Phytochemical Analysis and Antibacterial Activities of *Combretum Molle* Stem Bark Extract for Management of Respiratory Tract Infections

BY

KAMPI MARIA GORRET

(BU/UP/2019/1536)

A Research project report Submitted to the Department of Chemistry for the Partial Fulfillment of the Requirements for the Award of the Bachelors of Science Education degree at Busitema University

May 2023

Declaration

I, Kampi Maria Gorret, declare that the research dissertation is my own original work otherwise cited, and where such has been the case, references have been stated and that the same work has not been submitted for any award in any other university or other tertiary institute of higher education

Signature..... Date..24/05/23.....

Approval

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

Dr. Owor Richard Oriko

Signature..... Date..24/05/23.....

Dedication

This report is dedicated to my parents and grandparents Ms. Kadondo Robinah and Mr. Mugabi Edward, my siblings Waiswa Micheal Mugabi, Kafuko Phillip, Mugabi David and Mpatogera Gertrude and my uncles Mr. Byakika Emmanuel Paddy, Mr. Kafuko Mosses, Mr. Ntabewra Hebert and Mr. Ibale Stephen Richard who have supported me in all aspects of life in my entire life journey of pursing my career dream as a professional teacher.

Acknowledgment

I would like to express my sincere appreciation and gratitude to my supervisor Dr. Owor Richard Oriko for having guided me and supported me during each step of my research. My great appreciation also goes to the Busitema University Research and Innovation Fund (BURIF) for the financial support towards my work. My great thanks also go to the almighty God for the gift of life, the good health, protection and the wisdom entirely at school and throughout my life journey as a student. In great capacity I would like to thank the mighty fraternity of Busitema University, faculty of science and education, Nagongera campus which has been a great foundation in producing quality teachers with a sense of integrity.

With great value and respect to the department of Chemistry, and all the lecturers namely, Dr. Kamoga Omar, Dr. Oriko Richard Owor, Mr. Musagala Peter, Mr. Egor Moses, Dr. Andima Moses, Dr. Kigozi Moses, Mr. Katwesigye Richard and Ms. Nakijoba Lydia, for your entire support from the start of the course to the end.

I also thank my supervisor Dr. Oriko Richard Owor who always guided me and provided the necessary support towards the completion of my research review.

I would to also thank prof. Olema David, Dr. Suubi Margret, Dr. Artubin, Mr. Kalyango, Mr. Wadaga Nelson, Dr. Owor Richard Oriko and Mr. Musagala Peter for having mentored me and guided as I was perusing my career.

Special regards go to Chemistry students more so Nambala Oliver, Wabwire Jacob, Kyotalye Brain, Kiwuso Hassan, Mwagale Flavia, Kisakye Kezia Priscilla and all the students of the faculty of science and education for the great company and support during my entire stay in Nagongera. Thanks for being good to me may God bless you all.

Abstract

Respiratory tract infections affect both the upper and lower respiratory parts of the respiratory system. It's caused by the bacterium called *Streptococcus pneumoniae.* 9 million Ugandans are reported to have symptoms of respiratory tract infections after every two weeks in the primary health care centres and over 2 million people die each year. Traditionalists and herbalists in Uganda have adapted to using *Combretum molle* regulate the cases of respiratory tract infections. Therefore, this Study was to investigate the phytochemical composition of *C.molle* for antibacterial activities, analyze the phytochemical composition of *C.molle* and to evaluate the efficacy of *C.molle* stem back extract against antibacterial activities. The crude extract of *C.molle* 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 alkaloids, flavonoids, phenols, Quinones, tannins, Saponins and Glycosides while terpenoids and steroids were not present in the crude extract. An herbal syrup was formulated and named CODEM 40. This could top to greater protection and assistance to people in managing respiratory tract infections. Therefore, additional studies and elucidation of the active compounds so as to provide a new or principal component for production of new drugs can be opened up further studies to investigate the toxicity of *C.molle* herbal products before their recommendation for use.

TABLE OF CONTENT

Table of Contents

List of Acronyms

C.molle; Combretum Molle

S. pneumoniae: Streptococcus pneumoniae

WHO; world health organization

CMS; Carboxymethyl Cellulose

RTIs; Respiratory Tract Infections

HCl; Hydrochloric acid

AQ; Aqueous extract

OE; Organic extract

DNA; Deoxyribose Nucleic Acid

List of figures

List of tables

CHAPTER ONE: INTRODUCTION

1.1 Background

Respiratory tract infections (RTIs) are diseases associated with fever, sore throat, blocked nose, cough, colds, malaise and running nose. It is always caused either viruses like *Rhinovirus* and *Corona* virus or bacteria like *Streptococcus*, *Staphylococcus* and *Moraxella* (Chrian, Erasto, & Otieno, 2011). Globally, *Streptococcus* and *Staphylococcus* are the commonest bacteria that cause the majority of respiratory tract infections (Wiemken, Peyrani, & Ramirez, 2012).

A respiratory tract infection is one of the diseases that report the biggest number of patients in primary health care centers of Uganda. The commonest that affects the populations include pneumonia, asthma, and tuberculosis. These mainly affect extreme ages that is to say very young and very old (Wiemken et al., 2012). In Uganda, over 9 million people are infected by respiratory tract infections with about 2 million deaths every year. The majority of these infections occur in low income countries where the increased cases of infections are attributed to inaccessibility to immunization, medication and inability of health care systems to provide care thus leading to avoidable death (Boloursaz et al., 2013). In Uganda, 3 million infants (< 5 years) have been identified to be victims of respiratory tract infections every year (Kiguli et al., 2021). Entirely about 9 million Ugandans are reported to have symptoms of RTIs after every two weeks in the primary health care centres and over 2 million people die each year (Erku & Aberra, 2018).
There are numerous drugs prescribed for management of respiratory tract infections and they are in category of analgesics (paracetamol and ibuprofen), antihistamine (diphenhydramine), antibiotics (penicillin and amoxicillin) and anticholinergics (ipratropium and scopolamine) (WHO, 2001). In spite of the present drugs, RTIs cases are still in increase (Schuetz et al., 2013) For example in Uganda, there were 19 millions Ugandans suffering from respiratory tract infections in 2020, 18millions in 2021, 20millions in 2022. This is has contributed to COVID-19 (Vihta et al., 2022)

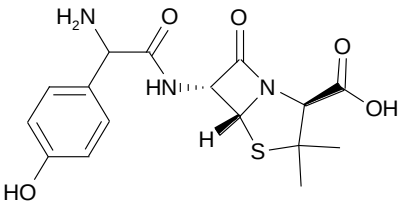
The surge up of the many victims suffering from RTIs is linked to resistance of the pathogens the available drugs (Cimolai, 2021).The cumulative rates of antibiotic resistance in community pathogens have focused the attention of researchers and clinicians on this public health problem(Ferri, Ranucci, Romagnoli, & Giaccone, 2017). There has been resistance to penicillin in

the past decade with a very high-level resistance to antibiotics(Brusselaers, Vogelaers, & Blot, 2011). For example *staphylococcus aureus* has become resistant to penicillin and Streptococcus pnuemoniae has also become resistant to penicillin (Rammelkamp & Maxon, 1942) and *Streptococcus pnuemoniae* has also become resistant to penicillin (Reinert, 2009)

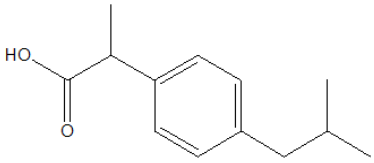
Resistance of the bacteria to drugs has become a natural biotic occurrence that is linked to a range of factors like limited access to effective drugs, un-restricted laws governing the production of drugs and absence of income to pay for suitable and first-class medications (Planta, 2007). As a consequence, there is an urgent need for alternative therapy for management of RTIs (Dias & Bagnato, 2020)

Therefore most Ugandans have resorted to usage of medicinal plants as an alternative in management of respiratory tract infections in both rural and urban areas in Uganda since they are accessible and available at any time of the day.(Lamorde et al., 2010; Tugume & Nyakoojo, 2019). The medicinal plants used in management of RTIs in Uganda include: *Citrus limon, Biden pilosa, garlic* and *Combretum molle* (Kaggwa et al., 2022).

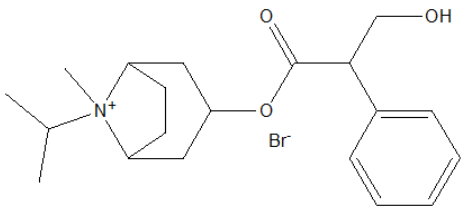
The search for new drugs with improved antibacterial activity to substitute those that have become inactive is therefore necessary(Wang, Hu, & Shao, 2017).This study was purposed to study the phytochemical and antibacterial property of *C.molle*.



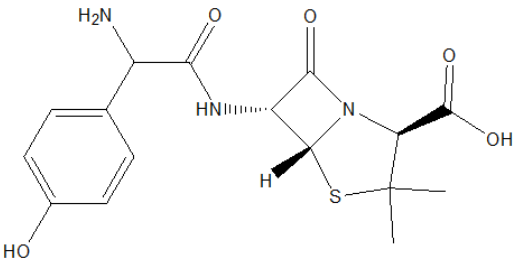
Amoxicillin



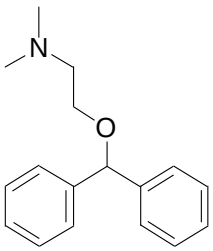
Ibuprofen



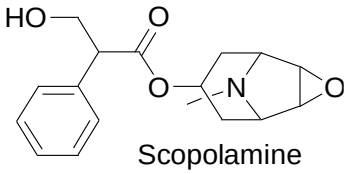
Ipratropium



Amoxicillin



Diphenhydramine



Scopolamine

1.2 Problem statement

Prevalence of respiratory tract infections is very high globally and it has reported many cases in primary health care units (Jin et al., 2020). Approximately 8.8 million Ugandans are reported to have signs of respiratory tract infections in every two weeks in the primary health care Centers(Snidal, Barnard, Atuhairwe, & Amor, 2015). over 3 million of these die each year of respiratory tract infections (Cummings et al., 2019).

This is ascribed to high charges for the treatment, inaccessibility to the drugs mainly for people in rural areas of Uganda (Robertson et al., 2019). Similarly, the most upsetting issue is the resistance of *S.pnuemoniae* to the approved drugs(Ventola, 2015).*Streptococcus pneumoniae's* failure to respond to the treatment has led to extended ailment and greater risk of death(Friedman, Temkin, & Carmeli, 2016).

Treatment dissatisfactions also result into to extended occurrences of contamination, surging up a big numbers of sick people in the public thus rendering the general population to the risk respiratory tract infections(Umuhoza et al., 2021). It has be sited that disease resistance is emerging to nearly all the currently existing drugs since bacterial pathogens are developing with new drugs of virulence and new designs of resistance to antimicrobial means(Organization, 2000).

New classes of drugs are progressively required outstanding to the cumulative population at risk and the rising prevalence of resilient pathogenic bacteria (O'Neill, 2016). This has called for other options that are affordable and accessible to Ugandans like medicinal plants(Ssenku et al., 2022). *C.molle* is one of the medicinal plant has been used in management of respiratory tract infections in various parts of Uganda (Schultz, Anywar, Wack, Quave, & Garbe, 2020)

C.molle based components are used as natural antimicrobials to treat bacterial pathogens and respiratory tract infection. The antimicrobial compounds from *C.molle* may prevent the bacteria

by several mechanisms than the currently used drugs which may have medical significance in treatment of resistant bacterial strains (Seukep, Sandjo, Ngadjui, & Kuete, 2016).However, there is scarce information on the efficacy of *C.molle* on the management of RTIs, this has promoted the researcher to conduct one.

1.3 Objectives

1.3.1 General objectives

Investigate the phytochemical composition of *C.molle* for antibacterial activities

1.3.2 Specific objectives

1. Analyze the phytochemical composition of *C.molle*
2. Evaluate the efficacy of *C.molle* stem back extract on *S. pneumoniae*

1.4 Significance

S. pneumoniae has become resistant to some of the existing drugs like penicillin and amoxicillin; this is attributed to the mutations of *S. pneumoniae*. Therefore, there is need to develop new drugs to manage the wide spread of respiratory tract infections which is 3 million infants (< 5 years) have been identified to be victims of respiratory tract infections every year (Kiguli et al., 2021). Entirely about 9 million Ugandans are reported to have symptoms of respiratory tract infections after every two weeks in the primary health care centres and over 2 million people die each year in Uganda (Erku & Aberra, 2018).

Therefore, *C.molle* can be exploited as a source of antibiotics. Thus, this study was aimed at providing an alternative source for management respiratory tract infections from *C.molle* and to justify the information about the phytochemical composition of *C.molle* stem bark extract.

CHAPTER TWO: LITERATURE REVIEW

1.1 Description of a medicinal plant

Medicinal plants have been used for centuries as remedies for human diseases and offer a new source of biologically active chemical compounds as antimicrobial agents(Das, Tiwari, & Shrivastava, 2010).

Medicinal plants are the richest bio-resources of drugs of traditional medicinal systems, modern medicines, food supplements, and folk medicines, pharmaceuticals, intermediate and chemical entitled for synthetic drugs (Majeed & Rehman, 2021).

Basing on estimations, (14- 28)% of higher plant species function as medicine and 74% of pharmacologically active plant obtained compounds were found after making a pursue on medicinal use of plants (Ncube, Afolayan, & Okoh, 2008)

Depending on medicinal plants have the ability be a source of both traditional and modern medicines. Herbal medicine has been shown to have profound utility with about 80% of rural population dependence on it for their primary health care(Mahomoodally, 2013). Around 80-90 billion of the population in the world rely on traditional medicine for their primary health care(Sen & Chakraborty, 2017).

Plant materials give the models for (50 -60)% of Western pharmaceutical industries and other uses that proposed capable useful biological action (Pan et al., 2013).The desire of using plant extracts for treatment of microbial infections has been increasing from late 1990s when the typical antibiotics became ineffective with undesirable toxic effects(Lewis, 2013).

2.2 *Combretum molle*

C.molle is called velvet bush willow in English, baster rooibos in Afrikaans and umBondwe in Zulu. It belongs to the genus Combretum(I. E. Cock & Van Vuuren, 2020).

C. molle germinates from fresh seeds, grows as a shrub to a height of about 13 meters with a rounded crown. With a bark that is grey when young and black when old, with simple leaves that are hairy when immature and smooth when mature. It flowers from September to November(Shorrocks & Bates, 2015).

Combretaceae consists of 20 genera and 300 species (Eloff, Katerere, & McGaw, 2008). These species include: *Combretum apiculatum*, used to treat snake bites and bloody diarrhea, (Roy, Gorai, Acharya, & Roy, 2014). *Combretum hereroene*, used to treat bilharzia and infertility in women (McGaw et al., 2001)and *C.molle* is used to treat fever, dysentery, diarrhea, vomiting and fever (Papo, Van Vuuren, & Moteetee, 2022).Different organs such as leaves, roots and stem bark of *C. molle* are predominantly used (Nyenje, 2011)

Traditional physicians hire species of Combretaceae for medicinal uses like bacterial, fungal, viral and parasitic infections(I. Cock, 2015). C. molle has been stated to have antifungal, antimicrobial, anti-parasitic, antioxidant, and anti-inflammatory activity (Kaggwa et al., 2022)

However, there isn't a sufficient awareness study which has proven the stem bark of *C.molle* for its antibacterial activity against *S. pneumoniae* which has created potential health harms in our communities as a cause of respiratory tract infections. And since there is scarce information about the efficacy *C.molle* stem bark extract in the management of respiratory tract infections, this prompted the researcher to conduct one.

2.2.1 Structures of *Combretum molle*

Figure 1 **Structures of *Combretum molle***

2.2.2 PHTOCHEMICAL SCREENING OF *COMBRETUM MOLLE*

Medicinal plants produce aromatic secondary metabolites which synthesize phytochemical compounds that have no effect on the human body these include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids(Shakya, 2016)

Academic fields have screened plant material for antimicrobial properties, various solvents for example ethanol, methanol, petroleum ether, chloroform, hexane, ethyl acetate, DCM(dichloromethane) and water has been used to extract active compounds(Houghton & Raman, 2012)

Using the thin layer chromatography, *C.molle* methanolic plant extract was found to have phytochemical compounds like phenolic, flavonoids, tannins and Saponins (Ntshanka M. N, 2020).

According to the phytochemical screening carried out by Bassene in 2012, *C.molle* was found to have tannins, flavonoids, alkaloids, Saponins, phenolic compounds and heterosides cardiotonics(Koevi et al., 2015). Phytochemical compounds like flavonoids, glycosides, tannins and Saponins have made *C.molle* a good inhibitor of microbial growth(Kulawe, Abubakar, & Abubakar, 2019).

2.3 Respiratory Tract Infections

A respiratory tract infection disturbs respiratory system which is responsible for breathing. These infections affect your sinuses, throat and lungs (de Menezes et al., 2021). RTIs are classified as upper respiratory tract infections and lower respiratory tract infections (Sandelowsky, Ställberg, Nager, & Hasselström, 2011). Upper RTIs include: Common cold, Epiglottitis, Laryngitis and sore throat A patient exhibits symptoms like fever, hoarse noise, fatigue, red eyes and running nose. It usually last for one to two week (Zoorob, Sidani, Fremont, & Kihlberg, 2012). The lower RTIs distress the airways and lungs. They last longer and are more serious. They include: bronchitis this causes coughing and fever and bronchiolitis which mainly affect children, chest infection and pneumonia(Sandelowsky et al., 2011).

2.4 *Streptococcus pneumoniae*

S. pneumoniae is a Gram-positive, non-spore-forming cocci which are about 0.5-1.2 µm in size (Ghosh & Mandal, 2018). It often grows in chains and pairs that oxidase and catalase as negative (Raluca & Mariana).It colonizes the upper respiratory tract of 5-15% of normal individuals(Baggett et al., 2017). *S. pneumoniae* is very infectious as it kills the host defense system(Aberdein, Cole, Bewley, Marriott, & Dockrell, 2013). Its responsible for causing respiratory tract infections It does the spreading by produces toxins that are harmful to its host and has numerous protein surfaces with physical structures that play a vital role in pathogenesis (Brooks & Mias, 2018). *S. pneumoniae* withstand both aerobic and anaerobic conditions. Thus making responsible for millions of deaths worldwide. (Brooks & Mias, 2018).

2.4.1 STRUCTURE OF *STREPTOCOCCUS PNEUMONIAE*

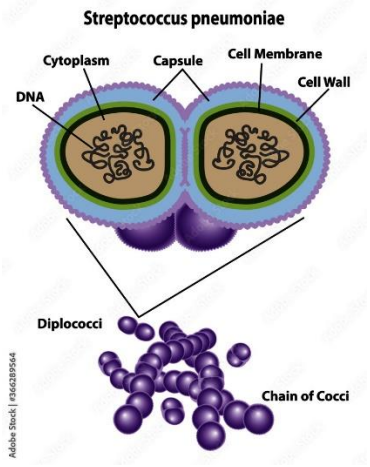


Figure 2* structure of *streptococcus pneumoniae

2.5 Mode of transmission of Respiratory tract infections

RTIs can be got when a virus or bacteria enters your respiratory system.(Lappin et al., 2017) Can get an affected if they interact with infect or tremor hands with the infected person touch their mouth, nose or eyes. These germs from the hands go in and contaminate the body (Sarwar & Imran, 2021).

2.6 Mechanisms Bacterial resistance against prescribed drugs

Bacterial resistance has its basis at the hereditary level where changes in the genetic character of the formerly prone bacteria takes place via mutation or overview of novel hereditary evidence (Martinez, 2014)

Hereditary mechanism encompasses the growth of antibiotic resistance inclining to be linked to the degree of easiness of the DNA existing in the microorganism becoming resistant to the ease per which it can obtain DNA from other microorganisms (Michael-Kordatou, Karaolia, & Fatta-Kassinos, 2018).

Biological Mechanisms of Resistance depends on the gene which is transmitted to a bacterium, the development of antibiotic resistance occurs when the gene is able to express itself and produce a tangible biological effect resulting in the loss of activity of the antibiotic (Fesseha, Kahsey, & Kidanemariam, 2019).

Antibiotic destruction or antibiotic transformation. This destruction or transformation occurs when the bacteria produces one or more enzymes that chemically degrade or modify the antimicrobial, making them inactive against the bacteria (Nadeem et al., 2020).

2.7 Diagnosis of Respiratory tract infections

RTIs are diagnosed by blood culture this performed on patients with community acquired pneumonia who require hospitalization. It can also be diagnosed by antigen test of Legionella pneumophila serogroup 1 antigen detection in urine, serological test and amplification test(Woodhead et al., 2005)

The doctor can diagnose the infection based on the symptoms the patient has by carrying out a physical examination on the nose, ears and throat of the patient(Gabrielian et al., 2002). It can be through carrying out a chest X-ray, lung scan or performing a lung function test to see how the lungs work (Hassantabar, Ahmadi, & Sharifi, 2020). Streptococcus throat is usually diagnosed over a throat culture and Serological tests(Spellerberg & Brandt, 2022).

2.8Treatment of respiratory tract infections

The drugs prescribed for management of RTIs are in categories of analgesics (paracetamol and ibuprofen), antihistamine (diphenhydramine), antibiotics (penicillin and amoxicillin) and anticholinergics (ipratropium and scopolamine) (WHO, 2001).

CHAPTER THREE: MATERIAL AND METHODOLOGY

3.1 Plant material

C.molle was collected from its natural habitat in Nagongera town council in Tororo district. It was identified and verified at Makerere University Herbarium by Ms. Gauden Nantale, a lecturer in Biology department at Busitema University Nagongera Campus. The stems bark extract of the plant was used for the study. The stem bark was dried in the shade. The dried plant samples were grounded into powder using an electric mortar. After grinding, the sample was stored in an air tight container to control bacterial growth.

3.2 Extraction

3.2.1 Aqueous extract (AE)

This was done using decoction method. Briefly, approximately 100 g of shade-dried powder of stem bark of *C.molle* was boiled in water (300 mL) for three hours. The mixture was cooled and filtered using a sieve to remove suspended particles and the filtrate was concentrated to obtain a paste. The paste was dried in the oven to obtain a powder.

3.2.2 Organic extract (OE)

The shade dried and ground powder of Combretum molle stem bark extract (300g) was extracted using dichloromethane/methanol (1:1, v/v) at room temperature. The extract was concentrated under reduced pressure on a rotary evaporator to obtain crude.

3.3 Preliminary Phytochemical analysis;

The aqueous, ethyl acetate and hexane extracts of whole plant of *Combretum molle stem bark extract* 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 are as follows;

3.3.1 Test for Alkaloids

1. **Wagner's Test:** 1ml of the Wagner's reagent was added to 1ml of a 1ml of an acidic solution of crude extract. The formation of a reddish-brown precipitate indicated the presence of alkaloids.

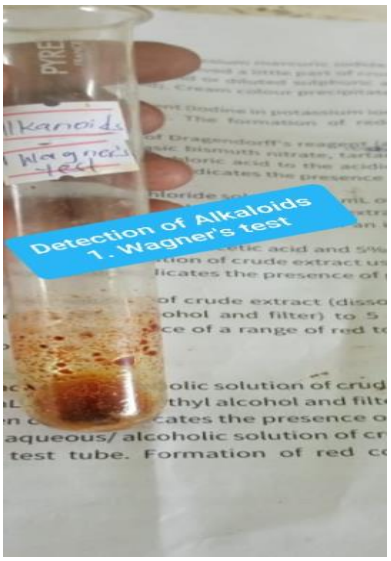


Figure 3 Wagner's Test for Alkaloids of C. molle

2. **Dragendorrf's reagent Test:** 2ml of Dragendorrf's reagent were and 2ml of dilute hydrochloric acid were added to the acidic solution of crude extract. An orange red colour precipitate indicated the presence of alkaloids

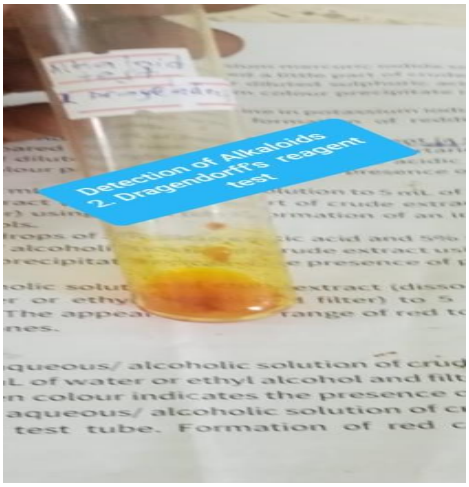


Figure 4 **Dragendorrf's reagent Test for alkaloids**

3.3.2 Test for Flavonoids:

Ferric chloride test: 3 drops of neutral ferric chloride solution was added to 1ml of an alcoholic solution of the crude extract in a test tube. Formation of a blackish red colour indicated the presence of flavonoids.

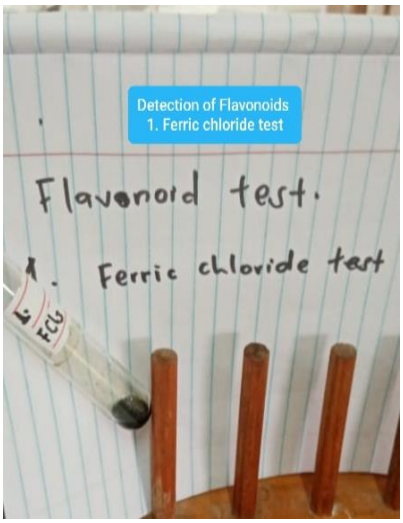


Figure 5 Ferric chloride test for Flavonoids

Zinc-HCl reduction test: A pinch of zinc dust and 3drops of concentrated HCl was added to 1ml of an alcoholic solution of crude extract in a test tube. The appearance of magenta colour indicates the presence of flavonoids.

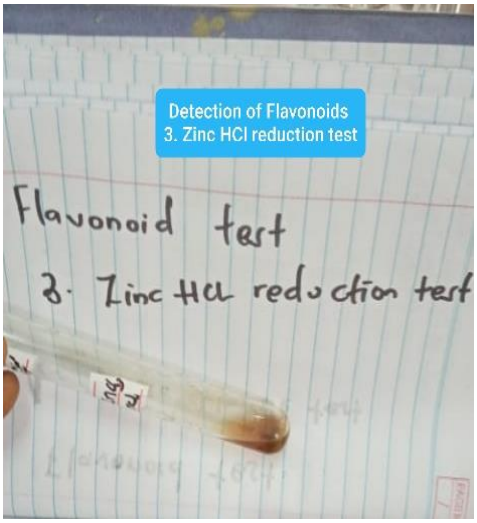


Figure 6 Zinc-HCl reduction test for Flavonoids

Lead-Acetate test: 3 drops of aqueous basic lead acetate solution was added to 1ml of the alcoholic extract in a test tube. The appearance of a reddish-brown bulky precipitate indicated the presence of flavonoids.

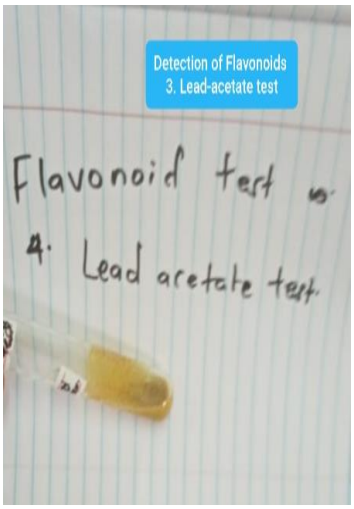


Figure 7 Lead-Acetate test for Flavonoids

3.3.3 Tests for Phenols:

1. **Ferric chloride test:** 1ml of ferric chloride solution was added to 5ml of an alcoholic crude extract of Combretum molle in a test tube. Formation of an intense colour indicated the presence of phenols.

Figure 8 ferric chloride test for phenols

3.3.4 Test for Quinones:

To 5ml of the aqueous solution of the crude extract were added to 5ml of alcoholic potassium hydroxide solution. The appearance of a red to blue coloration indicated the presence of quinones.

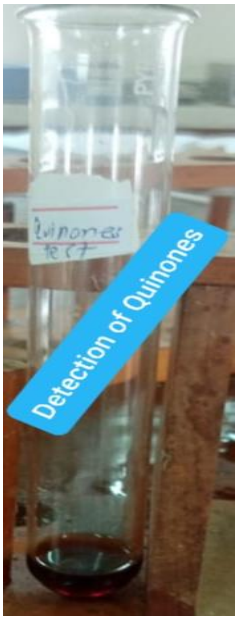


Figure 9 Test for Quinones:

3.3.5 Test for Tannins:

1. **Ferric Chloride test:** 3drops of ferric chloride solution was added to 1ml of the aqueous solution of the crude extract in a test tube. Formation of blackish precipitate indicated the presence of tannins.

Figure 10; Ferric Chloride test for Tannins

2. **Lead acetate test:** 3drops of aqueous basic lead acetate solution was added to 1ml of aqueous solution of crude extract using a test tube. Formation of a reddish-brown bulky precipitate indicated the presence of tannins.

Figure 11 Lead acetate test for tannins

3.3.6 Test for Saponins:

The plant extract (5mL) of Combretum molle was taken in a test tube and shaken vigorously for about 5 minutes to obtain a stable froth. Formation of frothing which persisted on warming confirmed the presence of Saponins.

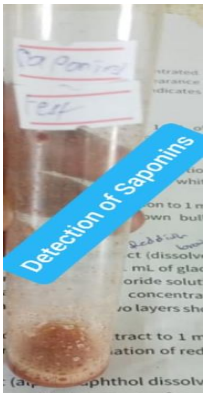


Figure 12 test for Saponins

3.4 Total flavonoid content:

This method was based on the formation of the flavonoids - aluminum complex which had an absorptivity maximum at 415nm. 100µl of Combretum molle stem bark extract was mixed in methanol (10 mg/mL) with 100µl of 20 % aluminum chloride 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 nm. Blank samples were prepared from 100 mL of Combretum molle stem bark extract and a drop of acetic acid were 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. . The calibration curve was constructed using the standard quercetin solution prepared at concentration of 20, 40, 60, 91 and 113 µg/ml as shown in figure 13

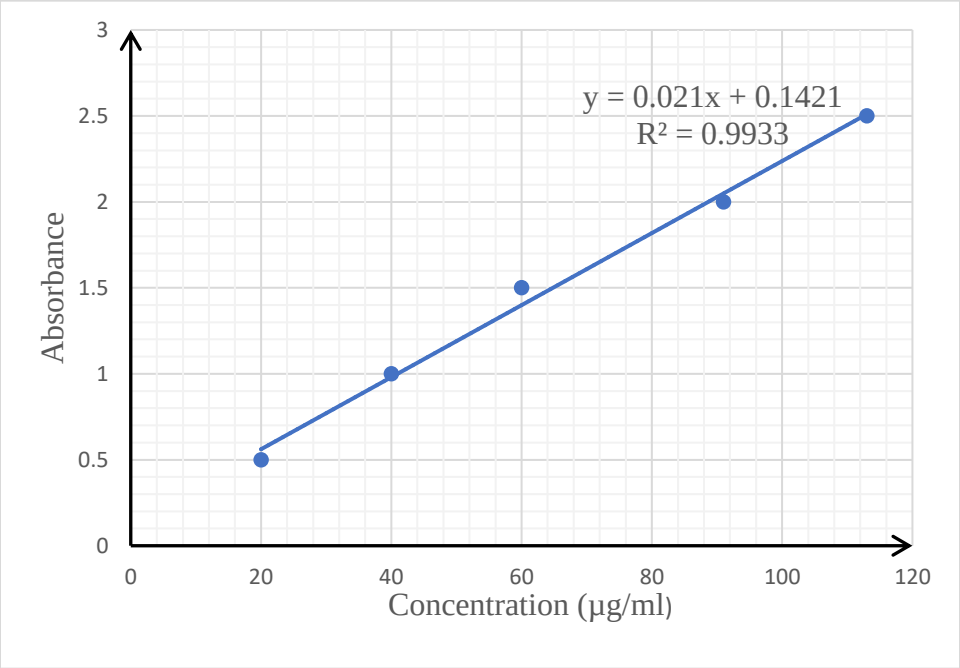


Figure 13 A calibration curve for total flavonoid using standard quercetin solution.

Total flavonoid content was determined using the equation below;

$$Total\ \ flavonoid(\mu g/mg) = \frac{Cx1000}{10} = \frac{0.0829x1000}{10} = 8.3$$

C= concentration $(\mu g/mg)$ derived from the calibration curve

3.5 Total alkaloid content:

Combretum molle (5g) of the sample was weighed and placed into a 250 mL beaker. 200 mL of 10% acetic acid in ethanol was added and beaker was covered with aluminum foil and allowed to stand for 4 hours. The extract was filtered and concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide solution was added drop wise 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 with the alkaloid was dried and weighed. The weight was recorded.

3.6 Total phenolic content:

The total phenolic concentration was measured using the Folin-Ciocalteau method. 100 µl aliquot of 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 phenol reagent was added. After incubation for 30 minutes at room temperature in darkness, the absorbance was read at 720nm using spectrophotometer. The total phenolic contents of the samples were expressed as mg gallic acid equivalent per gram (mg GAE/g). Gallic acid or ascorbic acid was used as a positive control. A calibration curve was constructed using standard ascorbic acid solution of concentrations 0.05, 0.1, 0.15 and 0.2mg/ml.

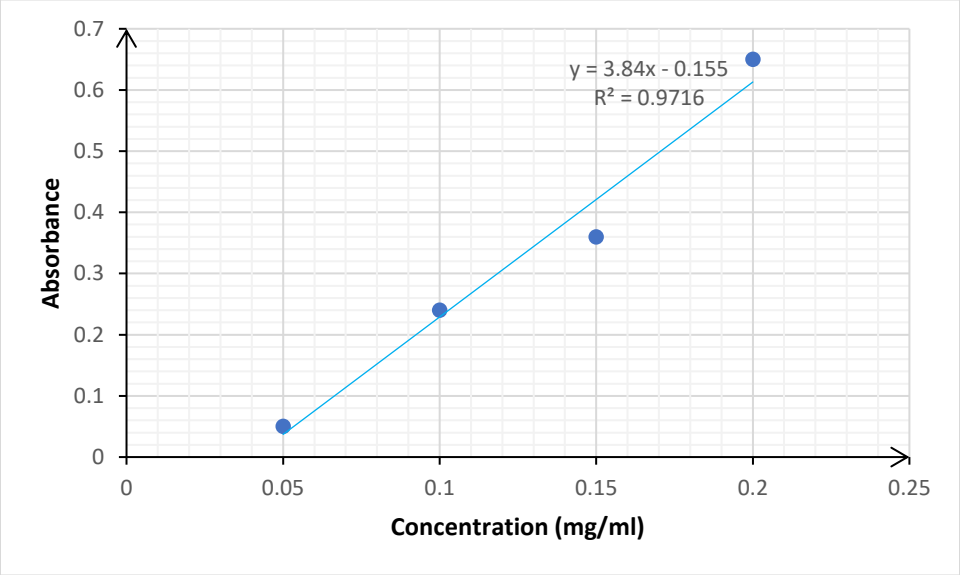


Figure 14 A calibration curve for determination of total phenolic content of RT using standard ascorbic acid.

The total phenolic content was determined using the equation below

$$Total\ \ phenolics\ (\mu g/mg) = \frac{Cx1000}{10} = \frac{0.051x1000}{10} = 5.1$$

3.7 Total tannins

Combretum molle (500g) of the sample 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 50mL volumetric flask and was made up to the mark. 5 mL of the filtrate was pipetted into a test tube and was mixed with 2 mL of 0.1 M FeCl3 in 0.I N HCl & 0.008 M potassium Ferro cyanide. The absorbance at 120 nm within 10 minutes was measured and recorded. The calibration curve was constructed using the standard ascorbic acid solution prepared at concentration of 0.1, 0.2, 0.33 and 0.5mg/ml (figure 15).

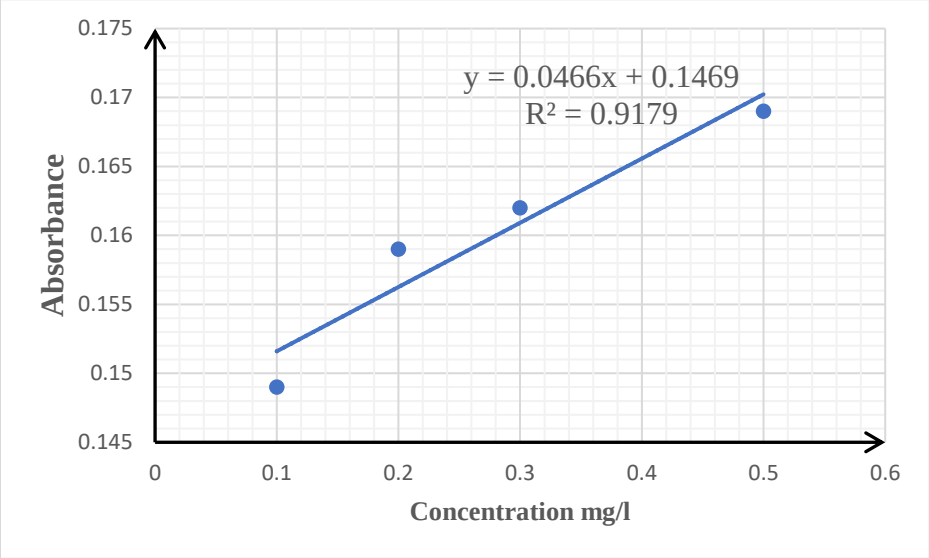


Figure 15 A calibration curve for determination of total tannins using standard ascorbic acid

The total tannins content was determined using the equation below

$$Total\ \ flavonoid(\mu g/mg) = \frac{Cx1000}{10} = \frac{0.37x1000}{10} = 37$$

3.9 Formulation of a *Combretum molle* poly-herbal syrup.

25g of *Combretum molle* powder was mixed with 15g of *Desmodium triflorum* powder, 50 ml of distilled water was added to the mixture then stirred using the magnetic shaker machine for 45minutes.

The mixture was then filtered and the filtrated was kept and the residue deposited away.

30g of glucose was added to the filtrated and mixed together in a 500ml measuring cylinder, 100g of sodium sorbitol and CMC were added then stirred using an electric stirrer.

200ml of glycerin was added to the mixture then shook and kept in a refrigerator.

Table 1 ***THE PHYSIOCHEMICAL EVALUATION OF SYRUP FORMULATED***

PARAMENT	**RESULT**
Density	0.891g/ml
pH Determination	
pH meter	6.35
Organoleptic	
Color	Light brown
Odor	Pleasant
Taste	Sweet
Appearance	Turbid

3.9.1 post formulation properties of CODEM 40

This was done in order to investigate the physiochemical properties of syrup. The post formulation properties tested like smell, color, taste, and appearance as illustrated in table 1.

3.9.2 PH test

To determine the alkalinity, acidity and neutral nature of the syrup formulated.

3.9.3 Density determination

This was done to determine the state of CODEM 40 and to ensure the consistency and quality control during the production of the syrup. It was also determined to characterize and estimate the composition of the syrup. And determine its weight.

CHAPTER FOUR: RESULTS AND DISSCUSION

4.1 Phytochemical screening

The phytochemical screening of *C. molle* stem bark extract indicated the presence of alkaloids, glycosides, flavonoids, phenols, Saponins, tannins and Quinones. However, steroids and terpenoids were absent as represented in the Table 2 and Figure 16.

Table 2: phytochemical screening of the extracts of *C.molle*

Phytochemical components	**Tests**	**Present (+) and absent (-)**
Alkaloids	Wagner's test	+ +
	Dragendorrf's reagent test	+ +
Flavonoids	Ferric chloride test	+ +
	Zinc-HCl reduction test	+ +
	Lead acetate test	+ +
Phenols	Ferric chloride test	+ +
Quinones	5ml of aqueous crude extract and 5ml of KOH	+ +
Tannins	Ferric chloride test	+ +
	Lead acetate	+ +
Saponins	Crude extract was mixed with 20ml of water and agitated	+ + + +
Glycosides	Keller kiliani test	+ +
	Sulphuric acid test	+ +
Terpenoids	Trim-Hill	-
Steroids	Salkowski	-
	Liebermann-Burchard	-

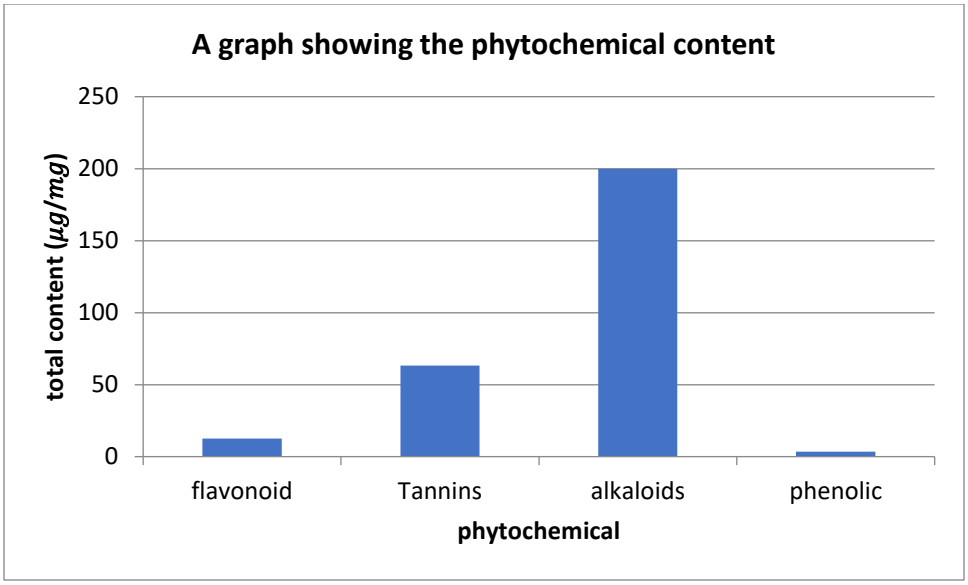


Figure 16 A graph showing the phytochemical content

The antibacterial activity of C. molle is attributed to the presence of alkaloids, glycosides, flavonoids, phenols, Saponins, tannins and Quinones which may act as synergistically to hinder the growth the bacteria. Alkaloids contain nitrogen and have pharmacological properties. Flavonoids have polyphenolic compounds are known to contain antioxidant, anticancer and anti-inflammatory properties. Tannins are polyphenolics compounds that have caustic properties. Phenols are aromatic compounds that have antioxidant properties (Koevi et al., 2015). However, the antibacterial evaluation was not done but the present literature reveals that *C. molle* is a good remedy for antibacterial activity

4.2 properties of CODEM 40

C. molle extract was found to possess phytochemical constituents with therapeutic values and therefore it was used to formulate herbal remedy branded as CODEM 40. CODEM 40 is an herbal syrup containing C. molle extract as a main active ingredient together with *Desmodium triflorum* which has reported contain unique phytochemicals and shown to possess antibacterial activities. Physiochemical properties of the herbal remedy (CODEM 40) are presented in Table 3.

Table 3 ***properties of CODEM 40***

Property	Observation	Conclusion **or** comment

PH	Litmus paper turn from blue to pale yellow	Slightly acidic and almost neutral
Smell	Very pleasant	Good
Taste	Sweet	Good
Texture	Turbid	Fair

CHAPTER FIVE: CONCLUSION AND RECCOMENDATION

5.1 CONCLUSION

The results from this study showed that the extract from the stem bark of *C. molle* contains alkaloids, glycosides, flavonoids, phenols, Saponins, tannins and Quinones. This study therefore, contributes to justifying the traditional use of the *C.molle* for the management of respiratory tract infections with formulation of an herbal syrup named CODEM 40. Therefore, new research outcomes could top to greater protection and assistance to people who use *C.molle* to manage RTIs that are contributing to an improved access to health care and thus an excellent lifespan.

In conclusion, the phytochemical analysis of *C.molle* stem bark extract and the formation of the herbal syrup CODEM 40 could offer a promising approach for management of RTIs.

5.2 RECOMMENDATIONS

It is recommended there to do additional studies on extra cleansing and elucidation of the active compounds so as to provide a new or principal components for production of new drugs, antimicrobial activities against other organisms including bacteria and fungi in order to find the wide spectrum activity of the plant extract and fractions and further studies on the toxicity and mechanism of action of the plant extract and fractions to ensure a perfect product of CODEM 40 with tested toxicity properties.

REFERENCES

Aberdein, J., Cole, J., Bewley, M., Marriott, H., & Dockrell, D. (2013). Alveolar macrophages in pulmonary host defence–the unrecognized role of apoptosis as a mechanism of intracellular bacterial killing. *Clinical & Experimental Immunology, 174*(2), 193-202.

Ally, M. H. S. Antimicrobial Properties of Velvet Bush Willow (Combretum molle) Crude Bark Extracts on Selected Bacteria Species.

Baggett, H. C., Watson, N. L., Deloria Knoll, M., Brooks, W. A., Feikin, D. R., Hammitt, L. L., . . . Madhi, S. A. (2017). Density of upper respiratory colonization with Streptococcus pneumoniae and its role in the diagnosis of pneumococcal pneumonia among children aged< 5 years in the PERCH study. *Clinical Infectious Diseases, 64*(suppl_3), S317-S327.

Beceiro, A., Tomás, M., & Bou, G. (2013). Antimicrobial resistance and virulence: a successful or deleterious association in the bacterial world? *Clinical microbiology reviews, 26*(2), 185-230.

Boloursaz, M. R., Lotfian, F., Aghahosseini, F., Cheraghvandi, A., Khalilzadeh, S., Farjah, A., & Boloursaz, M. (2013). Epidemiology of lower respiratory tract infections in children. *Journal of Comprehensive Pediatrics, 4*(2), 93-98.

Brooks, L. R., & Mias, G. I. (2018). Streptococcus pneumoniae's virulence and host immunity: aging, diagnostics, and prevention. *Frontiers in immunology, 9,* 1366.

Brusselaers, N., Vogelaers, D., & Blot, S. (2011). The rising problem of antimicrobial resistance in the intensive care unit. *Annals of intensive care, 1,* 1-7.

Chrian, M., Erasto, P., & Otieno, N. J. (2011). Antimycobacterial activity and cytotoxicity effect of extracts of Hallea rubrostipulata and Zanthoxylum chalybeum. *Spatula DD, 1*(3), 147-152.

Cimolai, N. (2021). The complexity of co-infections in the era of COVID-19. *SN Comprehensive Clinical Medicine, 3*(7), 1502-1514.

Cock, I. (2015). The medicinal properties and phytochemistry of plants of the genus Terminalia (Combretaceae). *Inflammopharmacology, 23,* 203-229.

Cock, I. E., & Van Vuuren, S. F. (2020). The traditional use of southern African medicinal plants for the treatment of bacterial respiratory diseases: A review of the ethnobotany and scientific evaluations. *Journal of Ethnopharmacology, 263,* 113204.

Cummings, M. J., Tokarz, R., Bakamutumaho, B., Kayiwa, J., Byaruhanga, T., Owor, N., . . . Lutwama, J. J. (2019). Precision surveillance for viral respiratory pathogens: virome capture sequencing for the detection and genomic characterization of severe acute respiratory infection in Uganda. *Clinical Infectious Diseases, 68*(7), 1118-1125.

Das, K., Tiwari, R., & Shrivastava, D. (2010). Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. *Journal of medicinal plants research, 4*(2), 104-111.

de Menezes, B. R. C., Rodrigues, K. F., Schatkoski, V. M., Pereira, R. M., Ribas, R. G., do Amaral Montanheiro, T. L., & Thim, G. P. (2021). Current advances in drug delivery of nanoparticles for respiratory disease treatment. *Journal of Materials Chemistry B, 9*(7), 1745-1761.

Dias, L. D., & Bagnato, V. S. (2020). An update on clinical photodynamic therapy for fighting respiratory tract infections: a promising tool against COVID-19 and its co-infections. *Laser Physics Letters, 17*(8), 083001.

Eloff, J., Katerere, D., & McGaw, L. (2008). The biological activity and chemistry of the southern African Combretaceae. *Journal of Ethnopharmacology, 119*(3), 686-699.

Erku, D. A., & Aberra, S. Y. (2018). Non-prescribed sale of antibiotics for acute childhood diarrhea and upper respiratory tract infection in community pharmacies: a 2 phase mixed-methods study. *Antimicrobial Resistance & Infection Control, 7*(1), 1-7.

Ferri, M., Ranucci, E., Romagnoli, P., & Giaccone, V. (2017). Antimicrobial resistance: A global emerging threat to public health systems. *Critical reviews in food science and nutrition, 57*(13), 2857-2876.

Fesseha, H., Kahsey, R., & Kidanemariam, F. (2019). An Insight Review on Antibiotic Resistance and Its Challenges. *Ind. J. Pure App. Biosci, 7*(6), 19-28.

Friedman, N. D., Temkin, E., & Carmeli, Y. (2016). The negative impact of antibiotic resistance. *Clinical Microbiology and Infection, 22*(5), 416-422.

Gabrielian, E., Shukarian, A., Goukasova, G., Chandanian, G., Panossian, A. G., Wikman, G., & Wagner, H. (2002). A double blind, placebo-controlled study of Andrographis paniculata fixed combination Kan Jang in the treatment of acute upper respiratory tract infections including sinusitis. *Phytomedicine, 9*(7), 589-597.

Ghosh, B., & Mandal, S. (2018). Perspectives to DENV and GAS infections and associated serum LDH profiles. *EC Microbiology, 14*.

Hassantabar, S., Ahmadi, M., & Sharifi, A. (2020). Diagnosis and detection of infected tissue of COVID-19 patients based on lung X-ray image using convolutional neural network approaches. *Chaos, Solitons & Fractals, 140*, 110170.

Houghton, P., & Raman, A. (2012). *Laboratory handbook for the fractionation of natural extracts*: Springer Science & Business Media.

Jin, Y.-H., Cai, L., Cheng, Z.-S., Cheng, H., Deng, T., Fan, Y.-P., . . . Huang, Q. (2020). A rapid advice guideline for the diagnosis and treatment of 2019 novel coronavirus (2019-nCoV) infected pneumonia (standard version). *Military medical research, 7*(1), 1-23.

Kaggwa, B., Kyeyune, H., Munanura, E. I., Anywar, G., Lutoti, S., Aber, J., . . . Kamba, P. F. (2022). Safety and Efficacy of Medicinal Plants Used to Manufacture Herbal Products with Regulatory Approval in Uganda: A Cross-Sectional Study. *Evidence-based complementary and alternative medicine, 2022*.

Kiguli, S., Olopot-Olupot, P., Alaroker, F., Engoru, C., Opoka, R. O., Tagoola, A., . . . Mogaka, C. (2021). Children's Oxygen Administration Strategies And Nutrition Trial (COAST-Nutrition): a protocol for a phase II randomised controlled trial. *Wellcome open research, 6*.

Koevi, K.-K. A., Millogo, V., Fokou, J. B. H., Sarr, A., Ouedraogo, G. A., & Bassene, E. (2015). Phytochemical analysis and antioxidant activities of Combretum molle and Pericopsis laxiflora. *International Journal of Biological and Chemical Sciences, 9*(5), 2423-2431.

Kulawe, D., Abubakar, A., & Abubakar, Z. (2019). The activities of the aqueous crude extracts of the leaf, root and bark of Combretum molle against selected test organisms. *International Journal of Scientific and Research Publications (IJSRP), 9*(10), p9428.

Lamorde, M., Tabuti, J. R., Obua, C., Kukunda-Byobona, C., Lanyero, H., Byakika-Kibwika, P., . . . Ryan, M. (2010). Medicinal plants used by traditional medicine practitioners for the treatment of HIV/AIDS and related conditions in Uganda. *Journal of ethnopharmacology, 130*(1), 43-53.

Lappin, M., Blondeau, J., Boothe, D., Breitschwerdt, E., Guardabassi, L., Lloyd, D., . . . Turnidge, J. (2017). Antimicrobial use guidelines for treatment of respiratory tract disease in dogs and cats: Antimicrobial Guidelines Working Group of the International Society for

Companion Animal Infectious Diseases. *Journal of Veterinary Internal Medicine, 31*(2), 279-294.

Lewis, K. (2013). Platforms for antibiotic discovery. *Nature reviews Drug discovery, 12*(5), 371-387.

Mahomoodally, M. F. (2013). Traditional medicines in Africa: an appraisal of ten potent African medicinal plants. *Evidence-based complementary and alternative medicine, 2013*.

Majeed, M., & Rehman, R. U. (2021). Phytochemistry, Pharmacology, and Toxicity of an Epiphytic Medicinal Shrub Viscum album L.(White Berry Mistletoe). *Medicinal and Aromatic Plants: Healthcare and Industrial Applications*, 287-301.

Martinez, J. L. (2014). General principles of antibiotic resistance in bacteria. *Drug Discovery Today: Technologies, 11*, 33-39.

McGaw, L., Rabe, T., Sparg, S., Jäger, A., Eloff, J., & Van Staden, J. (2001). An investigation on the biological activity of Combretum species. *Journal of Ethnopharmacology, 75*(1), 45-50.

Michael-Kordatou, I., Karaolia, P., & Fatta-Kassinos, D. (2018). The role of operating parameters and oxidative damage mechanisms of advanced chemical oxidation processes in the combat against antibiotic-resistant bacteria and resistance genes present in urban wastewater. *Water research, 129*, 208-230.

Międzybrodzki, R., Hoyle, N., Zhvaniya, F., Łusiak-Szelachowska, M., Weber-Dąbrowska, B., Łobocka, M., . . . Górski, A. (2021). Current updates from the long-standing phage research centers in Georgia, Poland, and Russia. *Bacteriophages: Biology, Technology, Therapy*, 921-951.

Nadeem, S. F., Gohar, U. F., Tahir, S. F., Mukhtar, H., Pornpukdeewattana, S., Nukthamna, P., . . . Massa, S. (2020). Antimicrobial resistance: more than 70 years of war between humans and bacteria. *Critical Reviews in Microbiology, 46*(5), 578-599.

Ncube, N., Afolayan, A., & Okoh, A. (2008). Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. *African journal of biotechnology, 7*(12).

Ntshanka M. N, E. I. P., Mthunzi F. M, Moloto M. J, Mubiayi K. P. Investigation into the Phytochemical Profile, Antioxidant and Antibacterial Potentials of Combretum Molle and Acacia Mearnsii Leaf Parts. Biomed Pharmacol J 2020;13(4). (2020).

Nyenje, M. E. (2011). *Phytochemical Analysis And Bioactivity Of The Stem Bark Of Combretum Molle On Some Selected Bacterial Pathogens.* University of Fort Hare,

O'Neill, J. (2016). Tackling drug-resistant infections globally: final report and recommendations.

Organization, W. H. (2000). Overcoming antimicrobial resistance. *Overcoming antimicrobial resistance.*

Pan, S.-Y., Zhou, S.-F., Gao, S.-H., Yu, Z.-L., Zhang, S.-F., Tang, M.-K., . . . Fong, W.-F. (2013). New perspectives on how to discover drugs from herbal medicines: CAM's outstanding contribution to modern therapeutics. *Evidence-based complementary and alternative medicine, 2013*.

Papo, L., Van Vuuren, S., & Moteetee, A. (2022). The ethnobotany and antimicrobial activity of selected medicinal plants from Ga-Mashashane, Limpopo Province, South Africa. *South African Journal of Botany, 149*, 196-210.

Planta, M. B. (2007). The role of poverty in antimicrobial resistance. *The Journal of the American Board of Family Medicine, 20*(6), 533-539.

Raluca, P., & Mariana, B. DIFFERENTIAL DIAGNOSIS BETWEEN STAPHYLOCOCCI AND STREPTOCOCCI BY THE LATEX AGGLUTINATION TEST.

Rammelkamp, C. H., & Maxon, T. (1942). Resistance of Staphylococcus aureus to the Action of Penicillin. *Proceedings of the Society for Experimental Biology and Medicine, 51*(3), 386-389.

Reinert, R. (2009). The antimicrobial resistance profile of Streptococcus pneumoniae. *Clinical Microbiology and Infection, 15*, 7-11.

Robertson, N. M., Nagourney, E. M., Pollard, S. L., Siddharthan, T., Kalyesubula, R., Surkan, P. J., . . . Kirenga, B. J. (2019). Urban-rural disparities in chronic obstructive pulmonary disease management and access in Uganda. *Chronic Obstructive Pulmonary Diseases: Journal of the COPD Foundation, 6*(1), 17.

Roy, S., Gorai, D., Acharya, R., & Roy, R. (2014). Combretum (combretaceae): Biological activity and phytochemistry. *American Journal of Pharm Research, 4*(11).

Sandelowsky, H., Ställberg, B., Nager, A., & Hasselström, J. (2011). The prevalence of undiagnosed chronic obstructive pulmonary disease in a primary care population with respiratory tract infections-a case finding study. *BMC family practice, 12*(1), 1-9.

Sarwar, A., & Imran, M. (2021). Prioritizing infection prevention and control activities for SARS-CoV-2 (COVID-19): a multi-criteria decision-analysis method. *Journal of healthcare leadership*, 77-84.

Schuetz, P., Muller, B., Christ-Crain, M., Stolz, D., Tamm, M., Bouadma, L., . . . Tubach, F. (2013). Procalcitonin to initiate or discontinue antibiotics in acute respiratory tract infections. *Evidence-Based Child Health: A Cochrane Review Journal, 8*(4), 1297-1371.

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

Sen, S., & Chakraborty, R. (2017). Revival, modernization and integration of Indian traditional herbal medicine in clinical practice: Importance, challenges and future. *Journal of traditional and complementary medicine, 7*(2), 234-244.

Seukep, J. A., Sandjo, L. P., Ngadjui, B. T., & Kuete, V. (2016). Antibacterial and antibiotic-resistance modifying activity of the extracts and compounds from Nauclea pobeguinii against Gram-negative multi-drug resistant phenotypes. *BMC Complementary and Alternative Medicine, 16*(1), 1-8.

Shakya, A. K. (2016). Medicinal plants: Future source of new drugs. *International journal of herbal medicine, 4*(4), 59-64.

Shorrocks, B., & Bates, W. (2015). *The biology of African savannahs*: Oxford University Press, USA.

Snidal, S. J., Barnard, G., Atuhairwe, E., & Amor, Y. B. (2015). Use of eCompliance, an innovative biometric system for monitoring of tuberculosis treatment in rural Uganda. *The American journal of tropical medicine and hygiene, 92*(6), 1271.

Spellerberg, B., & Brandt, C. (2022). Laboratory diagnosis of Streptococcus pyogenes (group A streptococci).

Ssenku, J. E., Okurut, S. A., Namuli, A., Kudamba, A., Tugume, P., Matovu, P., . . . Walusansa, A. (2022). Medicinal plant use, conservation, and the associated traditional knowledge in rural communities in Eastern Uganda. *Tropical Medicine and Health, 50*(1), 39.

Tugume, P., & Nyakoojo, C. (2019). Ethno-pharmacological survey of herbal remedies used in the treatment of paediatric diseases in Buhunga parish, Rukungiri District, Uganda. *BMC Complementary and Alternative Medicine, 19*(1), 1-10.

Umuhoza, T., Bulimo, W. D., Oyugi, J., Musabyimana, J. P., Kinengyere, A. A., & Mancuso, J. D. (2021). Prevalence of human respiratory syncytial virus, parainfluenza and adenoviruses in East Africa Community partner states of Kenya, Tanzania, and Uganda: A systematic review and meta-analysis (2007–2020). *PloS one, 16*(4), e0249992.

Ventola, C. L. (2015). The antibiotic resistance crisis: part 1: causes and threats. *Pharmacy and therapeutics, 40*(4), 277.

Vihta, K. D., Pouwels, K. B., Peto, T. E., Pritchard, E., Eyre, D. W., House, T., . . . Cook, D. (2022). Symptoms and Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Positivity in the General Population in the United Kingdom. *Clinical Infectious Diseases, 75*(1), e329-e337.

Wang, L., Hu, C., & Shao, L. (2017). The antimicrobial activity of nanoparticles: present situation and prospects for the future. *International journal of nanomedicine, 12*, 1227.

WHO. (2001). *Cough and cold remedies for the treatment of acute respiratory infections in young children*. Retrieved from

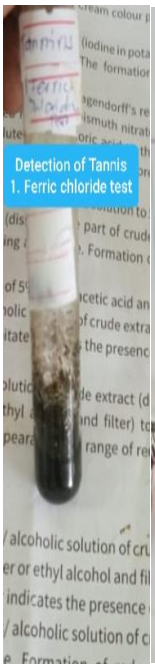
Wiemken, T. L., Peyrani, P., & Ramirez, J. A. (2012). Global Changes in the Epidemiology of Community-Acquired Pneumonia. *Semin Respir Crit Care Med, 33*(03), 213-219.

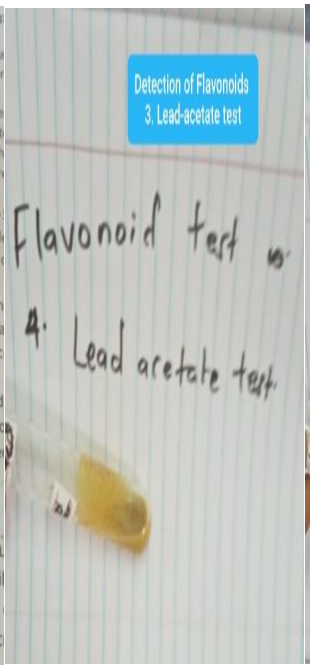
Woodhead, M., Blasi, F., Ewig, S., Huchon, G., Leven, M., Ortqvist, A., . . . Verheij, T. J. (2005). Guidelines for the management of adult lower respiratory tract infections. *European Respiratory Journal, 26*(6), 1138-1180.

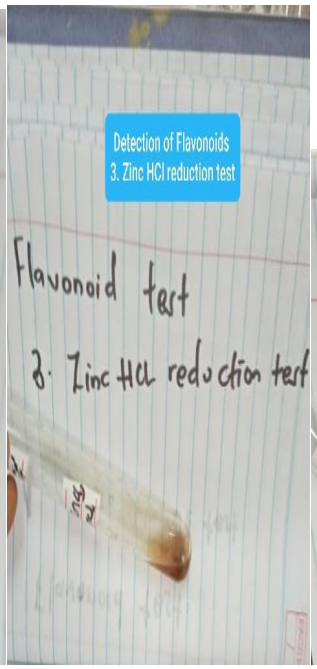
Zoorob, R., Sidani, M. A., Fremont, R. D., & Kihlberg, C. (2012). Antibiotic use in acute upper respiratory tract infections. *American family physician, 86*(9), 817-822.

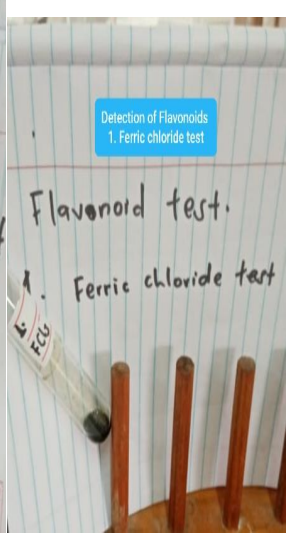
Appendix

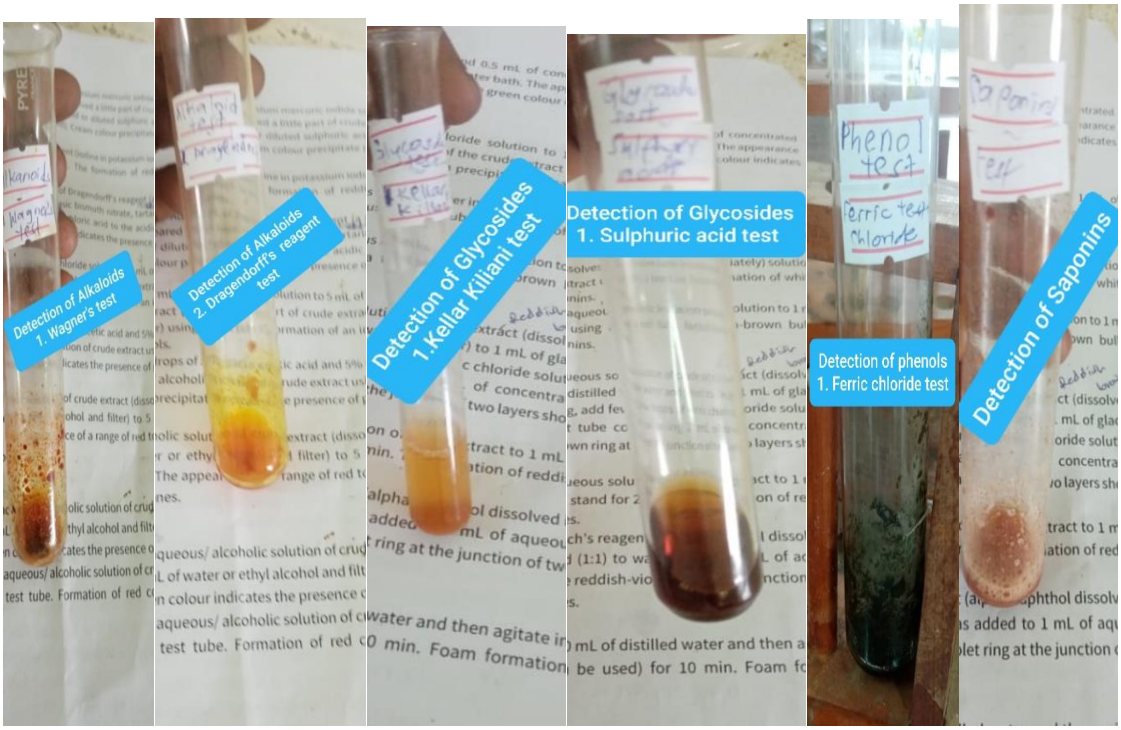

Tannis test
2. Lead acetate test


Detection of Tannis
1. Ferric chloride test


Detection of Flavonoids
3. Lead-acetate test
Flavonoid test
4. Lead acetate test.


Detection of Flavonoids
3. Zinc HCl reduction test
Flavonoid test
3. Zinc HCl reduction test


Detection of Flavonoids
1. Ferric chloride test
Flavonoid test.
1. Ferric chloride test


Detection of Alkaloids
1. Wagner's test
Detection of Alkaloids
2. Dragendorff's reagent test
Detection of Glycosides
1.Kellar Kiliani test
Detection of Glycosides
1. Sulphuric acid test
Detection of phenols
1. Ferric chloride test
Detection of Saponins


Detection of Quinones