluable kindness that they accorded me since the beginning of my studies until now.

My thanks are addressed to INES/RUHENGERI, especially all staff of Biotechnologies Department for having trained us since our arrival until the end of our studies. It is with a great pleasure that I take this opportunity to thank all Laboratory technicians of INES RUHENGERI that helped us in carrying out all required experiments.

Lastly, I thank all my friends and classmates whose ideas, moral and financial contribution to my studies will never be forgotten in my life.

NGABONZIMA Gaspard

ABSTRACT

Sexually transmitted diseases are more frequent all over the World including Rwanda, our beloved country. Long time ago, the medicinal plants have been used by practitioners to treat different types of diseases including sexually transmitted diseases. In that case, we carried out this research work to reveal the impact of medicinal plant named *Phytolacca dodecandra* whose vernacular name is “Umuhoko” in reduction of some sexual related diseases. Normally, this plant has been used in treatment of different diseases such as diarrhea, injuries, respiratory infections, typhoid fever, bacterial and fungal related diseases.

Using the methanolic extracts of leaves and bark of *Phytolacca dodecandra*, we obtained the following results:

-The extracts of leaves of *Phytolacca dodecandra* have the antimicrobial activity on *Neisseria gonorrhoea* and *Candida albicans* at inhibitory minimum volume of 5µl at 3mm of inhibition diameter zone and 10µl at 2mm of inhibition diameter zone respectively.

-The extracts of Barks of *Phytolacca dodecandra* have the antimicrobial activity on *Neisseria gonorrhoea* and *Candida albicans* at inhibitory minimum volume of 10µl at 2mm of inhibition diameter zone and 15µl at 1mm of inhibition diameter zone respectively.

Thus, the obtained results allow us to conclude that the use of leaves and Bark extracts of *Phytolacca dodecandra* in treatment of diseases caused by *Neisseria gonorrhoea* and *Candida albicans* is efficient.

RÉSUMÉ

Les maladies sexuellement transmissibles sont plus fréquentes dans le monde entier y compris notre pays préféré le Rwanda. Les plantes médicinales ont été utilisées longtemps par les tradipraticiens pour soigner les différents types de maladies dont les maladies sexuellement transmissibles. Dans ce cas, nous avons mené un travail de recherche pour révéler l'impact d'une plante médicinale nommée *Phytolacca dodecandra* dont le nom vernaculaire est « umuhoko » pour traiter les maladies sexuellement transmissibles telles que *Gonorrhoea* et *Candidiose*

Normalement, cette plante a été utilisée dans le traitement de différentes maladies à savoir la diarrhée, les plaies, les infections respiratoires, la fièvre typhoïde, les maladies bactériennes et les maladies fongiques.

En utilisant les extraits méthanoïques des feuilles et de l'écorce de *Phytolacca dodecandra*, nous avons obtenus les résultats suivants :

-Les extraits de feuilles de *Phytolacca dodecandra* ont une activité antimicrobienne sur *Neisseria gonorrhoea* et *Candida albicans* au volume minimal inhibiteur de 5 μ l avec 3mm de diamètre de zone d'inhibition et 10 μ l avec 2mm de diamètre de zone d'inhibition respectivement.

-Les extraits de l'écorce de *Phytolacca dodecandra* ont une activité antimicrobienne sur *Neisseria gonorrhoea* et *Candida albicans* au volume minimal inhibiteur de 10 μ l avec un diamètre de zone d'inhibition de 2mm et 15 μ l avec 1mm de diamètre de zone d'inhibition respectivement.

De ce fait, les résultats obtenus nous permettent de conclure que l'utilisation des extraits bruts de feuilles et de l'écorce de *Phytolacca dodecandra* dans le traitement des maladies causées par *Neisseria gonorrhoea* et *Candida albicans* est efficace.

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LIST OF ACRONYMS AND ABBREVIATIONS

%:	Percentage
+	positive
>:	More than
1 st :	First
CHUK :	Centre Hospitalier Universitaire de Kigali
Gr:	Gram
Gram ⁺ :	Gram positive
Gram-:	Gram negative
http:	Hyper transfer text protocol
INES :	Institut d'enseignement Supérieur
ml:	milliliter
MSA:	Mannitol Salt Agar
N ^o :	Number
°C :	Celsius degree
PCA:	Potato Count Agar
PDA:	Potatoes Dextrose Agar
Vol:	Volume
www:	World Wide Web
µl:	microlitre

1. INTRODUCTION

In different countries of the world, plants are used in traditional medicine which is known as the knowledge, skills and practices of holistic health care, recognized and accepted for its role in the maintenance of health and the treatment of diseases. This traditional medicine is commonly based on indigenous, theory, beliefs and experiences that are handed down from generation to generation (Duraffourd *et al.*, 1997).

Currently, more value is attributed to the natural remedy and modern medicine used for medicinal plants, because they allow the best result in long term (PAMPLONA, 2001).

World health organization (WHO) estimates that approximately 80% of the people in the developing countries including Rwanda rely to the traditional medicine for primary needs, of which a major portion involves the use of plant extracts or their active principal (FRANCIWORTH *et al.*, 1985).

In that case, *Phytolacca dodecandra* known as Umuhoko (Plant of phytolaceae family) should have the antimicrobial activity against *Neisseria gonorrhoeae* and *Candida albicans*, the bacterium and fungus responsible of *Gonorrhoea* and *Candidiosis*.

2. PROBLEM STATEMENT

Many years ago, the human being faced the serious problems. Those problems may be economically encountered, politically known and socially effectuated. The most important among those problems is related to multiple diseases. Some of them are sexually transmitted diseases that tend to affect many people in rural area as well as in urban area.

In their research, R. Morissette and J. PECHERIEA (2003) identified that *Candida albicans* and *Neisseria gonorrhoea* were frequent from 65% to 85%. It was also confirmed by ANNO'FELL VERHOEVEN and FRANK (1985) that *Neisseria gonorrhoea* and *Candida albicans* are the first causes of *Gonorrhoea* and *Vulvovaginitis*, respectively, to human being.

Nowadays, these sexual related diseases still being a big challenge in our society. A great number of affected people are ranging from 21 to 31 years with the frequency of 41.1% as it has been confirmed by Y-J-GOLVAN (2003), PAUL SINGLETON (1999) and VERHOEVEN (1782). These harmful microorganisms are the major cause of bad health that conducts to the death.

Some plants are also misused in our country during the treatment of different diseases. *Phytolacca dodecandra* is found among those plants. This research should contribute to the resolution of all those problems.

3. SIGNIFICANCE OF THE STUDY

This research work known as “Contribution to the study of antimicrobial activity of bark and leaves extracts of *Phytolacca dodecandra* (umuhoko) against *Neisseria gonorrhoeae* and *Candida albicans*” revealed a great importance in different ways.

First of all, the diseases related to the infection by microorganisms, essentially those caused by *Neisseria gonorrhoeae* and *Candida albicans* will be reduced using the local and non expensive treatment.

The medicinal plants will be taken into consideration. Hence, the temptations to their elimination or their mishandling will no longer exist. This is especially applied to *Phytolacca dodecandra* (Umuhoko) which shows an important value in terms of antimicrobial activity.

4. HYPOTHESES

-The crude extracts of *Phytolacca dodecandra* could have the antimicrobial effect on bacteria named *Neisseria gonorrhoeae* and fungus called *Candida albicans*.

-The crude extracts of leaves and bark of *Phytolacca dodecandra* could react differently on *Neisseria gonorrhoea* and *Candida albicans*

5. OBJECTIVES OF THE STUDY

5.1. General objective

To promote the use of *Phytolacca dodecandra* in traditional medicine

5.2. Specific objectives

-To prepare the different extracts from *Phytolacca dodecandra*

-To demonstrate the antimicrobial activity of leaves and bark crude extracts of *Phytolacca dodecandra* on microorganisms *Neisseria gonorrhoea* and *Candida albicans*.

-Compare the efficiency of antimicrobial activity of leaves and bark crude extracts of *Phytolacca dodecandra*.

6. METHODOLOGY

During this research conducted at INES/Ruhengeri Laboratories, different scientific methods have been used. They were accompanied by different techniques that helped us to achieve our goals. They include experimental method, analytical method, statistic methods, synthetic method and sampling method.

7. DELIMITATION

7.1. Time delimitation

This research work covers a period from June 2012 to November 2012

7.2. Space delimitation

This research work concerns the region of Musanze District and the experiments have been carried out in INES-RUHENGERI especially in Laboratory of Microbiology. The strains of *Neisseria gonorrhoea* and *Candida albicans* were isolated from CHUK.

7.3. Domain delimitation

The regarded domain of this research work is the microbiology field where this work permitted us to relate the acquired knowledge of Microbiology, Molecular Biology and Plant Systematic.

8. WORK SUBDIVISION

The activities organization is subdivided into two main parts:

- The first part concerns the literature review.
- The second part regards the experimental activity, the analysis of the obtained result, the interpretation, discussion and the conclusion.

PART ONE: LITERATURE REVIEW

CHAPTER I: GENERALITIES

1.1. Medicinal plants

Medicinal plants are various plants used in herbalism and thought by some to have medicinal properties. Few plants or their phytochemical constituents have been proven to have medicinal effects by rigorous science or have been approved by regulatory agencies such as the United States Food and Drug Administration or European Food Safety Authority. (Samuel Mathew, 1998)

Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects, fungi and herbivorous mammals. Many of these phytochemicals have beneficial effects on long-term health when consumed by humans, and can be used to effectively treat human diseases. At least 12,000 such compounds have been isolated so far; a number estimated to be less than 10% of the total. These phytochemicals are divided into primary metabolites such as sugars and fats, which are found in all plants; and secondary metabolites – compounds which are found in a smaller range of plants, serving a more specific function. For example, some secondary metabolites are toxins used to deter predation and others are pheromones used to attract insects for pollination. It is these secondary metabolites and pigments that can have therapeutic actions in humans and which can be refined to produce drugs, examples are insulin from the roots of dahlias, quinine from the cinchona, morphine and codeine from the poppy, and digoxin from the foxglove. Chemical compounds in plants mediate their effects on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs; thus herbal medicines do not differ greatly from conventional drugs in terms of how they work. This enables herbal medicines to be as effective as conventional medicines, but also gives them the same potential to cause harmful side effects.

Hundreds of medicinal plants are items of commerce, however relatively small countries are used in formulated herbal remedies. Several formulations like herbal teas, extracts, decorations, infusions, are prepared from medicinal plants (KRAISINTU, 1997).

Most cultures have a tradition of using plants medicinally. In Europe, apothecaries stocked herbal ingredients for their medicines. In the Latin names for plants created by Linnaeus, the word *officinalis* indicates that a plant was used in this way. For example, the marsh mallow has the classification *Althaea officinalis*, as it was traditionally used as an emollient to soothe ulcers. Ayurvedic medicine, herbal medicine and traditional Chinese medicine are other examples of medical practices that incorporate medical uses of plants. Pharmacognosy is the branch of modern medicine about medicines from plant sources. Plants included here are those that have been or are being used medicinally, in at least one such medicinal tradition.

Herbs are used for cleansing, elimination and detoxification. Some are used to stimulate the body's self-healing capabilities to counteract physical symptoms, while others may be used as a tonic to help the body's immunity (WALTER V. Reid, 1993).

Modern medicine now tends to use the active ingredients of plants rather than the whole plants. The phytochemicals may be synthesized, compounded or otherwise transformed to make pharmaceuticals. Examples of such derivatives include Digoxin, from digitalis; capsaicin, from chili; and aspirin, which is chemically related to the salicylic acid found in white willow. The opium poppy continues to be a major industrial source of opiates, including morphine. Few traditional remedies, however, have translated into modern drugs, although there is continuing research into the efficacy and possible adaptation of traditional herbal treatments (www.wikipediafoodanddrugadministration, consulted on 25 September 2012).

1.2. *Phytolacca dodecandra*

Scientific classification

Kingdom: *Plantae*

(unranked): *Angiosperms*

(unranked): *Eudicots*

(unranked): *Core eudicots*

Order: *Caryophyllales*

Family: *Phytolaccaceae*

Genus: *Sarcoca*

Species: *P. dodecandra*

Phytolacca dodecandra, commonly known as endod,

The morphology of *Phytolacca dodecandra*, locally called endod, was examined using herbarium specimens from throughout its natural range in Africa and analyzed by principal component and principal coordinate analyses as well as contour mapping of the coordinate scores. Examination of *P. dodecandra* from throughout its range in Africa revealed that a few pubescent-leaved plants were found from Ethiopia. *Phytolacca dodecandra* was found to be very variable in its morphology, both within a region and among regions. Major regional trends discovered were differentiation of: 1) the east and West Africa populations; 2) Madagascar plants from the mainland populations; 3) the Gabon plants from other West African plants; and 4) the Uganda, Burundi, Zimbabwe, and South African plants from other regions. The figure 1 shows the plant *Phytolacca dodecandra* extracted from Gacaca sector in Musanze District.



Figure 1: *Phytolacca dodecandra*

Source: Photo taken by the author, on 15 July 2012

1.3. Bacterial growth

Bacterial growth is the division of one bacterium into two daughter cells in a process called binary fission. Providing no mutational event occurs the resulting daughter cells are genetically identical to the original cell. Hence, "local doubling" of the bacterial population occurs. Both daughter cells from the division do not necessarily survive. However, if the number surviving exceeds unity on average, the bacterial population undergoes exponential growth. The measurement of an exponential bacterial growth curve in batch culture was traditionally a part of the training of all microbiologists; the basic means requires bacterial enumeration (cell counting) by direct and individual (microscopic, flow cytometry), direct and bulk (biomass), indirect and individual (colony counting), or indirect and bulk (most probable number, turbidity, nutrient uptake) methods (<http://en.wikipedia.org/wiki/Bacterial-growth.png>, retrieved on 30/09/2012).

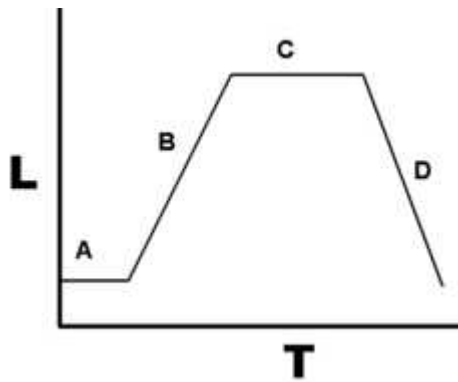


Figure 2: Bacterial growth

A: Lag phase

B: Logarithmic or exponential phase

C: Stationary phase

D: Death phase

Growth is shown as $L = \log(\text{numbers})$ where numbers is the number of colony forming units per ml, versus T (time.)

1. During lag phase, bacteria adapt themselves to growth conditions. It is the period where the individual bacteria are maturing and not yet able to divide. During the lag phase of the bacterial growth cycle, synthesis of RNA, enzymes and other molecules occurs (http://en.wikipedia.org/wiki/Bacterial_growth#cite_3, consulted on 29 September 2012).
2. Exponential phase (sometimes called the log phase or the logarithmic phase) is a period characterized by cell doubling. The number of new bacteria appearing per unit time is proportional to the present population. If growth is not limited, doubling will continue at a constant rate so both the number of cells and the rate of population increase doubles with each consecutive time period. For this type of exponential growth, plotting the natural logarithm of cell number against time produces a straight line. The slope of this line is the specific growth rate of the organism, which is a measure of the number of divisions per cell per unit time. The actual rate of this growth (i.e. the slope of the line in the figure) depends upon the growth conditions, which affect the frequency of cell division events and the probability of both daughter cells surviving. Under controlled conditions, cyanobacteria can double their population four times a day. Exponential growth cannot continue indefinitely, however, because the medium is soon depleted of nutrients and enriched with wastes (http://en.wikipedia.org/wiki/Bacterial_growth#cite_note_bacanove2008_2, consulted on 29 September 2012).
3. The "stationary phase" is due to a growth-limiting factor; this is mostly depletion of a nutrient, and/or the formation of inhibitory products such as organic acids. An awkward but unfortunately widespread explanation is that the stationary phase results from a situation in which growth rate and death rate have the same values (newly formed cells per time = dying cells per time but this is not logical, and it is better to forget this. Such an explanation would not be in accordance with the observed substrate depletion and also could never explain the rather "smooth," horizontal linear part of the curve during the stationary phase. Death of cells as a function of time is rather unpredictable and very

difficult to explain. Another not really logical explanation of the stationary phase is that “there isn’t anymore enough space for the cells. However; under the microscope you will see that there is still plenty of water between the cells. Only in an agar colony with densely packed cells space is obviously limiting (http://en.wikipedia.org/wiki/Bacterial_growth#cite_3, consulted on 29 September 2012).

4. At death phase, bacteria run out of nutrients and die. This basic batch culture growth model draws out and emphasizes aspects of bacterial growth which may differ from the growth of macrofauna. It emphasizes clonality, asexual binary division, the short development time relative to replication itself, the seemingly low death rate, the need to move from a dormant state to a reproductive state or to condition the media, and finally, the tendency of lab adapted strains to exhaust their nutrients. In reality, even in batch culture, the four phases are not well defined. The cells do not reproduce in synchrony without explicit and continual prompting (as in experiments with stalked bacteria) and their exponential phase growth is often not ever a constant rate, but instead a slowly decaying rate, a constant stochastic response to pressures both to reproduce and to go dormant in the face of declining nutrient concentrations and increasing waste concentrations (http://en.wikipedia.org/wiki/Bacterial_growth#cite_note_4, consulted on 29 September 2012).

1.3.1. *Neisseria gonorrhoeae*

Scientific classification

Kingdom: *Bacteria*

Phylum: *Proteobacteria*

Class: *Beta Proteobacteria*

Order: *Neisseriales*

Family: *Neisseriaceae*

Genus: *Neisseria*

Species: *N. gonorrhoeae*

Binomial name

Synonyms *Gonococcus* Neisser 1879

Neisseria gonorrhoeae cultured on two different media types and presented in stereoscopic. The figure 2 shows the strains of *Neisseria gonorrhoea*.

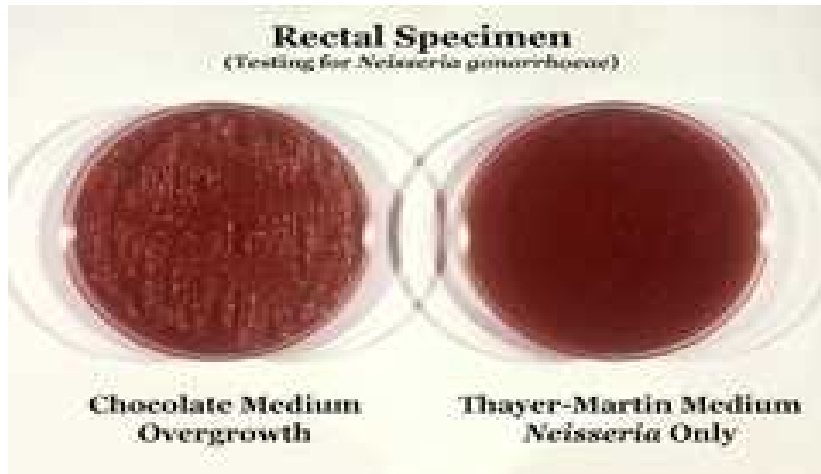


Figure 3: *Neisseria gonorrhoea* strains

Source:(http://en.wikipedia.org/wiki/File:Neisseria_gonorrhoea_01.png, consulted on 01 October 2012)

Neisseria gonorrhoea, also known as *gonococci* (plural), or *gonococcus* (singular), is a species of Gram-negative coffee bean-shaped diplococci bacteria responsible for the sexually transmitted infection gonorrhea

N. gonorrhoea was first described by Albert Neisser in 1879. This microorganism is an obligate human parasite, not found in other animals, isolated from the genitourinary tract, joint fluid, anorectal swabs or exudates from the eyes by breaking pus or discharge on freshly prepared chocolate agar incubating at 37° C.

Neisseria gonorrhoeae is a species of Gram-negative cocci, usually seen in pairs with the adjacent sides flattened, it is pathogenic to humans who are its only natural host, it is responsible for the disease *Gonorrhea*.

Gonorrhea is a sexually transmitted disease of worldwide importance. The highest attack rate in both men and women occurs between 15 and 29 years of age. Host-related factors such as the number of sexual partners, contraceptive practices, sexual preference, and population mobility contribute to the incidence of Gonorrhea. *Neisseria gonorrhoeae* can grow and multiply easily in warm, moist areas, including the cervix, uterus, and fallopian tubes in women, and in the urethra in women and men. The bacterium can also grow in the mouth, throat, eyes, and anus.

Gonorrhea is spread through contact with the penis, vagina, mouth, or anus. Ejaculation does not have to occur for gonorrhea to be transmitted or acquired. Gonococci adhere to columnar epithelial cells, penetrate them, and multiply on the basement membrane. Adherence is facilitated through pili and opa proteins (K. R. ANEJA, 2003)

Neisseria gonorrhoeae typically infects the mucous membranes causing infections such as urethritis, cervicitis, salpingitis, pelvic inflammatory disease, proctitis, conjunctivitis and pharyngitis.

In males there is typically a 2-3 day incubation period after which a purulent discharge from the urethra and dysuria develops. Around 95% of infected males are symptomatic. Rare complications include prostatitis, epididymitis, and periurethral abscesses. *Neisseria gonorrhoeae* primarily infects the cervix in women however the symptoms of gonorrhea are often mild and most women who are infected have no symptoms. Even when a woman has symptoms, they can be so non-specific as to be mistaken for a bladder or vaginal infection. Symptoms include vaginal discharge, dysuria, and abdominal pain. Complications from an ascending infection include abscesses of the ovarian tubes and pelvic inflammatory disease. Around 10%-20% of infected women develop these complications. In 1%-3% of infected women and a lower percentage of infected men the bacterium disseminates via the blood causing bacteremia and arthritis.

Gonorrhea can also be spread from mother to baby during delivery. This can cause blindness, joint infection, or a life-threatening blood infection in the infant.

Antibiotics can successfully cure gonorrhea but because many people with gonorrhea also have Chlamydia, antibiotics for both infections are usually given together.

1.3.1.1. Microbiology

N. gonorrhoeae are motile (twitching motility) and possess type IV pili to adhere to surfaces. The type IV pili operate mechanistically similar to a grappling hook

Neisseria are fastidious Gram-negative cocci that require nutrient supplementation to grow in laboratory cultures. Specifically, they grow on chocolate agar with carbon dioxide. These cocci are facultatively intracellular and typically appear in pairs (diplococci), in the shape of coffee beans. Of the eleven species of *Neisseria* that colonize humans, only two are pathogens. *N. gonorrhoeae* is the causative agent of *gonorrhea* (also called "The Clap," which is derived from the French word "clapier," meaning "brothel") and is transmitted via sexual contact

Neisseria is usually isolated on Thayer-Martin agar (or VPN agar)—an agar plate containing antibiotics (vancomycin, colistin, nystatin, and TMP-SMX) and nutrients that facilitate the growth of *Neisseria* species while inhibiting the growth of contaminating bacteria and fungi. Further testing to differentiate the species includes testing for oxidase (all clinically relevant *Neisseria* show a positive reaction) and the carbohydrates maltose, sucrose, and glucose test in which *N. gonorrhoeae* will only oxidize (that is, utilize) the glucose.

N. gonorrhoeae is naturally competent for DNA transformation as well as being capable of conjugation. Both of these concepts allow for the DNA of *N. gonorrhoeae* the ability to undergo conformational changes. Especially dangerous to the health industry is the ability to conjugate since this can lead to antibiotic resistance.

Symptoms of infection with *N. gonorrhoeae* differ depending on the site of infection. Note also that 10% of infected males and 80% of infected females are asymptomatic.

(http://en.wikipedia.org/wiki/File:Neisseria_gonorrhoea_01.png, consulted on 01 October 2012)

Infection of the genitals can result in a purulent (or pus-like) discharge from the genitals which may be foul smelling. Symptoms may include inflammation, redness, swelling, and dysuria

N. gonorrhoeae can also cause conjunctivitis, pharyngitis, proctitis or urethritis, prostatitis and orchitis.

Infection of the genitals in females with *N. gonorrhoeae* can result in pelvic inflammatory disease if left untreated, which can result in infertility. Pelvic inflammatory disease results if *N. gonorrhoeae* travels into the pelvic peritoneum (via the cervix, endometrium and fallopian tubes). Infertility is caused by inflammation and scarring of the fallopian tube. Infertility is a risk to 10 to 20% of the females infected with *N. gonorrhoeae*.

1.3.1.2. Treatment and Prevention

If *N. gonorrhoeae* is resistant to the penicillin family of antibiotics, then ceftriaxone (a third-generation cephalosporin) is often used. Sexual partners should also be notified and treated.

Transmission can be reduced by the usage of latex barriers, such as condoms or dental dams, during intercourse, oral and anal sex, and by limiting sexual partners. (http://en.wikipedia.org/wiki/File:Neisseria_gonorrhoea_01.png, consulted on July 25, 2012)

1.3.1.3. Vaccine

Due to the relative frequency of infection and the emerging development of antibiotic resistance in strains of *N. gonorrhoeae*, vaccines are thought to be an important goal in the prevention of infection. However, there have been relatively low emphases on research to such a vaccine in the medical literature and few human clinical trials for prospective vaccines. The ability to develop an effective vaccine has been limited by the lack of acquired immunity to infection to model a vaccine after and the current lack of commitment in effort and resources.

1.3.2. *Candida albicans*

The figure 4 shows the strains of *Candida albicans* extracted from e human body.

Candida albicans



Figure 4: *Candida albicans* strains

Source:(http://en.wikipedia.org/wiki/File:Candida_albicans_2.jpg, consulted on 05 September 2012)

Scientific classification

Kingdom:	<i>Fungi</i>
Phylum:	<i>Ascomycota</i>
Subphylum:	<i>Saccharomycotina</i>
Class:	<i>Saccharomycetes</i>
Order:	<i>Saccharomycetales</i>
Family:	<i>Saccharomycetaceae</i>
Genus:	<i>Candida</i>
Species:	<i>C. albicans</i>

Binomial name

Candida albicans

(C.P. Robin and Berkhout, 1923)

Synonyms

Candida stellatoidea

Oidium albicans

Candida albicans is an opportunistic fungus (or form of yeast) that is the cause of many undesirable symptoms ranging from fatigue and weight gain, to joint pain and gas.

The Candida yeast is a part of the gut flora, a group of microorganisms that live in your mouth and intestine. When the Candida population starts getting out of control it weakens the intestinal wall, penetrating through into the bloodstream and releasing its toxic byproducts throughout the body (http://en.wikipedia.org/wiki/File:Candida_albicans_2.jpg, consulted on 01st October 2012)

Candida albicans is a diploid fungus that grows both as yeast and filamentous cells and a causal agent of opportunistic oral and genital infections in humans. Systemic fungal infections (fungemias) including those by *C. albicans* have emerged as important causes of morbidity and mortality in immunocompromised patients (e.g., AIDS, cancer chemotherapy, organ or bone marrow transplantation). *C. albicans* biofilms may form on the surface of implantable medical devices. In addition, hospital-acquired infections by *C. albicans* have become a cause of major health concerns.

Treatment

Treatment commonly includes:

- amphotericin B, caspofungin, or fluconazole for systemic infections
- fluconazole or caspofungin for oral or esophageal infections
- topical azole for vaginal infections

Other Treatments

- Aspirin can be made into a chalky paste by mixing with a lotion and applied as a mask to the affected area. After letting the paste dry, the area can be exfoliated with a cloth or pumice (Bath sponges and loofahs should be avoided) - NOTE: Inhibition of *C. albicans* biofilm with Aspirin is shown as dose related. Therapeutic doses (100 μ M and 1 mM) of

Aspirin may inhibit growth by over 70%. Whereas, higher concentrations have shown inhibition of over 90% after 48 hours.

PART TWO: EXPERIMENTAL WORK

CHAPTER II: MATERIALS AND METHODS

2.1. Tools and Apparatus

2.1.1. Tools:

- | | |
|--------------------|--------------------|
| -Micropipette | -Aluminium foil |
| -Petri dishes | -Precision balance |
| -Spoons | -Erlenmeyer |
| -Mortar and Pestle | -Test tube |
| -Funnel | -Funnel |
| -Spatulas | -Cotton pads |
| -Filter paper | |

2.1.2. Apparatus:

- | | |
|--------------|---------------------|
| -Microscope | -Autoclave |
| -Thermometer | -Rotavapor |
| -Ovens | -pHmeter |
| -Thermostat | -Electronic balance |
| -Incubator | -Laminar flow hood |

2.1.3. Reagents:

- Water
- Distilled Water
- Alcohol 95% (methanol)
- Chloroform
- Extracts of *Phytolacca dodecandra*
- Iodine gram
- Safranin
- Gentian Violet (Crystal Violet)
- Peptonated water
- Microorganisms (*Neisseria gonorrhoea* and *Candida albicans*)
- Culture media

2. 2. Methods

2.2.1. Sample preparation

The different extracts required from our medicinal plant “*Phytolacca dodecandra*” have been sterilized and crushed into powder to be used in the culture media for detection of the antimicrobial activity.

The strains of *Neisseria gonorrhoea* and *Candida albicans* have been isolated from pus of patients in CHUK.

2.2.2. Data collection

Using different tools and apparatus mentioned above, all required data have been collected and noted down for their suitable analysis.

2.2.3. Procedure

- Harvest the plant material
- Wash it with distilled water
- Dry it in an oven 40°C during 48 hours
- Crush the plant material using mortar and pestle
- Mix the obtained powder with methyl alcohol for the maceration
- Filtrate the obtained mixture
- Subject to evaporation by using the rotavapor for the removal of methanol
- Apply this procedure to all required organs
- Isolate the strains of *Neisseria gonorrhoeae* and *Candida albicans* from pus of patient
- Culture the crude extracts on appropriate media after incubation in Oven at 37°C for 24 hours
- Using the Petri dishes, perform a culture of microorganisms (*Neisseria gonorrhoeae* and *Candida albicans* on the prepared crude extracts.
- Report the observation and discuss the result.

CHAPTER III: PRESENTATION, ANALYSIS AND DISCUSSION OF RESULTS

3.1. Presentation and analysis of results

3.1.1. Extraction of *Phytolacca dodecandra*

After harvesting, washing leaves and bark parts of *Phytolacca dodecandra* using distilled water, we proceeded by drying and crushing of those organs. The obtained powder of both leaves and bark were subjected to the maceration by using methanol as solvent and the results are summarized in the table below:

Table 1: Results of extraction of *Phytolacca dodecandra*

The name of the plant	Used organ of plant	Used quantity of powder in g	Volume of methanol in ml	Quantity of crude extracts obtained in g	Percentage
<i>Phytolacca dodecandra</i>	Leaves	100	500	17.54	17.54%
	Bark	100	500	17.95	17.95%

The results of table 1 show that the used solvent has extracted a considerable quantity of crude extracts. This implies that our medicinal plant *Phytolacca dodecandra* possesses a non negligible quantity of its parts soluble in methanol. Thus, the obtained quantity of leaves and bark crude extracts of *Phytolacca dodecandra* are 17.54g and 17.95g respectively.

3.1.2. Sterility control of prepared *Phytolacca dodecandra* extracts

Different volumes of Leaves and Bark crude extracts of *Phytolacca dodecandra* were subjected to the culture media (PDA and PCA) and incubated in an oven at 37°C for 24 hours. The outcomes are summarized in the table 2.

Table 2: Results of sterility testing of Leaves and Bark crude extracts of *Phytolacca dodecandra* on *Neisseria gonorrhoea*

Volume Of crude extracts in μl	Microorganism and Plant organs	<i>Neisseria gonorrhoea</i>	
		Leaves	Bark
5		-	-
10		-	-
15		-	-
20		-	-
25		-	-
30		-	-
35		-	-
40		-	-
45		-	-
50		-	-
55		-	-
60		-	-

These results show that leaves and bark crude extracts of *Phytolacca dodecandra* do not contain any gram-negative bacteria *Neisseria gonorrhoea*. Thus, there is no contamination during handling, and this allows us to use this extracts for further testing.

Table 3: Results of sterility testing of Leaves and Bark crude extracts of *Phytolacca dodecandra* on *Candida albicans*

Volume Of crude extracts in μ l	Microorganism and Plant organs	<i>Candida albicans</i>	
		Leaves	Bark
5		-	-
10		-	-
15		-	-
20		-	-
25		-	-
30		-	-
35		-	-
40		-	-
45		-	-
50		-	-
55		-	-
60		-	-

These results show that leaves and Bark crude extracts of *Phytolacca dodecandra* do not contain any fungus *Candida albicans*. Thus, there is no contamination during handling, and this allows us to use this extracts for further testing.

3.1.3. Growth control of culture media

The growth control Test is the assessment of the ability of the culture media to grow up bacteria.

Potato Dextrose Agar and Potato Count Agar are culture media used for the sensitivity test. The necessary requirements are obliged to grow up bacteria.

First colonies of *Neisseria gonorrhoea* and *Candida albicans* were cultured at Chocolate Agar and incubated in an oven at 37°C.

All samples of culture media tested enabled growth of bacteria.

3.1.4. Evaluation of antimicrobial activity of crude extracts of *Phytolacca dodecandra*

After having realized that leaves and bark crude extracts of *Phytolacca dodecandra* do not contain any kind of contamination, we judged very important to perform different tests for each of the regarded organs.

3.1.5. Results of different experiments of sensitivity tests.

These tests involve the study of effect of leaves and bark crude extracts of *Phytolacca dodecandra* on two microorganisms (*Neisseria gonorrhoea* and *Candida albicans*) with different volumes. The effect of leaves and bark crude extracts of *Phytolacca dodecandra* on microorganisms of our interest can then be determined.

The table 4 shows all species of our interest relative sensitivity to *Phytolacca dodecandra* crude extracts of different volume.

Table 4: Relative Diameter (in mm) of inhibitory zone of leaves and bark extracts *Phytolacca dodecandra* against *Neisseria gonorrhoea*

Vol in μl	Diameter in mm on <i>N. gonorrhoea</i> :	
	Leaves	Bark
5	3	0
10	4	2
15	6	2
20	7	4
25	8	5
30	10	8
35	12	9
40	14	10
45	15	12
50	16	15
55	16	17
60	16	17

The table 3 shows that the crude extracts of leaves and Bark of *Phytolacca dodecandra* are active against the growth of *Neisseria gonorrhoea* considering the obtained inhibitory zone.

The minimum inhibitory volume for leaves is 5 μl and the corresponding diameter of inhibitory zone is 3mm while for bark is 10 μl on the corresponding diameter of inhibitory zone is 2mm.

The figure 5 shows the variation of inhibition zone with leaves and bark crude extracts of the plant *Phytolacca dodecandra* against the microorganism *Neisseria gonorrhoea* using different volumes of those crude extracts.

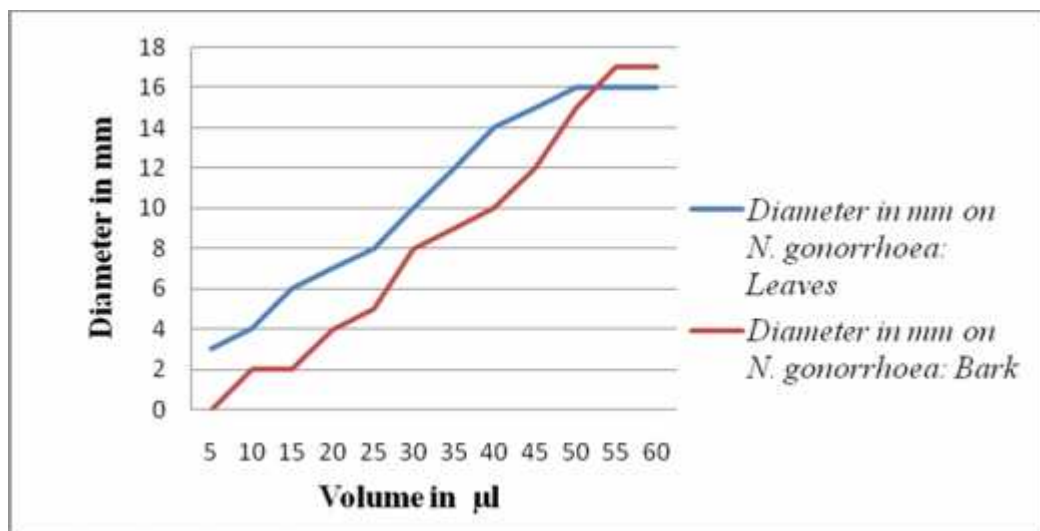


Figure 5: Variation of inhibition zone with leaves and bark crude extracts of *Phytolacca dodecandra* on *Neisseria gonorrhoea*

The figure 5 implies that the leaves and bark crude extracts of *Phytolacca dodecandra* revealed the antimicrobial effect on the gram-negative bacteria known as *Neisseria gonorrhoea*. It is generally remarked that for a minimum volume of 10 μl , *Neisseria gonorrhoea* is inhibited at the corresponding diameter of 3mm.

Table 5: Relative Diameter of inhibitory zone of leaves and bark extracts *Phytolacca dodecandra* against *Candida albicans*.

Vol in μl	Diameter in mm on <i>Candida albicans</i>	
	Leaves	Bark
5	0	0
10	2	0
15	5	1
20	5	2
25	6	2
30	8	4
35	9	6
40	10	8
45	10	10
50	12	13
55	14	15
60	14	15

The table 5 shows that the crude extracts of leaves and Bark of *Phytolacca dodecandra* are active against the growth of *Candida albicans* considering the obtained inhibition zone.

The minimum inhibition volume for leaves is 10 μ l and the corresponding diameter of inhibitory zone is 2mm while for bark is 15 μ l and the corresponding diameter of inhibitory zone is 1 mm.

The figure shows the variation of inhibition zone with leaves and bark crude extracts of the plant *Phytolacca dodecandra* against the microorganism *Candida albicans* using different volumes of those crude extracts.

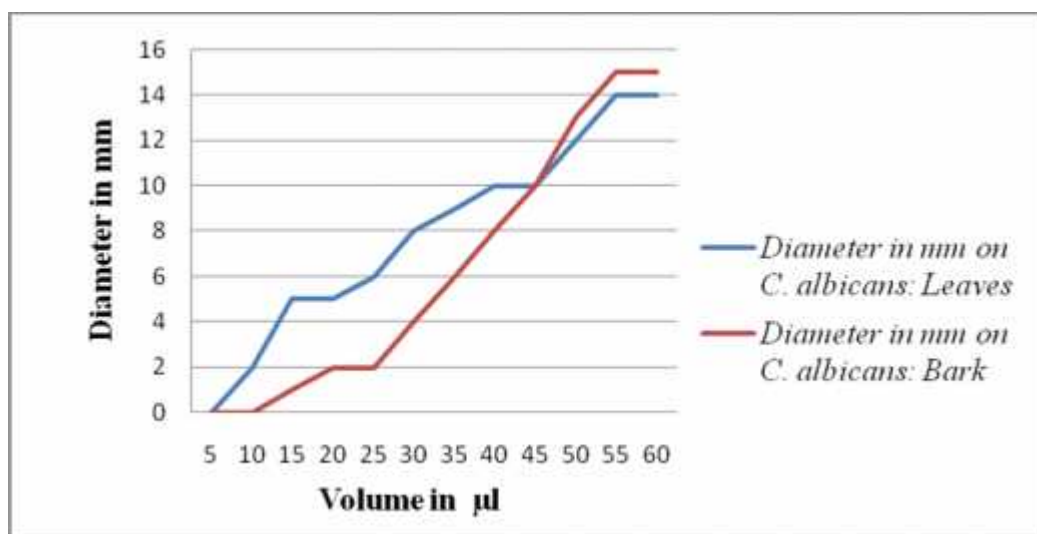


Figure 6: Variation of inhibition zone with leaves and bark crude extracts of *Phytolacca dodecandra* on *Candida albicans*

The figure 6 implies that the leaves and bark crude extracts of *Phytolacca dodecandra* revealed the antimicrobial effect on the fungus known as *Candida albicans*. It is generally realized that for a minimum volume of 15 μ l, *Candida albicans* is inhibited at the corresponding diameter of 3mm.

3.2. Results discussion

We conducted a scientific research at INES/RUHENGERI Laboratories. This research was entitled “contribution to the study of antimicrobial activity of crude extracts from leaves and bark of *Phytolacca dodecandra* against *Neisseria gonorrhoea* and *Candida albicans*,

When we performed several laboratory tests including the antibiogram test and several points have been remarked.

The crude extracts from leaves of *Phytolacca dodecandra* showed a positive result on gram-negative bacteria like *Neisseria gonorrhoea* at the minimum inhibitory volume of 5µl with the diameter of 3mm considering the inhibition zone. The crude extracts from leaves of *Phytolacca dodecandra* showed also a positive result on fungus *Candida albicans* at the minimum inhibitory volume of 10 µl with the diameter of 2mm in terms of inhibition zone.

The crude extracts from barks of *Phytolacca dodecandra* showed a positive result on gram-negative bacteria like *Neisseria gonorrhoea* at the minimum inhibitory volume of 10µl with the diameter of 2mm considering the inhibition zone. The crude extracts from leaves of *Phytolacca dodecandra* showed also a positive result on fungus *Candida albicans* at the minimum inhibitory volume of 15µl with the diameter of 1mm in terms of inhibition zone.

These results may be due to the active substances such as phenols, Quinones, Flavonoids and others biochemical substances contained in *Phytolacca dodecandra*. They imply that the crude extracts of *Phytolacca dodecandra* possess the significant effect at more than 15 µl of inhibitory volume with the corresponding inhibition zone diameter of 1mm (BRUNETON, 1987)

This enhances that crude extracts from leaves of *Phytolacca dodecandra* are more efficient than crude extracts of barks of *Phytolacca dodecandra*. The obtained results are similar to that found by THOMAS in 1998 where he has shown that from the concentration of 5 µl to 70 µl, the crude extracts of *Phytolacca dodecandra* are more active and demonstrate the high antimicrobial effects.

As it is known, the crude extracts of a plant with a minimum volume ≤ 100 µl is admitted to have the significant result in terms of anti-infection (IKEDAF, 1990).

In that case, the crude extracts from leaves and barks of *Phytolacca dodecandra* showed the antimicrobial effect against gram-negative bacterium like *Neisseria gonorrhoea* and fungus *Candida albicans*. (ROBE and VAN STADE, 1997).

After all, the crude extracts of *Phytolacca dodecandra* are known to be toxic. (FURSTERNBERG and HECKER, 1977). Precautions must be taken while manipulating leaves and bark crude extracts of this medicinal plant in order to avoid problem. However, traditional medicine should be controlled and well organized in order to reduce the harmful effects that may be caused by overdosing, mishandling and misconception of some medicinal plants like *Phytolacca dodecandra*.

We finally remark that, using methanolic extracts of *Phytolacca dodecandra*, the gram-negative bacterium *Neisseria gonorrhoea* and fungus like *Candida albicans* are inhibited.

CHAPTER 4: CONCLUSION AND RECOMMENDATIONS

4.1. Conclusion

This research work conducted at INES/RUHENGERI was aimed at identifying the effect of microorganisms *Neisseria gonorrhoea* and *Candida albicans* on leaves and bark crude extracts of *Phytolacca dodecandra*.

The objectives of this scientific work were to prepare extracts of *Phytolacca dodecandra*, to demonstrate the effects of leaves and bark crude extracts on microbes named *Neisseria gonorrhoea* and *Candida albicans*, and finally to compare the efficiency of sensibility of the two organs on these two microorganisms.

The laboratory results have shown that:

-Leaves crude extracts of *Phytolacca dodecandra* are more sensitive than bark extracts of *Phytolacca dodecandra* in presenting the antimicrobial activity against *Neisseria gonorrhoea* as well as *Candida albicans* when the volume decreases.

-Bark extracts of *Phytolacca dodecandra* have shown the high sensitivity in terms of antimicrobial activity against *Neisseria gonorrhoea* when the volume increases differently to the fungus *Candida albicans*.

The obtained results allowed us to ensure that our objectives are achieved. Hence, the hypotheses are also verified according to the theory clarified by IKEDAF (1990) stating that the crude extracts of a plant with a minimum volume 100µl is admitted to have the significant result in terms of anti-infection

The antimicrobial effect of methanol extracts on these microorganisms may be due to the ability of the active properties of plant *Phytolacca dodecandra* and also other secondary metabolites reported to be antimicrobial.

We generally conclude that the leaves and bark crude extracts of *Phytolacca dodecandra* have the antimicrobial effects against the growth of microorganisms *Neisseria gonorrhoea* and *Candida albicans*.

4.2. Recommendations

Due to the antimicrobial activities of *Phytolacca dodecandra* against microorganisms *Neisseria gonorrhoea* and *Candida albicans*, several recommendations may be formulated:

***TO THE MINISTRY OF HEALTH:**

- Organize several trainings of both Biotechnologists and traditional practitioners in Parasitology.
- Recruit enough skill full Biotechnologists in all medical Laboratories and Health centers.

***To INES RUHENGERI**

- search for enough Laboratory materials in domain of Microbiology like the culture media and other important materials
- increase the capacity of students in domain of Microbiology by providing the trainings to students in medical institutions.

***TO FUTURE RESEARCHERS**

- Perform deep research on the antimicrobial activities leaves and bark extracts of *Phytolacca Dodecandra* against other microorganisms
 - Identify the substance responsible of the harmful effect of *Phytolacca dodecandra* on human body.
 - Study the antimicrobial activity of *Phytolacca dodecandra* against *Neisseria gonorrhoea* and *Candida albicans* using other organs like flowers and fruits.
- .

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APPENDICES

Appendix 1: Table that shows the Laboratory results of leaves and bark crude extracts of *Phytolacca dodecandra* against *Neisseria gonorrhoea* and *Candida albicans*.

Organ & Quantity Volume in μl	Leaves		Bark	
	<i>Neisseria gonorrhoea</i>	<i>Candida albicans</i>	<i>Neisseria gonorrhoea</i>	<i>Candida albicans</i>
5 μl	3	0	0	0
10 μl	4	2	2	0
15 μl	6	5	2	1
20 μl	7	5	4	2
25 μl	8	6	5	2
30 μl	10	8	8	4
35 μl	12	9	9	6
40 μl	14	10	10	8
45 μl	15	10	12	10
50 μl	16	12	15	13
55 μl	16	14	17	15
60 μl	16	14	17	15

Appendix 2: Rotavapor machine used to obtain crude extracts

