Evaluation of Phytochemical Constituents of *Rotheca Myricoides* (Hocsht) For the Management of Syphilis in Humans and Formulation of An Herbal Remedy.

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DECLARATION

I Nambala Oliver declare that this research dissertation is my original work and has not been submitted elsewhere for the award of a degree. Where other people's work has been used, this has probably been acknowledged and cited according to the university policy.

Signature ..... Date ..4../05/2023

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APPROVAL

This research dissertation has been submitted for examination and has been approved by my supervisor

Dr. Oriko Richard Owor

Signature

Date ..5...../..5.... /2023

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DEDICATION

This report is dedicated to my parents Mr. Bataala George and Ms.Lyagga Prossy,Aunt Damali Nahire who have always supported me spiritually, physically and financially in the entire walk in pursuing my dream. Special thanks also to Mr. Hahysha Noah Wambigi and his wife Christine,Mr Hagobi Nathan for supporting me financially. Not forgetting Aunt Migamba Agnes and my cousin brother Kwamusi Benjamin and and my siblings Hashyha Boaz,Kaleele Andrew, Nabikamba Briayer,Konso Elizabeth and Igumba Magala who have always supported financially,advised and guided me in my academic journey. This research is also dedicated to my colleagues in the same race: Kiwuso Hassan, Kyaligonza Wilson,Kyotalye Brian,Mugaba Marium, Kampi Maria, Mudume Ishaika and Chebijira Mercy for their special time and support for the success of my research.

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LIST OF ABBREVIATIONS AND ACRONYMS

STI:	**Sexually transmitted infection**
TP:	***Treponema pallidum***
RM:	***Rotheca myricoides***
OE:	**Organic extract**
RME:	***Rotheca myricoides* Extract**
mL:	**milli litre**
mg:	**milligram**

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ABSTRACT

Syphilis is a multistage acute and chronic sexually transmitted infection (STI) that is caused by the bacterium *Treponema pallidum subspecies pallidum* that produces skin and mucous membrane lesions at the onset of the infection. Syphilis globally claims over 58% of the population per year in the world as per 2021.*Rotheca myricoides* is used locally for various medicinal purposes by traditionalists and herbalists in Uganda. Plants products have been used since ancient times in the management of various conditions. *Rotheca myricoides* (Hochst) is used in the management of diabetes, treatment of malaria, cancer, gastrointestinal disorders, asthma, spleen enlargement, persistent fever, headache, dysentery, diarrhea, syphilis, snakebite, impotence, eye problems, typhoid, cough, tonsillitis, rheumatism gonorrhea. The objective of this study is to evaluate the phytochemical constituents of *Rotheca myricoides* and its efficacy on the management of syphilis. The crude extract of *Rotheca myricoides* was subjected to preliminary phytochemical screening and antimicrobial tests. The phytochemical tests were carried out using standard methods of analysis and these investigations revealed the presence of tannins, saponins, flavonoids, phenols, quinones and alkaloids while terpenoids, glycosides and steroids were not present in the crude extract. Herbal syrup was formulated by mixing different active ingredients and a few of its physiochemical properties were determined. The toxicity profile of *R. myricoides* traditional therapy must first be determined as while as the appropriate quantity for the full dose. The bacterial assay should be done to find the minimum inhibitory concentration of *R. myricoides* extract on *T. pallidum*

CHAPTER ONE: INTRODUCTION

1.1 Background

Syphilis is a multistage acute and chronic sexually transmitted infection (STI) that is caused by the bacterium *Treponema pallidum* (Rothschild, 2005; L. V. Stamm, 2010) that produces skin and mucous membrane lesions at the onset of the infection. The primary site of syphilis is the genitalia, although primary lesions also occur on the outside of the genital (Peeling, Mabey, Herring, & Hook, 2006). Many syphilis cases are transmitted sexually, although it may also be transmitted vertically from the infected mother to the newborn child (Goh, 2005). Syphilis can also be transmitted by direct contact with skin lesions, saliva, and blood. Genital and oral sex are both implicated in the transmission of syphilis with humans as its only known natural host (Horváth, 2011). The presence of syphilis is accompanied by additional sexually transmitted infections in approximately 10% of the population with syphilis and syphilis-associated genital ulceration which increases the risk for *Human Immunodeficiency Virus* (HIV) (Rothschild, 2005). Syphilis can seriously complicate pregnancy and may result in stillbirths, spontaneous abortions, intrauterine growth restriction, and prenatal death, as well as serious injury in newborn-infected children (Genç & Ledger, 2000).

The manifestations of syphilis are classically divided into stages of occurrence, with each stage having its specific signs and symptoms related to time and antigen-antibody responses (Little, 2005). The stages are primary, secondary, latent, tertiary, and congenital. In the chronic stage, cardiovascular(aortic aneurysm, mucinous myocarditis), bone, viscera, and neurological diseases are produced (Román & Román, 1986).

Currently, penicillin G has been the treatment of choice for more than half a century (Clement, Okeke, & Hicks, 2014), and other drugs such as doxycycline, azithromycin, and erythromycin among others. Population increase, prohibitive costs for treatment, side effects of some synthetic drugs, and inadequate supplies of drugs coupled with the development of drug resistance to syphilis are among the challenges facing the current treatment strategies for syphilis (Okaiyeto, Nwodo, Mabinya, & Okoh, 2018). Despite the many years of using penicillin without failure and resistance, there was a need to introduce the use of second-line oral antibiotics for patients who are allergic to penicillin G(Clement et al., 2014) However, some bacteriostatic antibiotics such as

erythromycin and azithromycin that were introduced have showed some failure in treating syphilis due to mutations (Katz & Klausner, 2008; Wu et al., 2014).

Today, up to 90% of the African population uses traditional medicine to help meet their healthcare needs(Wachtel-Galor & Benzie, 2011) due to prohibitive costs, and the failure of some antibiotics to manage several diseases. Such plants include *Rotheca myricoides(R.M)* that is used for fertility (Mire, 2016), management of diabetes(Fang & Zhang, 2021), and other diseases such as typhoid, cough, eye problems, gonorrhea(Moshi, Otieno, & Weisheit, 2012) and syphilis in traditional medicinal practices. Therefore, this study was aimed at investigating the efficacy of *Rotheca myricoides* extract on the management of syphilis.

1.2 Problem statement.

The prevalence of infective syphilis is significantly rising particularly in developed countries (Tefera & Kim, 2019), with the male population being severely affected (Schmidt, Carson, & Jansen, 2019). Syphilis is a multistage acute and chronic sexually transmitted infection (STI) (Rothschild, 2005) that produces skin and mucous membrane lesions at the onset of the infection. The majority of syphilis cases are transmitted sexually although it may also be transmitted vertically from an infected mother to the newborn child (Goh, 2005). Chronically syphilis can also cause cardiovascular (aortic aneurysm, mucinous myocarditis) and neurological diseases such meningitis if not well managed. Despite the availability of penicillin G and other antibiotics like erythromycin and azithromycin, individuals in less developed countries such as Uganda still face great difficulty in accessing the appropriate medication (Little, 2005). This has resulted in the majority of them acquiring syphilis-related STIs, genital ulceration, a great risk for the *Human Immunodeficiency Virus*(Little, 2005), and a greater risk of stillbirth. Besides, there is also an emergency of resistance of the *T. pallidum* to the antibiotics rendering them less effective (Katz & Klausner, 2008; L. V. Stamm, 2010)

However, in traditional medicinal practices, medicinal plants have been used to manage syphilis. Among such plants include *R.myricoides* which is used for fertility (Mire, 2016), management of diabetes10:12 AM, and other diseases such as typhoid, cough, eye problems, gonorrhea(Moshi et al., 2012) and syphilis in traditional medicinal practices. Since there is scarce data on the efficacy

of *Rotheca myricoides* extract on the management of syphilis, this has prompted the researcher to conduct one.

1.3 Objectives.

1.3.1 General objective.

To evaluate the phytochemical constituents of *Rotheca myricoides* for the management of syphilis.

1.3.2 Specific objectives.

1. To identify the phytochemical constituents in the extracts of *Rotheca myricoides.*
2. To evaluate the efficacy of the extracts of *R. myricoides* on syphilis bacterium *T. pallidum*.

1.4 Justification.

Bacteriostatic antibiotics such as erythromycin and azithromycin that were introduced as second-line oral antibiotics for patients who are allergic to penicillin G have shown failure in treating syphilis, this is due to mutations (Katz & Klausner, 2008; Wu et al., 2014), there is need to develop new antibiotics to manage the widespread of syphilis that globally claims over 58% of the population per year in the world as per 2021(Organization, 2021). *R. myricoides* can be exploited as a source of antibiotics like the way quinine was extracted from the bark of L.*Cinchona* in 1820 (Leete, 1969) as a malaria antibiotic.

The study is therefore aimed at evaluating the efficacy of *R. myricoides* extract on the management of syphilis. If *R. myricoides* extract is found effective on the management syphilis, herbal formulation can be made and provide an alternative remedy for the management of syphilis

CHAPTER TWO: LITERATURE REVIEW.

2.1 Botany of *Rotheca myricoides.*

Rotheca myricoides belongs to order Lamiales in family Lamiaceae, genus *Rotheca* and Species *Myricoides(Rattray & Van Wyk, 2021). Rotheca myricoides* is an evergreen shrub(Mire, 2016), it grows 4 meters tall and 2.5 meters bound(L. K. Kamau, 2018). It has oval shaped leaves and masses of pale blue flowers. Commonly grows in tropical and warm temperate regions of the world,(Patel, Acharya, & Acharya, 2014), and able to survive at the altitude range of 900-1680 meters. It is native to African countries and calculated elsewhere(Ayinkamiye, 2020). This species may be found in rocky areas, edges of evergreen forests and streams(NYACHWAYA, 2019).

Figure 1. Image of *Rotheca myricoides* (Hocsht) steane & Mabb (Harrison, 2009)

2.2 Taditional uses of *Rotheca myricoides.*

Generally, plant species of the genus *Rotheca* have been used traditionally by many people as herbal medicine across Africa to manage several diseases(L. N. Kamau, Mbaabu, Mbaria, Gathumbi, & Kiama, 2016; Tefera & Kim, 2019).They include *Rotheca serrata* that is believed to exhibit excellent antihistaminic, antiallergic, antinociceptive, and anti-inflammatory properties(Patel et al., 2014), *Rotheca glandulosum* has showed hypoglycaemic and lipid-lowering properties(Jadeja, Thounaojam, Ramachandran, & Devkar, 2009). *Rotheca myricoides* is used in the management of diabetes in the lower Eastern part of Kenya, where a decoction is prepared by boiling the leaves and a cupful (250ml) is taken daily(Keter & Mutiso, 2012). Other ethnobotanical uses of *Rotheca myricoides* include the treatment of malaria(Mukungu, Abuga, Okalebo, Ingwela, & Mwangi, 2016). Cancer(Esubalew et al., 2017), gastrointestinal disorders, asthma, spleen enlargement, persistent fever, headache, d5ysentery, diarrhea, syphilis, snakebite, impotence, eye problems, typhoid, cough, tonsillitis, rheumatism gonorrhea(Moshi et al., 2012). Since no research has been done on the efficacy of *Rotheca myricoides* on the management of syphilis, this prompted the research to conduct one.

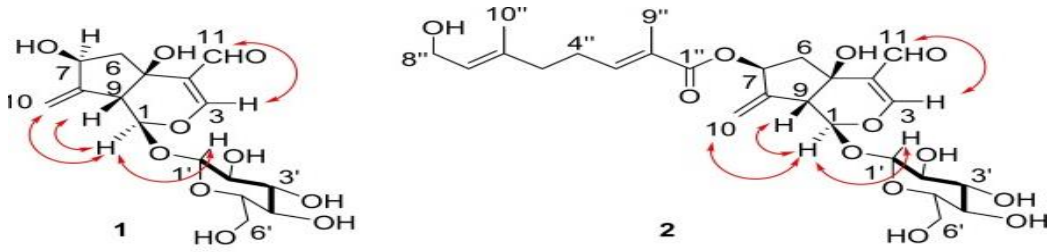
2.3 phytochemistry of *Rotheca myricoides.*

2.3.1 Phytochemical screening of *R. myricoides* (Hochst) Steane and Mabb.

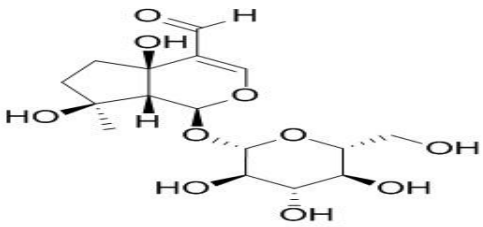
Thin layer chromatographic phytochemical analysis of 35 selected medicinal plants by use of dichlorimethane, methanol, and water (1:1) of the whole plant extract of *Rotheca myricoides* (Hochst) revealed the presence of alkaloids, anthraquinones, xanthins, valepotriates, cardioactive glycosides, flavonoids, coumarin drugs,linans, saponins and Arbutin drugs(Ochwang'I et al., 2016). Phytochemical screening of the crude *Rotheca myricoides* root extract using dichlorimethane (1:1) and methanol revealed the presence of phenolic glycosides. (Esatu et al., 2015).

2.3.2 compounds isolated from *Rotheca myricoides.*

The methanol extract of the root bark of rotheca myricoides subjected to column chromatography on silica gel, and selected fractions were subsequently isolated by preparative reversed-phase HPLC to give the two new iridoid glycosides myricoidoside A (1) and B (2) as well as euphroside and 7-O-cinnamoyl-5-hydroxygardaloside.



Euphroside



Chromatographic fractionation of a non-basic residue resulted in the isolation of two new homodetic cyclohexapeptides named cleromyrine I and II(Bashwira et al., 1989). Alkaloids such as myricoidine 1 and dihydromyricoide 2 were isolated from *Rotheca myricoides(Bashwira & Hootele, 1988).*

2.4 Pharmacology/ biological activities.

Pharmacologically its extracts exhibited hypoinsulinemic, hypoglycemic and hypolipidemic activities on type 2 diabetes rat model(Chege, Waweru, Frederick, & Nyaga, 2019).Rotheca myricoides is also believed to exhibited antimicrobial,antituberclosis(Njeru et al., 2016) and antimutagenic properties(Verschaeve & Van Staden, 2008).

2.5 Bacteria tested on *Rotheca myricoides.*

Methanolic solvent fraction of *Rotheca myricoides have* been investigated for Antimicrobial against array of microorganisms such as *Staphylococcus* aureus, *Cryptococcus neoformans*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella sonnei*, Methicillin-resistant *S. aureus*, Candida *albicans*, and *Mycobacterium tuberculosis* by disc diffusion and microdilution techniques. *(Njeru et al., 2016)*

2.6 Diseases that have been tested against *Rotheca myricoides*.

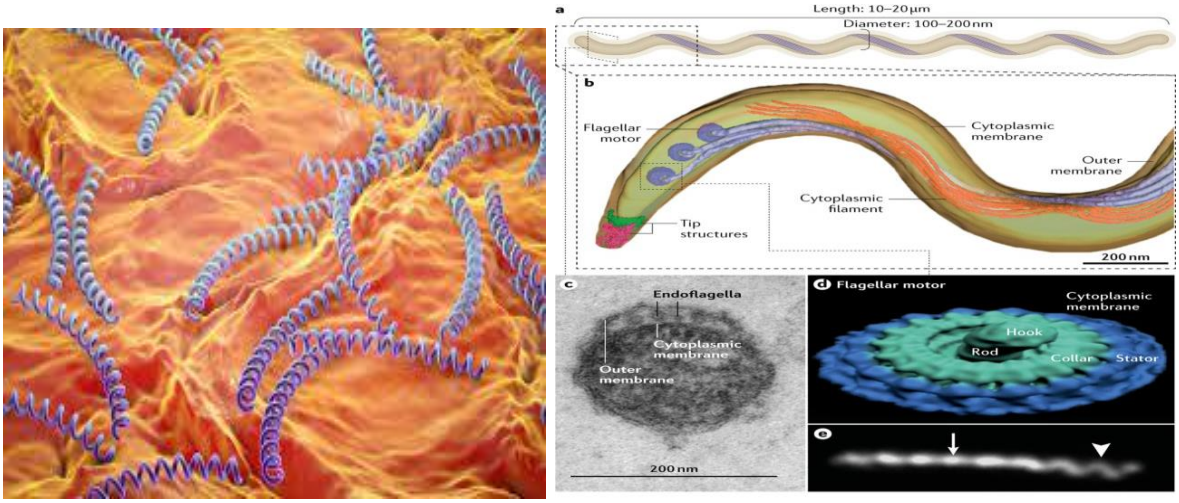
These include**;** malaria(Mukungu et al., 2016), Cancer(Esubalew et al., 2017), gastrointestinal disorders, asthma, spleen enlargement, persistent fever, headache, dysentery, diarrhea, syphilis, snakebite, impotence, eye problems, typhoid, cough, tonsillitis, rheumatism gonorrhea(Moshi et al., 2012). It is also used in the management of diabetes in the lower Eastern part of Kenya, where a decoction is prepared by boiling the leaves and a cupful (250ml) is taken daily(Keter & Mutiso, 2012)**.**

2.7 *Treponema pallidum.*

Treponema pallidum, is a spirochete bacterium with various subspecies that causes the diseases syphilis which include; endemic syphilis, yaws(Giacani & Lukehart, 2014; Marks, Lebari, Solomon, & Higgins, 2015) among others. It is a helically coiled usually 6–15 μm long and 0.1–0.2 μm wide. *T. pallidum* has a minimal metabolic activity due to lack of either tricarboxylic acid cycle or oxidative phosphorylation(Norris, Cox, & Weinstock, 2001). *Treponema pallidum* consists of three subspecies, *T. P endemicum*, *T. P pallidum pertenue and T.P pallidum* each of which has a distinct associated disease(Štaudová et al., 2014).

T.P pallidum is a motile, gram-negative spirochaete(Kumar Jaiswal et al., 2017), the etiologic agent of syphilis that appears antigenically inert and lacks detectable protein as judged by immunocytochemical and biochemical techniques commonly used to identify the outer membrane constituents of gram-negative bacteria(Walker, Zampighi, Blanco, Miller, & Lovett, 1989) .It is usually acquired by close sexual contact, entering the host via breaches in squamous or columnar epithelium(Hughes & Sawleshwarkar, 2023) and has incubation period of 21 days ranging between 10 to 90 days. It is transmitted to a fetus by transplacental passage during the late stages of pregnancy which gives rise to congenital syphilis(Rowe, Newberry, & Jnah, 2018). Its helical structure allows it to move in a corkscrew motion through mucous membranes or enter minute breaks in the skin(MINDEL & ESTCOURT, 2000). In females the initial lesion is usually on the labia, the walls of the vagina, or the cervix while in men it is on the glans of the penis. It gets into contact with the host's blood and lymph systems via mucous and tissue membranes. In more severe cases, it may gain access to the host by infecting the skeletal bones and central nervous system of the body(Radolf, 1996).

Figure 2. Images showing *Treponema pallidum bacteria*



(Lukehart & Marra, 2007)

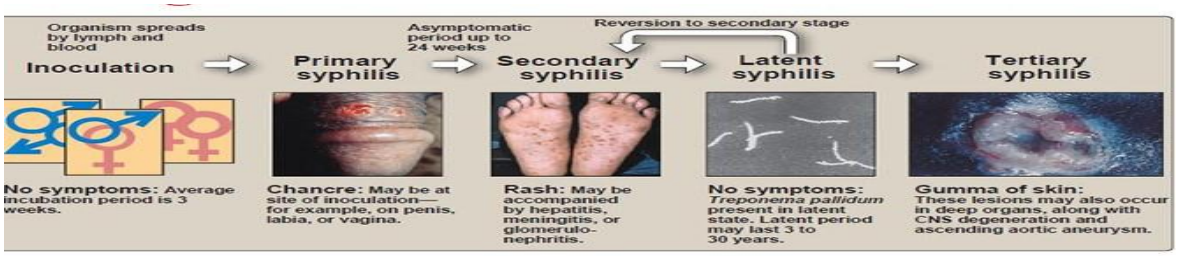
2.8 The problem and effects

Syphilis is a multistage chronic sexually transmitted infection (STI) caused by the bacterium *Treponema pallidum subspecies pallidum*, it produces skin and mucous membrane lesions at its onset(Little, 2005). It is diagnosed by identifying *Treponemes* using dark field microscopy or by direct fluorescent antibody stain, staining of *Treponeme*s in the histology specimen, and by serological tests(Goh, 2005). In dark field microscopy, *Treponemes* are identified by morphology and characteristic movements(Ambooken et al., 2022; Mehta, Saurav, & Balachandran, 2008)

Most cases of syphilis are transmitted sexually, although it may also be transmitted vertically from an infected mother to the newborn child which results in congenital syphilis(Goh, 2005), Oral sex and genital sex are both implicated in the transmission of syphilis, with the genitalia being its primary site although primary lesions also occur extragenitally(Peeling et al., 2006) If not managed well, syphilis results in acquiring syphilis-related STIs, genital ulceration, a great risk for the *Human Immunodeficiency Virus*(Little, 2005), and a greater risk of stillbirth. Chronically syphilis causes cardiovascular (aortic aneurysm, mucinous myocarditis) and neurological diseases such meningitis if not well managed.

Syphilis manifests itself in four major different stages. Each stage has its strange signs and symptoms. The stages are primary, secondary, latent and tertiary and congenital(Román & Román, 1986).

Figure 3. Image showing stages of syphilis and its effects.



2.9 Treatment of syphilis and failures of the treatment.

Currently, antibiotic treatment is a key component for managing syphilis since there is no vaccine to prevent the infection with *Treponema pallidum,* Benzathine penicillin G is the best antibiotic as of now(L. Stamm, 2015). However, second-line oral antibiotics such as Ceftriaxone, Tetracycline, Amoxicillin, Doxycycline, Azithromycin, and Erythromycin(Little, 2005; L. Stamm, 2015)among others have been introduced to manage syphilis especially in penicillin allergic patients. Despite the availability of penicillin G and other antibiotics like erythromycin and azithromycin(L. V. Stamm, 2010), individuals in less developed countries such as Uganda still face great difficulty in accessing the appropriate medication. Besides, there is also an emergency of resistance of the *T. pallidum strains* to the antibiotics rendering them less effective (Katz & Klausner, 2008; L. V. Stamm, 2010) and this has called for change in the approach to syphilis and also the rapid development of non-resistance antibiotics which requires further research such that more effective remedies are developed to manage such dangerous infections.

2.10 How syphilis is managed traditionally.

Traditionally, syphilis is managed using *R. Myricoides* where the whole plant parts are pounded, mixed with locally made ethanol (amalwa) and is taken as a remedy every day for a full week.

CHAPTER THREE: MATERIAL AND METHODOLOGY

3.1 Plant collection.

The whole pant of the *Rotheca myricoides* plant was collected from its natural habitat in Butaleja district, Busolwe town council, Nandahabi village, its identity verified at Makerere University herbarium by Ms. Carol Kawuma a lecturer in the Biology department at Busitema University Nagongera Campus and a voucher specimen deposited therein NO/06/22/01. The whole plant was used for the study. The plant was shade dried and ground into a powder using an electric mortar. After grinding, the plant sample was stored in an air-tight container to avoid bacteria growth.

3.2 Extract preparation.

3.2.1 Extraction with Organic solvents. (OE)

The shade-dried and ground powder of the whole plant of *R. myricoides* (50g) was placed in a container, and *R. myricoides* extract was extracted using dichloromethane/methanol (1:1, v/v) at room temperature. The extract was concentrated under reduced pressure on a rotary evaporator and the crude was obtained.

3.2.2 Preliminary phytochemical analysis of the crude extract of *Rotheca myricoides*

The crude extract of the whole plant of *R. myricoides* was dissolved in corresponding solvents to obtain their solutions unless stated in the procedure. Their solutions were subjected to different chemical tests separately for the identification of various active constituents which were as follows;

3.2.2.1 Test for Flavonoids.

3.2.2.1.1Ferric chloride test

To the methanolic solution of the crude *R. Myricoides* extract (2 mL), a few drops of neutral ferric chloride solution was added. The formation of blackish red precipitate colour indicated the presence of flavonoids**.**

3.2.2.1.2 Zinc-HCl reduction test.

To the methanolic solution of the crude *R. Myricoides* extract (2 mL), a pinch of zinc dust and a few drops of concentrated HCl using a test tube. Appearance of magneta colour indicated the presence of flavonoids**.**

3.2.2.1.3 Lead-Acetate test.

To the aqueous solution of the crude *R. Myricoides* extract (2 mL), a few drops of aqueous basic lead acetate solution was added using a test tube. The appearance of a reddish-brown bulky precipitate indicated the presence of flavonoids.

3.2.2.2 Test for tannins.

3.2.2.2.1 Ferric chloride test

To the aqueous solution of the crude *R. Myricoides* extract (2 mL), a few drops of neutral ferric chloride solution was added using a test tube. Formation of blackish precipitate colour indicated the presence of tannins.

3.2.2.2.2 Lead-Acetate test.

To the aqueous solution of the crude *R. Myricoides* extract (2 mL), a few drops of aqueous basic lead acetate solution was added using a test tube. The appearance of a reddish-brown bulky precipitate indicated the presence of tannins.

3.2.2.3 Test for Saponins.

A little part of the crude extract of *R. Myricoides* (5mL) was mixed with distilled water and then agitated in a graduated cylinder for 10 minutes. Formation of frothing which persisted on warming confirmed the presence of Saponins.

3.2.2.4 Test for Alkaloids.

3.2.2.4.1 Wagner's Test.

T**o** the crude methanolic extract of *R. Myricoides* (1mL), 1mL of Wagner's reagent (Iodine in potassium iodide). The formation of a reddish-brown precipitate showed the presence of alkaloids.

3.2.2.4.2 Dragendorff's reagent Test.

To the acidic crude extract solution of *R. Myricoides* (5mL), 2mL of Dragendorff's reagent (a solution of potassium bismuth iodide prepared from basic bismuth nitrate, glacial acetic acid and potassium iodide) was added followed by 2mL of dilute hydrochloric acid. An orange precipitate indicated the presence of alkaloids

3.2.2.5 Tests for Phenols.

T**o** the crude methanolic extract of *R. Myricoides* (5mL), 1mL ferric chloride solution was added using a test tube. The formation of muddy precipitate showed the presence of phenols.

3.2.2.6 Test for quinones.

The crude methanolic extract of *R. Myricoides* (5mL) was added to alcoholic sodium hydroxide solution. The appearance of a range of red to blue indicated the presence of quinones.

3.2.2.7 Total alkaloid content.

The sample (5000mg) was weighed and placed into a 250 mL beaker. 200 mL of 10% acetic acid in ethanol was added and the beaker covered with aluminum foil and allowed to stand for 4 hours. The extract was filtered and concentrated in a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide solution was added dropwise to the extract until the precipitation was complete. The whole solution was allowed to settle. The precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue was the alkaloid, which was dried, weighed and weight was recorded as 47mg.

3.2.2.8 Total flavonoid content.

The method was based on the formation of the flavonoids-aluminum complex which has an absorptivity maximum of 415nm. 40mg of the plant sample was added to a 250ml conical flask containing 10ml of water. 100µl of the plant extracts was mixed in methanol (10 mg/ml) with 100µl of 20 % aluminum trichloride in methanol. A drop of acetic acid was added, and then diluted with methanol to 5mL. After 40 minutes the absorption was read at 415.0nm. Blank samples were prepared from 200µl of plant extracts and a drop of acetic acid was added, and

then diluted to 5mL with methanol. The absorption of standard Quercetin solution (0.5 mg/mL) in methanol was measured under the same conditions and calibration curve was obtained and used to get the concentration of the sample, The calibration curve was constructed using standard quercetin solution prepared at concentrations of 0.5, 1.0, 1.5, 2.0 and 2.5mg/ml. The total flavonoids were determined using the equation below,

Total flavonoids (µg/mg) $= \frac{C*1000}{10} = \frac{0.0729*1000}{10}$ = 7.29

C is the concentration derived from the calibration curve in **Figure 4.**

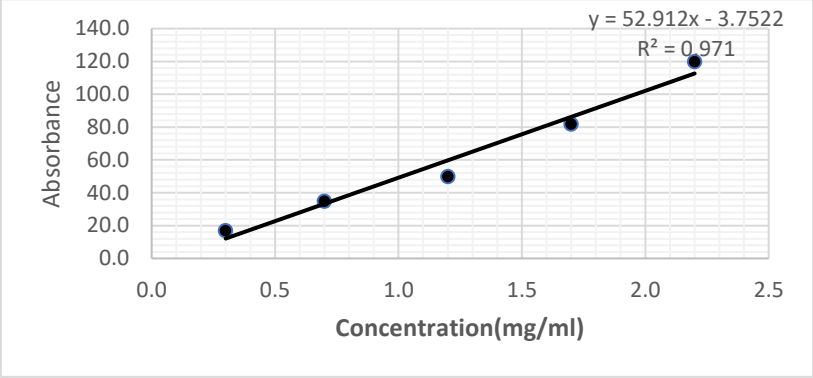


Figure 4 showing a calibration curve for determination of total flavonoids using standard quercetin solution.

3.2.2.9 Total phenolic content.

The total phenolic concentration was measured using the Folin-Ciocalteau method. In this procedure, 100 µl aliquot of the stock sample (extract concentration 1000 µg/mL of water) was mixed with 2.0 mL of 2% Na_2CO_3 and allowed to stand for 2 minutes at room temperature. Then 100 µl of 50% Folin-Ciocalteau's phenol reagent was added. After incubation for 30 min at room temperature in darkness, the absorbance is read at 720.0nm using a spectrophotometer. The total phenolic contents of the samples were expressed as mg ascorbic acid equivalent per gram. Ascorbic acid was used as a positive control. The calibration curve was constructed using standard ascorbic acid solution prepared at concentrations of 0.1, 0.2, 0.3, 0.4, 0.5and 0.6mg/ml (figure 5)

The total phenolic content was determined using the equation below,

Total phenols(µg/mg) $= \frac{C*1000}{10} = \frac{0.9714519*1000}{10}$ = 97.14519

C is the concentration derived from the calibration curve in figure 5

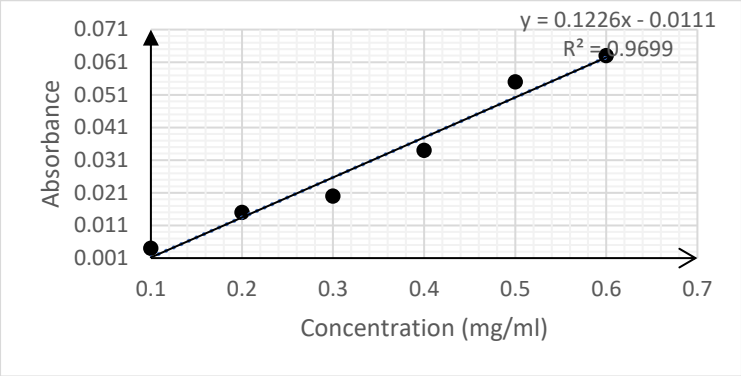


Figure 5 Showing the calibration curve for determination of total phenols using standard ascorbic acid solution.

3.2.2.10 Total tannins content.

The sample (5000mg) was weighed and transferred to a 50 mL plastic bottle. 50 mL of distilled water was added and shaken for 1 hour in a mechanical shaker. The mixture was filtered into a 50 ml volumetric flask and made up to the mark. 5 mL of the filtrate was pipetted into a test tube and were mixed with 2 mL of 0.1 M iron (iii) chloride in 0.1 M hydrochloric acid & 0.008 M potassium Ferro cyanide. The absorbance at 190nm within 10 minutes were measured and recorded and the calibration curve was plotted as shown in the figure 6. Ascorbic acid was used as a positive control. The calibration curve was constructed using standard ascorbic acid solution prepared at concentrations of 0.1, 0.2, 0.3, 0.4, and 0.5mg/ml (figure 6). The total phenolic content was determined using the equation below,

Total tannins (µg/mg) $= \frac{C*1000}{10} = \frac{39.04054*1000}{10}$ = 3904.054

C is the concentration derived from the calibration curve in figure 6

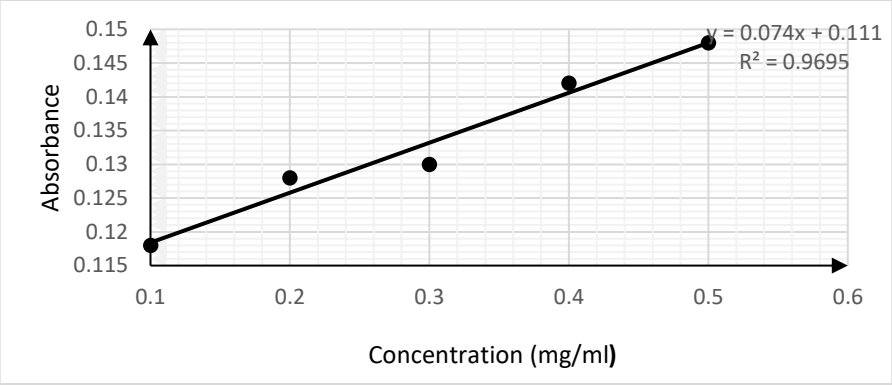


Figure 6 showing the calibration curve for determination of total tannins using standard ascorbic acid solution.

3.2.3 FORMULATION OF THE HERBAL SYRUP

All dried and grounded active ingredients **(***Rotheca myricoides and Tephrosia elganis***)** were mixed in the ratios of 250:150 mg in a clean conical flask containing distilled water in sufficient quantity. The mixture was shaken on a mechanical shaker at a temperature of 85^0c.The mixture was filtered and 25ml of the extract was obtained. The required quantity of other ingredients such as potassium sorbate(100mg) was dissolved in 10ml of distilled water. The solution was vortexed while adding Carboxymethyl cellulose(120mg) until it mixed uniformly was mixed,15ml of Glycerine was then added to make a 50ml herbal syrup (TROSTI150).

The table 1 below shows the summary of formulation procedure that was followed to make TROSTI150.

Table 1.

No	Ingredients	Uses	Quantity
1	Rotheca myricoides	Active ingredients	250mg
2	Trophrosia Elganis	Active ingredients	150mg
3	Potassium sorbate	Preservative	100mg
4	Carboxymethyl cellulose	Thickener (To increase the viscosity)	120mg

5	Water	Solvent	Quantity sufficient

CHAPTER FOUR: RESULTS AND DISCUSSION.

4.1 Results.

4.1.1 Phytochemicals characteristics of *R. Myricoides*.

The phytochemical analysis carried out on the crude *RME* showed that the whole plant was rich in pharmacologically useful phytochemicals such as Flavonoids, Tanins, Saponins, Alkaloids, Phenols and Quinones. Glycosides, TerPenoids and steroids were absent as shown in the table 2 below.

Table 2.

Phytochemicals	**Test**	**Present (+)/Absent (-)**
Flavonoids	Ferric chloride	++
	Zinc-HCl reduction	+
	Lead acetate	+
Steroids	Salkowski's test	-
	Liebermann-Burchard	-
Terpenoids	Trim –Hill	-
Tanins	Ferric chloride	++
	Lead acetate	++
Glycosides	Kellar Kiliani	–
	Suphuric acid	–
Saponins	Foam formation	+++
Alkaloids	Wagner's reagent	++
	Dragendroff's reagent	++
Phenols	Ferric chloride	+++
Quinones	Sodium hydroxide	+++

Key +: Weakly present, ++: Moderately present, +++: Strongly present.

Table 3. Total phytochemical content.

Total content	**Quantity** ($\mu g/mg$)**)**
Alkaloids	4700
Flavonoids	7.29
Phenolics	97.14519
Tannins	3904.054

4.2 Discussion of results.

Phytochemical analysis of the organic extract of *R. myricoides* revealed the presence of Flavonoids, Tanins, Saponins, Alkaloids, Phenols and Quinones as shown in table1 above. The constituents present in the extract have been reported to possess many therapeutic values.

4.2.1 Total alkaloid content.

5000mg of RM sample contained 47mg of total alkaloid content from the experimental results thus the concentration of the sample is 4700ug/mg.

4.2.2 Total tannin content.

The absorbance at 190 nm within 10 minutes was measured and recorded as 3 from a UV-vis photo spectrometer. Different concentrations of ascorbic acid were prepared from 0.5M and their absorbances obtained at the same wavelength and recorded and the calibration curve plotted which was used to determine the concentration of the sample as 3904.054ug/mg.

Tannins are believed to possess properties such as antioxidant, antimicrobial, anti-inflammatory, anticancer and virucides(Pizzi, 2021). Tanins are important in management of wound, diabetes, cardiovascular diseases diarrhea(Tcheghebe, Seukep, & Tatong, 2017).

4.2.3 Total phenolic content.

The total phenolic content was measured using the Folin-Ciocalteau method. 100 µl aliquot of stock sample. The standard calibration curve of ascorbic acid was used to determine the concentration of the sample as 97.14519 ug/mg. Phenolic compounds are important in defense

responses, such as antioxidant,anti-proliferative,anti-inflammatory and anti-aging activities (Lin et al., 2016) (Andersen, Lauridsen, & Skibsted, 2003)

4.2.4 Total flavonoids.

The absorption of standard quercetin solution (0.5 mg/mL) in methanol was measured under the same conditions at a wavelength of 415nm. The result was used to plot a calibration curve that was used to determine the concentration of total flavonoid content as 7.29 ug/mg. Flavonoids have many potential health benefits that demonstrate biological and pharmacological properties such as anti-inflammatory, antioxidant and antimicrobial activity(Jucá et al., 2020). Their antioxidant activities protect cells against oxidative damage and reduce the risk of developing certain types of cancers(Jacob & Burri, 1996). Flavonoids have also been reported to possess hypoglycemics and anti-diabetic effect(Brahmachari, 2011).

4.2.5 Saponins.

Saponins are glycosidic in nature and have expectorant and cardiotonic activity. Saponins have also been reported to have hypoglycemic and anti-diabetic effects which are very useful in the management of diabetes mellitus(Rajasree, Sibi, Francis, & William, 2016).

4.3 The physiochemical evaluation of the formulated herbal remedy

The physiochemical evaluation of the syrup formulated included the following properties;

Appearance, colour, dour, taste of the syrup and density as follows:

The syrup was turbid, reddish brown, it had a pleasant smell, sweetish with PH is 6.44 and density of 1.0314g/Ml.

Figure 7. Image showing the formulated herbal syrup (TROST150)

CHAPTER FIVE: CONCLUSION AND RECOMMENDATION.

5.1 Conclusion

To conclude, the phytochemical study revealed the presence of Flavonoids, Tanins, Saponins, Alkaloids, Phenols and Quinones which are compounds capable of causing diverse physiochemical and pharmacological properties. Their presence therefore seems to support the traditional use of *R. myricoides* in the management of various diseases such syphilis among others

5.2 Recommendation.

R. myricoides extract can then be used to make an herbal formulation as a natural remedy for management of syphilis. However, the toxicity profile of *R myricoides* traditional therapy must first be determined as while as the appropriate quantity for the full dose. The bacterial assay should be done to find the minimum inhibitory concentration of *R. myricoides* extract on *T. pallidum*. There is also need to isolate the bioactive ingredients in the plant extract that are responsible for management of the infections.

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