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QUANTITATIVE PHYTOCHEMICAL ANALYSIS OF *EUCLEA RACEMOSA* AS A POTENTIAL ANTIBACTRIAL HERBAL REMEDY FOR MANAGEMENT OF PNEUMONIA

BY

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A RESEARCH PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF CHEMISTRY FOR THE PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF THE DEGREE OF BACHELOR OF SCIENCE EDUCATION AT BUSITEMAUNIVERSITY

May 2024

1.1 DECLARATION:

I NANGOLI CRANIMER hereby declare that I am the sole author of this work to the best of my knowledge this work contains no material previously published by any other persons except where due acknowledgement has been made. This work contains no material which has been accepted as part of the requirements of any other academic degree; this has been acknowledged and cited according to the university policy

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Signature.

NANGOLI CRANIMER

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1.2 APPROVAL

SIGNATURE.....DATE ...13/5/2024.....

DR OWOR ORIKO RICHARD

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1.1 DEDICATION:

I dedicate my dissertation work to my family and many friends. A special feeling of gratitude to my loving parents, GIDUDU DAVID and NAMBOZO SARAH whose words of support and drive for determination resound in my ears. My brothers WOMUNGA EMMANUEL, WONIALA JOEL and WOTWALE APOLO have never left my side and are very special. Also dedicate this dissertation to my LECTURES OF CHEMISTRY who have supported me throughout the process. I will always appreciate all they have done, DR. ORIKO RICHARD OWOR, DR. KAMONGA for the many hours of advice on how to achieve this much in my research.

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1.4 ACRONYMS.

E.R – *Euclea racemosa*

S.P-*Streptococcus Pneumoniae*

CAP- Community Acquired Pneumonia

UNICEF- United States International Children's Emergency Funds

WHO- world health organization

NDA – National drug Authority

MIC - minimum inhibitory concentration

AST-Antimicrobial susceptibility testing

ABSTRACT.

Introduction: Pneumonia is a respiratory tract infection which is caused by microorganisms resulting into inflammations due to fluid filling in the air sac in either both or one of the lungs. Pneumonia kills more children than any other infectious disease, claiming the lives of close to 2,000 children everyday world wide.

Objective: To quantitatively analyze the Phytochemistry of *Euclea racemosa* for potential antibacterial activity and formulation of a herbal remedy for management of respiratory tract infections.

Materials and methods: The phytochemical analysis of *Euclea racemosa* that belongs to kingdom *plantae*, Order *Ericales*, family *Ebenaceae*, Genius *Euclea*, species *Euclea Racemosa*, was examined using organic extracts and aqueous extracts of the leaves of *Euclea racemosa*.

Results: The organic extracts used in investigation of phytochemical and confirmed alkaloids (214.87mg/g), flavonoids (30.3mg/g), tannins (136.5mg/g), phenols (30.2mg/g), glycosides, saponins and as well antibacterial activity investigated on *Escherichia coli, Pseudomonas aeruginosa and staphylococcus aureus* evidenced by the percentage rate inhibition.

Conclusion: The extracts showed high inhibition on *E.coli* than it was on *pseudomonas aeruginosa*, therefore the antimicrobial activity of E.racemosa is most effect on E.coli. The ability of E.racemosa to show antimicrobial activity may be attributed to the presence of flavonoids, alkaloids, phenolics, tannins, glycosides and saponins which were confirmed to be biologically active ingredients during the phytochemical analysis of the crude extract.

Chapter:1

1.1 INTRODUCTION

1.1.1 Background

Pneumonia is a respiratory tract infection which is caused by microorganisms resulting into inflammations due to fluid filling in the air sac in either both or one of the lungs. Pneumonia mainly affect children and the elderly or immune-compromised patients with over 60% of hospitalized patients being diagnosed with community acquired pneumonia. Pneumonia kills more children than any other infectious disease, claiming the lives of close to 2,000 children everyday world wide (Chan & Lake, 2013). Most of pneumonia cases are reported in developing nations. In West and Central Africa 1,620 cases per 100,000 children reported compared to USA where pneumonia prevalence among under-fives is between 16% and 33% (Rudan et al., 2008). In Uganda, prevalence of pneumonia was reported to be 53.7% at Mulago national Referral Hospital in 2019.

In Uganda pneumonia remains the second leading cause of all hospital admissions in children among children less than 5years of age. This places a substantial burden on the health systems as pneumonia accounts for the significant proportion of paediatric admissions(Organization, 2022). An economic burden is placed on already impoverished families. On average, Ugandan household spends shs.240, 000 per hospitalized episode of pneumonia. Therefore, there is urgent need to develop alternative antibiotics for management of pneumonia. Traditionally, medicinal plant have been used to manage pneumonia and its symptoms. Such plants includes *E.racemosa*, in this study, *E. racemosa* will be phytochemically investigated and its antibacterial properties will be evaluated.

1.2 STATEMENT OF THE PROBLEM.

Bacterial pathogens are potentially harmful microorganisms that have profound effect on humans and cause different infections for example the *Streptococcus pneumonia, Escherichia coli* that causes pneumonia(Abdel-Hamid et al., 2002). The World Health Organization estimated that pneumonia kills one child after every 43s globally(Wang et al., 2022).

World Health Organizations reported that Antimicrobial Resistance directly caused 1.27 million deaths worldwide in 2019 and triggered 4.95 million fatalities. The increasing incidence of bacterial resistance to antibiotics, including Penicillin, Amoxicillin, Erythromycin, and Ciprofloxacin, is a global concern(Organization, 2022). This calls for development of an alternative and more effective antibiotics which can possibly be derived by medicinal plants (Serwecinska, 2020). Medicinal plants, *E.racemosa* has been used locally in management of Pneumonia due to its antibacterial, antifungal properties. However, its phytochemical profile has not been investigated. Therefore, the study was intended to quantitatively investigate the phytochemical constituents of *E.racemosa* extracts and to formulate an herbal remedy for

management of Pneumonia and investigate its efficacy such that the plant can be used make a remedy to manage pneumonia

1.3 OBJECTIVES

1.3.1 General Objective

To investigate the phytochemicals in *E. racemosa* extracts for potential antibacterial activities and formulation of herbal remedy to relive pneumonia

1.3.2 Specific objective

1. To quantify the phytochemical constituents of *E.R* extracts
2. To evaluate the efficacy of *E.R* extracts against respiratory tract infections

1.4 Justification

Management of pneumonia used to be straightforward and penicillin generally was the antibiotic of choice (kaplan S. L, 1998). However rapid development and wide spread of penicillin resistance and multiple antibiotic resistance have occurred(Mahendra, 2019). Therefore there is need to develop a new antibiotics to manage the wide spread of bacterial infections which is claiming lives of over 4 million people per year in the world(Tomasz, 1997). *E.R* can be exploited as a source of an antibiotics like the way quinine was derived from cinchona tree. If the extract is found effective, herbal formulation can be made and provide an alternative remedy for management of bacterial pneumonia.

Chapter:2

2.1 LITERACTURE REVIEW

2.1.1 Phytology of *E.Racemosa*.

E.Racemosa belongs to kingdom *plantae*, Order *Ericales,* family *Ebenaceae,* Genius *Euclea,* species *Euclea Racemosa.* (Wagner, 2006)

E.Racemosa is described as shrub or a small tree 3-4 m BARK: Grey-black, rather smooth. LEAVES: usually opposite, shiny and leathery, dark green above but dull and pale below, long, oval, about 5cm, the tip rounded, narrowing to the base. The thick edge often curls right under. FLOWERS: small cream-white and sweet scented, in short sprays to 8cm besides leaves, male flowers with many stamens. FRUIT: Round and very small, less than 1cm, green at first, ripening purple-black with thin edible flesh around the seeds, two seeds per fruit. (Quattrocchi, 2012). *E.Racemosa* subsp. Schimperi is one the species among many categorical to top most medicinal plants used in Asia, Europe and African continents (GREEN, 2019). Research has been done on various Plants under the same Family of Ebenaceae and findings show that they have active components that have antibacterial, antifungal, ant-diabetic Properties, etc. (Abd EI Halim, 2014).

Figure 1: Young leaves of E.R

Figure 2: Mature leaves of E.R

Figure 1: Young fruits of E.R

2.1.2 Traditional uses of Euclea Racemosa

E.Racemosa has a wide usage culturally, medicinally and commercially. *E. Racemosa* is used in the treatment of wounds, teeth infections, eye disorders, head ache, pain, spasm and also smoking milk products(Gebremariam et al., 2015). The wood of *E.Racemosa* when burned produces a thick black smoke that was considered ideal for repelling insects and other pest. In Ethiopia the root/stem part of *E.Racemosa* is locally used as a toothbrush and to repel evil eye(Ayele et al., 2023).

E.Racemosa has been used in East Africa to treat various diseases including cancer. In Ethiopia leafs macerate is used to treat gonorrhea, eczema, and constipation. In Uganda the root bark are chewed for toothache and the cold decoction is drunk for malaria. In east Tanzania, the root decoction is used against cancer, abdominal pain and convulsive dysmenorrhea.(Dieter, 2000) Ethiopians treat the pots in which milk is kept with the smoke of E.Racemosa branches fire to prevent milk from curdling(Bati et al., 2024).

2.1.3 Phytochemistry of E.R

ROOTS. Preliminary phytochemical screening of different root extracts(methanol, chloroform, acetone) of *E.Racemosa* revealed negative test for alkaloids and carbohydrates in all extracts, flavonoids, glycosides, steroids detected in methanol extracts, phenols, saponins, triterpenes detected in both methanol and chloroform extracts, Tannins detected in acetone extracts(Lima et al., 2022). Showed presence of 1,4-napthoquinones showed in **figure 4**. 4S,8-Dihydroxy-6-methyl-1-tetrone, **figure 5**. 7-methyljuglones and its dimers disopyrin, **figure 6**. Isodiospyrin and **figure 7**. mamegakinon in the roots leaves and twigs(van der Vijver & Gerritsma, 1974). Phytochemical screening showed presence of triterpenoids and pentacyclic(Wube et al., 2005).

2.1.3.1 Compounds from E.Racemosa ssp schimperi

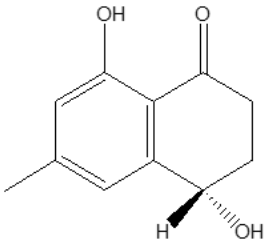


Figure 3: 4S, 8-Dihydroxy-6-methyl-1-tetrone

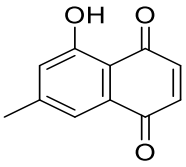


Figure 4: 5-Hydroxy-7-methyl-[1,4] naphoquinone

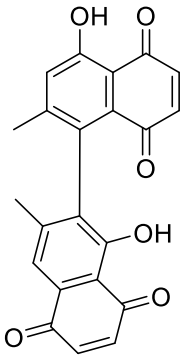


Figure 5: Isodiospyrin

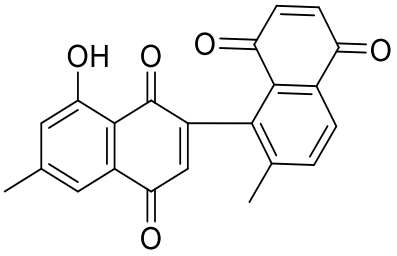


Figure 6: 4S,8-Hydroxy-2,6-dimethyl-[1,2] binaphthalenyl-5, 8,1,4-tetraone

2.1.4 Diseases and conditions that have been tested against *E.R*

These include; constipation, Gonorrhea, toothache, malaria, cancer, constipation, eczema(Wube et al., 2005). Eye disorders, abdominal pain, headache(Gebremariam et al., 2015).

2.1.5 Bacteria it has been tested against

E.Racemosa root extracts of methanol, chloroform and acetone have been investigated for their antibacterial activity by diffusion method(Gebremariam et al., 2015), all extracts were tested against seven bacteria strains; *staphylococcus aureus, streptococcus pneumonia, streptococcus pyogenes, Escherichia coli, klebsiella pneumonia, Pseudomonas aeruginosa and salmonella typhi*

and showed significant degree of effects on each strain(Saxena et al., 2020). E.Racemosa root extracts has been tested and proved to have in vivo laxative effects on conditions of constipation(Gardam, 2000). Its extracts as well have been tested for in vitro 12(S)-HETE inhibitory activities of napthoquinones extracted from roots of *E.Racemosa(Wube et al., 2005)*. The phytochemical screening of *E.Racemosa roots* was effectively achieved though there need for more research on other parts of *E.Racemosa* such as leaves, stem barks, screening their phytochemicals, verify whether the phytochemicals found in root bark are(or not) found in leaves of *E.Racemosa* and quantify for effectiveness. This is where the objective of my research was based.

2.2 Escherichia coli

Escherichia coli is scientifically classified into; Domain: Bacteria, Phylum: *Pseudomonadota*, Class: *Gammaproteobacteria*, Order: *Enterobacterales*, Family: *Enterobacteriaceae*, Genus: *Escherichia*, Species: *E.coli(Yu et al., 2021)* . *E. coli* is a small (0.5-1.0µm diameter) a Gram negative (G^{-ve}) facultative anaerobic, rod-shaped, coliform bacterium, of the genus *Escherichia* (Fracklam, 2001). They are usually found in groups and do not form spores and motile by peritrichous flagella(Kellogg et al., 2001). And their effect is due to blockage of the air sacs. The effect of this disease is highest in the youngest and the oldest section of the population in both more and less developed countries(Bogaert et al., 2004). *E.coli* was known as the major cause of pneumonia in the late 19th century (Watson et al., 1993)and it's a subject to many humoral immunity studies. And besides Pneumonia, *E.coli* is a causative agent for a range of serious human diseases including Bronchitis, bacterial meningitis, sepsis, otitis media and corneal ulcer.(Lawrence et al., 2015)

Figure 8: shows the outward view of lung invaded by Escherichia coli

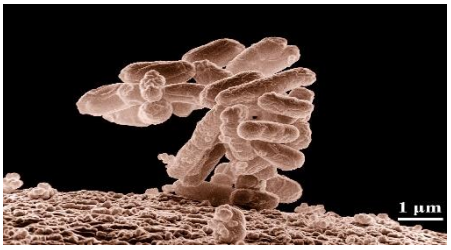
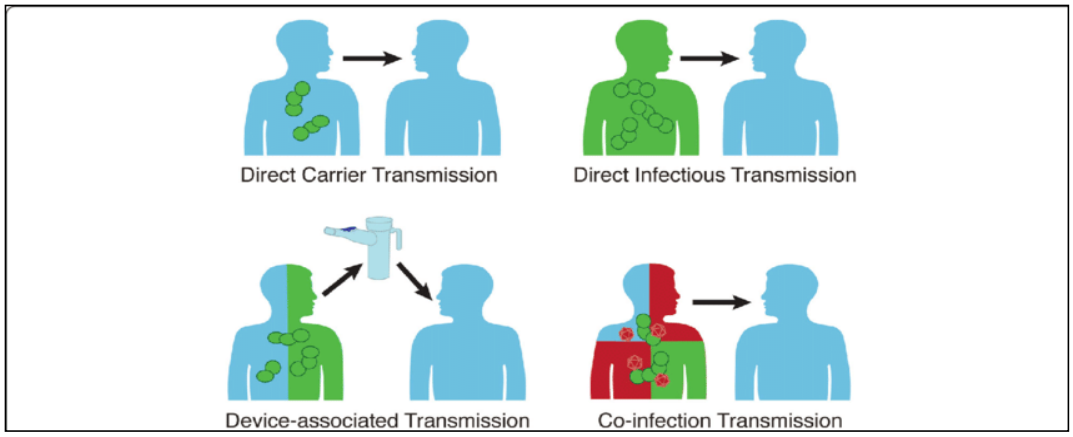


Figure 7: Microscopic View of S.P bacterium



E.coli is a regular colonizer of upper respiratory tract(Engholm et al., 2017) and resides asymptomatically in the health of carriers typically colonizing the respiratory tract, sinuses and nasal cavity(Bharti Rautela, 2020). However the *E.coli* primarily affects young children, older adults >65 years of age and individuals with impaired immune systems(Zisman et al., 2007).

E.coli spread between host through aerosols and potentially through the contamination of objects with mucosal secretions if the bacteria is living within a biofilm(Lynch III et al., 2013).

Figure 9: The figure shows how Pneumonia spreads from one person to another

Pneumonia is a disease can be generally diagnosed from chest therapy (X-rays) images by an expert radiologist by two known method of convolutional neural networks models Xception and Vgg16 for diagnosing of pneumonia. However the Xception network achieved more successful results in detecting pneumonia cases.(Unver H.M, 2019)

2.2.1 Treatment and the Failures in Treatment

Most cases of pneumonia can be managed in the outpatient setting. Severity assessment tools have been developed to help determine appropriate treatment setting(Grief & Loza, 2018). Pneumonia can be managed by antibiotics like any other bacterial caused disease. Earlier treatment of bacterial mainly based on immunotherapies, where vaccines and serums where used(Wynn, 1936) But later on the rate of mortality was high due bacterial resistance. Penicillin from chinchona tree and other such as Cephalosporins, streptomycin, Polymyxins etc. worked well as strong antibiotics and penicillin was nominated for the top therapeutic molecule.(Demain,

2011). Currently due to modifications, multidrug resistance patterns in Gram-positive and Negative bacteria are difficult to treat and may be even be untreatable with convectional antibiotics(Frieri et al., 2017). *E.coli* is a Gram-negative bacteria among the most resistant to antibiotics(Tomasz, 1997). This call for research on more antibiotics and this is one my objectives of my research to address the prevailing resistance of antibiotics and solve problems of bacterial infections Pneumonia inclusive. The antibiotic resistance in most bacteria a raise due to mutations, and through Plasmid- and transposon-specified genes.(Bryan, 1988)

Chapter:3

3.1 Materials and Methodology.

3.1.1 Plant material.

The leaves of the plant of *Euclea Racemosa* was collected from its natural habitat (village) Nagongera town council, Tororo District. It was identified, verified and quantified at Busitema university science laboratories. The leaves were dried in shade and the dried leaves samples of E. Racemosa were grounded into powder using an electric mortar. After grinding the sample was stored in an air tight container.

3.2 Extraction

3.2.1 Extraction with organic solvents (OE)

The shade-dried and ground powder of the leaves of *E.Racemosa* (mg) was extracted using methanol (1:1, v/v) at room temperature. The extracts were concentrated under reduced pressure on a rotary evaporator and the crude was obtained.

3.3 Preliminary Phytochemical analysis.

The crude extract of the leaves of *E.Racemosa* was dissolved in corresponding solvents to obtain their solutions. Their solutions were subjected to different chemical tests separately for the identification of various active constituents which was as follows;

3.3.1 Test for Flavonoids.

3.3.1.1 Ferric chloride test

To the methanolic solution of crude leave extracts of *E.Racemosa (*2mL), a few drops of neural ferric chloride solution was added. The formation of blackish red precipitate color will indicate the presence of flavonoids.

3.3.1.2 Zinc-HCl reduction test

To the methanolic solution of the crude leave extracts of *E.R (*2mL), a pinch of Zinc dust and few drops of concentrated HCL using a test tube. Appearance of magenta color indicated the presence of flavonoids.

3.3.1.3 Lead-Acetate test

To the aqueous solution of crude leave extracts of *E.R (*2mL), a few drops of aqueous basic lead acetate solution will be added using a test tube. The appearance of a reddish-brown bulky precipitate will indicate the presence of Flavonoids.

3.3.2 Test for Tannins.

3.3.2.1 Ferric Chloride

To 1 mL of aqueous solution of *E.R extracts* in a test tube (dissolve a little part of the *E.Racemosa* in 1 mL of distilled water and filter) will be added few drops of ferric chloride solution. Formation of a blackish precipitate indicates the presence of tannins.

3.3.2.2 Lead acetate test

To 1 mL of aqueous solution of *E.R* in a test tube, few drops of aqueous basic lead acetate solution will be added. Formation of reddish-brown bulky precipitate indicates the presence of tannins.

3.3.3 Detection of Saponins.

A little part of the *E.R extract* (2mL) will be mixed with 10mL of distilled water and then agitated in a graduated cylinder for 10 minutes. Formation of foam indicates the presence of saponins.

3.3.4 Detection of Quinones.

To 5mL of alcoholic sodium hydroxide, solution will be added 5mLof an alcoholic solution of *E.R extract*. The appearance of a range of red to blue coloration indicates the presence of quinones.

3.3.5 Detection of phenols.

3.3.5.1 Ferric chloride test

To 5mL of an aqueous/ alcoholic solution of E.R prototype in a test tube (dissolve little *E.Racemosa* leave powder in 5mL of water or methanol) was added 1mL of ferric chloride solution. Formation of an intense color indicates the presence of phenols.

3.3.5.2 Ellagic acid test

Add few drops of 5% glacial acetic acid and 5% sodium nitrite solution to 2 mL of an aqueous/ alcoholic solution of *E.Racemosa* using a test tube. Formation of muddy or brown precipitate indicates the presence of phenols

3.3.6 Test for Alkaloids solution.

3.3.6.1 Wagner's Test

To the crude methanolic extracts of *E.Racemosa* (1mL), 1mL of Wagner's reagent (Iodine in potassium Iodide) was added. The formation of a reddish-brown precipitate showed the presence of alkaloids

3.3.6.2 Dragendorff's reagent Test

To acid extracts of crude extract solution of *E.Racemosa* (5mL), 1mL of Dragendorff's reagent (a solution of potassium Bismuth iodide prepared from basic bismuth nitrate, glacial acetic acid and potassium iodide) will be added followed by 2mL of dilute hydrochloric acid. An Orange precipitate indicated the presence of Alkaloids

3.4 Quantification.

3.4.1 Total flavonoid content.

The total flavonoid content in the *E.R* will be determined by Aluminium chloride spectrophotometric method. Apigenin was used as a standard and flavonoid content was determined as Apigenin equivalent from the standard Apigenin solution at the concentrations of 2.81, 5.63, 11.25, 22.50 and 45.00 µg/ml that were prepared in methanol.

100 µl of each of the apigenin dilutions was mixed with 1500 µl of distilled water followed by 100µl of 5% sodium nitrate solution and allowed to stand for 6min. Then 150 µl of 10% aluminium chloride solution was added then allowed to stand for 5min after which 200 µl of 1M sodium hydroxide solution was added. The absorbance of this reaction mixture was measured at λmax 420nm using a single beam UV-VIS spectrophotometer (6705 JENWAY). The same procedure was repeated for methanolic extract solution of *E.Racemosa*. All the measurements were performed in triplicate for each analysis. The total flavonoid content will be determined from the linear equation of a standard curve prepared with apigenin and expressed as mg/g Apigenin equivalent (AE) of *E.Racemosa*.

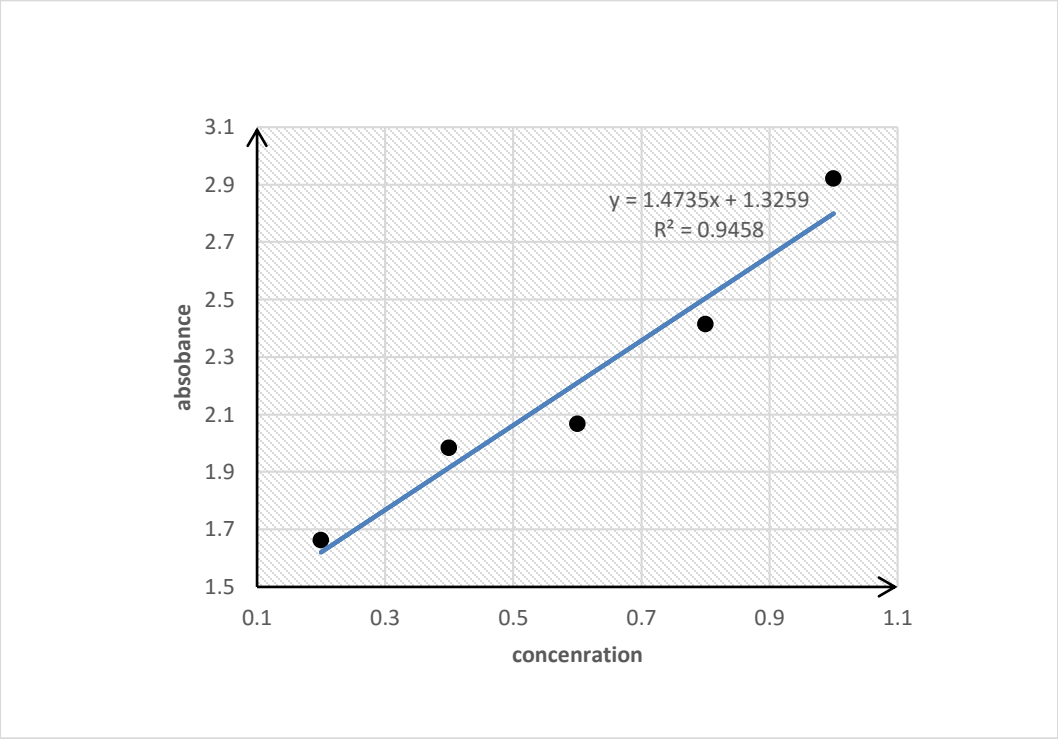


Figure 10: standard calibration of flavonoid

$$Total\ \ Flavonoids = \frac{x * DF * vol\ of\ 80\%\ methanol * volume\ added}{weight\ of\ the\ sample}$$

3.4.2 Total phenolic content

Folin-Ciocalteau method was used to estimate the total phenol content in *E.R*. In this method, a methanolic solution of the extract (1000 µg/ml) was added to 2500 µg of 10% Folin-Ciocalteau reagent dissolved in water and 2500 µg of 7.5% sodium carbonate. A blank was similarly prepared containing 500 µg methanol, 2500 µg of 10% Folin-Ciocalteau reagent dissolved in water and 2500 µg of 7.5% sodium carbonate. The samples was incubated in a thermostat at 45^0C for 45 minutes and the absorbance was determined using spectrophotometer at λmax 292nm using a single beam UV-VIS spectrophotometer (6705 JENWAY). The same procedure was repeated for the standard solution of propyl gallate. The calibration curve will be constructed using standard propyl gallate solution prepared at concentrations of 31.25, 62.50, 125.00, 250.00 and 500 µg/ml. The concentration of phenols will be read (µg/ml) from the calibration curve. Thereafter total

phenolic content in the plant will be expressed in terms of propyl gallate equivalent (mg of PGA/mg of extract).

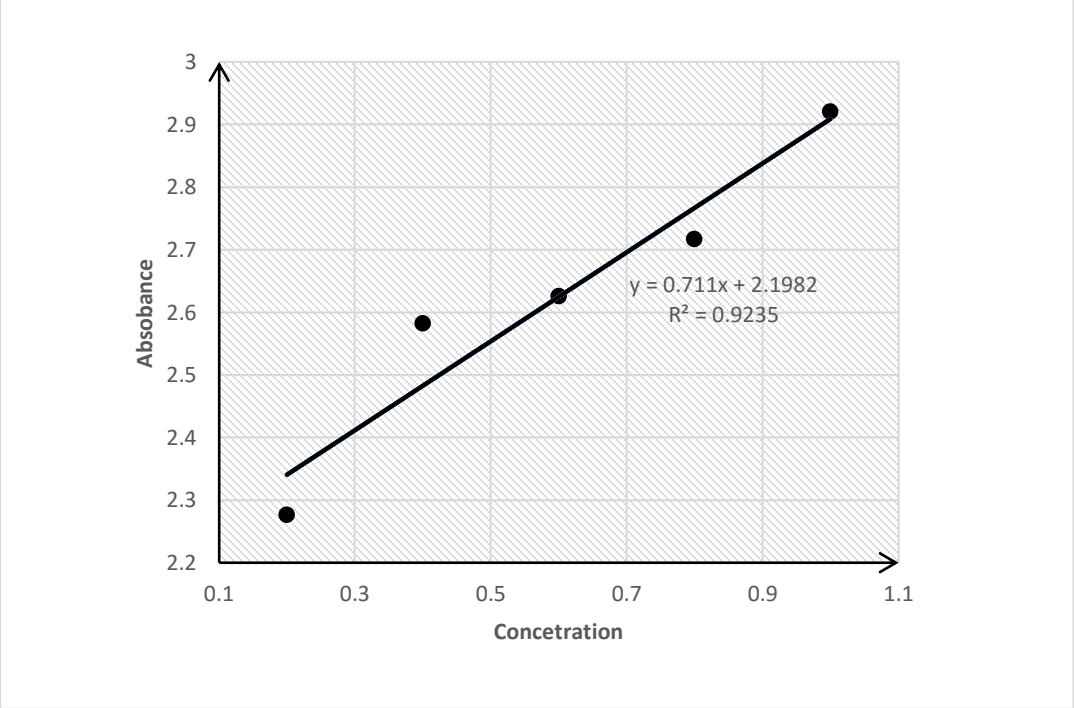


Figure 11: standard calibration curve of phenols

$$Total\ \ Phenols = \frac{x * DF * vol\ of\ 80\%\ methanol * volume\ added}{weight\ of\ the\ sample}$$

3.4.3 Total tannins

100 µL of 10 mg/mL extracts was added to a clean test tube containing 7.5mL distilled water. The Folin-Ciocalteau reagent (500 µL) was added to the mixture and vortexed thoroughly. 10mL of 35% solution of sodium carbonate ($NaCO_3$) was added to the mixture. The mixture in the test tube was transferred to 10 mL volumetric flask and the volume of the mixture made up to 10mL with distilled water. The mixture was shaken and kept at ambient temperature for 30 minutes in the dark. Tannic acid was used as a standard and reference standard solutions (31.3- 500 mg/ml) was prepared. The absorbance of the solution was measured at 650nm against a blank reagent a single beam UV-VIS spectrophotometer (6705 JENWAY). The calibration curve was constructed using

standard tannic acid solution prepared at concentrations of (31.3, 62.5, 125.0, 250.0 and 500 mg/ml). The concentration of phenols was read (mgTA/g) from the calibration curve.

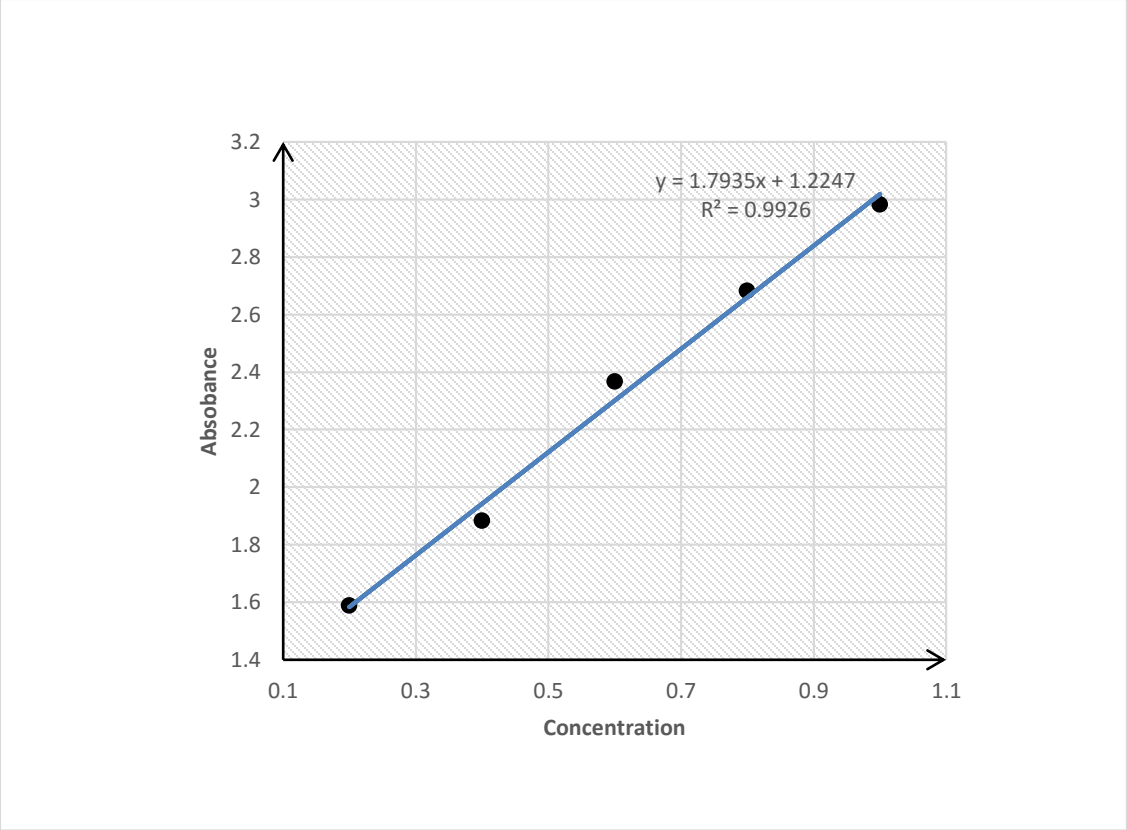


Figure 12: standard calibration curve of tannins

$$Total\ Tannins = \frac{x * DF * vol\ of\ 80\%\ methanol * volume\ added}{weight\ of\ the\ sample}$$

3.4.4 Total alkaloids

A solution of 1 mg/mL of plant extract was prepared using dimethyl sulfoxide (DMSO). 1 mL of 2 M HCl was added to 1 mL of DMSO dissolved extracts and the resulting mixture was filtered using filter paper. The filtrate was transferred to a 250 mL separating funnel and to this solution, 5 mL of 0.1% Bromocresol green (dissolved in methanol) was added followed by 5 mL of

phosphate buffer (pH 6.6). Chloroform (1 mL) was added into the separating funnel and the mixture was vigorously shaken, after which the funnel was allowed to stand to allow the mixture to separate into different layers. The lower layer was collected in a 10 mL volumetric flask. The process was repeated with 2, 3, and 4 mL of chloroform. Atropine was used to construct a standard curve using a concentration range of 1.0–0.0625 mg/mL. The absorbance of the sample and standard solutions was recorded at a wavelength of 470 nm against a reagent blank. The total alkaloid content was expressed as milligram atropine equivalent/ gram of extract (mg AE/g). All the measurements are evaluated in triplicate.

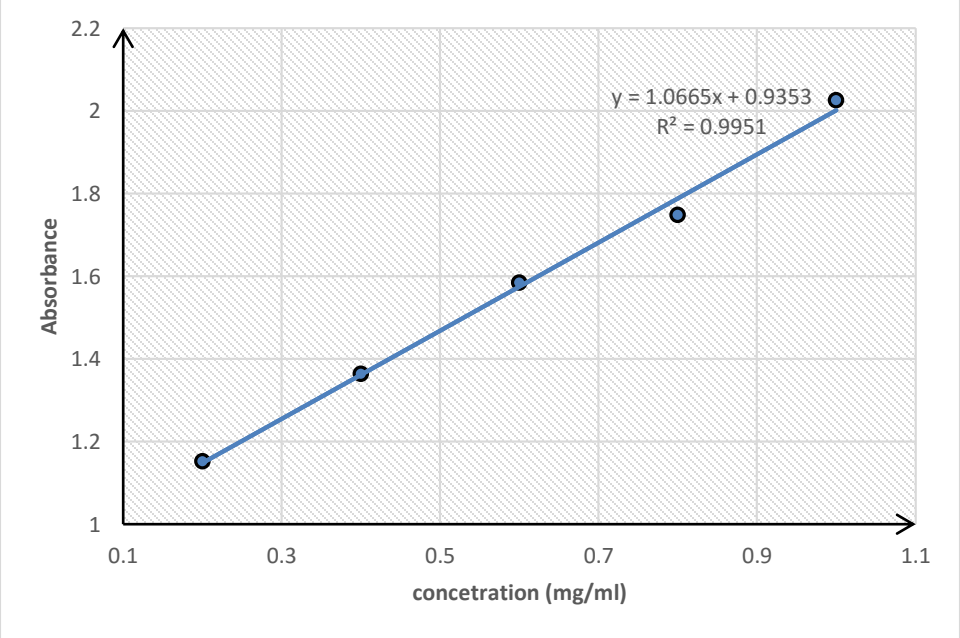


Figure 13: standard calibration curves of alkaloids

$$Total\ Alkaloids\ = \frac{x * DF * vol\ of\ 80\%\ methanol * volume\ added}{weight\ of\ the\ sample}$$

3.5 Bacteria Assay

3.5.1 Bacteria Susceptibility Testing

Procedures;

Disk diffusion method was used and required the following procedures;

A standard inoculum from bacterial culture was prepared by; choosing well-isolated colonies, creating a bacterial suspension (inoculum) and finally standardized the bacterial suspension using McFarland standards.

The bacterial suspension was inoculated on Marckocy and Nutritive agar as growth mediums for disk diffusion.

Disk soaked in sample extract (antimicrobial) was added and then incubation of the plates for 24 hours to allow disk diffusion.

The zone of inhibition was measured and recorded in terms of percentage rate of inhibition and finally interpretation of antimicrobial susceptibility test results was done.

Chapter:4 Results and Discussion

4.1 Pytochemicals charaacteristics of E.racemosa.

The phytochemical analysis carried out on the crude of *E.racemosa* showed that the whole plant was rich in pharmacologically important phytochemicals mainly Tannins, Saponins, Alkaloids, Flavonoids, Phenols and Quinones.

Phytochemicals	**Test**	**Present (+) / Absent (-)**	**Total content (mg/g)**
Flavonoids	Ferric chloride	+++	30.3
	Lead acetate	+++	
Tannins	Ferric chloride	++	136.5
	Lead acetate	+++	
Glycosides	Sulphuric acid	+	ND
	Kellar Kiliani	-	
Saponins	Foam formation	++	ND
Alkaloids	Wagner's reagent	+++	214.87
	Dragendorff's reagent	++	
Phenols	Ferric chloride	++	30.2

Key; weakly present (+), moderately present (++), strongly present (+++), absent (-)

4.2 Measuring the zone of inhibition

Bacteria	Concentration (MIC) (Mg/ml)	Actual zone of inhibition(mm)	% inhibition IR = (V_0-V)/ V_0 X 100
Escherichia coli	2	3	37.5%
	1	3	37.5%
	0.5	3	37.5%
	0.25	4	44.4%
Pseudomonas aeruginosa	2	2	28.6%
	1	1	16.7%
	0.5	2	28.6%
	0.25	1	16.7%
Staphylococcus aureus	2	NI	0%
	1	NI	0%
	0.5	NI	0%
	0.25	NI	0%
Black	2	NI	0%
	1	NI	0%
	0.5	NI	0%
	0.25	NI	0%

NI- No Inhibition

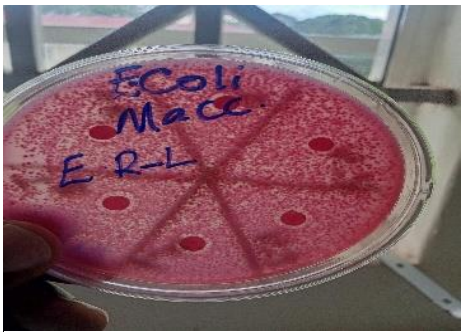


Figure 14: antibacterial activity on E.coli

Figure 15: antibacterial activity on Pseudomonas aeruginosa

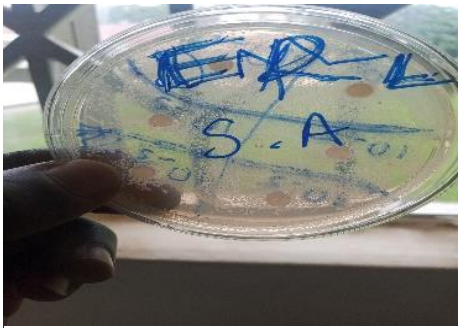


Figure 16: antibacterial activity on staphylococcus aureus

Chapter:5

Conclusion and recommendations

5.1 Conclusion

The results of this research suggests that the plant contains particularly flavonoids, alkaloids, phenolics, tannins, glycosides and saponins which justifies its use in traditional medicine. Therefore there is a fair correlation between traditional medicine remedy and the antimicrobial activity of *Euclea racemosa*. *E.Racemosa* extracts showed that *E.Racemosa* has antimicrobial activity on *Escherichia coli*, *pseudomonas aeruginosa*. However, the extracts showed high inhibition on *E.coli* than it was on *pseudomonas aeruginosa*, therefore the antimicrobial activity of E.racemosa is most effect on E.coli. The ability of E.racemosa to show antimicrobial activity may be attributed to the presence of flavonoids, alkaloids, phenolics, tannins, glycosides and saponins which were confirmed to be biologically active ingredients during the phytochemical analysis of the crude extract.

5.2 Recommendation

Results from this study showed the presence of flavonoids, alkaloids tannins, phenols, glycosides, and saponins. These phytochemicals are believed to attribute to the observed antibacterial activities in figures 14, fingure15 and figure 16, therefore I recommend the next researcher to do the following;

1) Formulation of a herbal remedy for management of respiratory tract infections
2) Antibacterial capabilities of crude extract of this plant when the concentration is doubled relative the one used (2mg/ml)

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