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PHYTOCHEMISTRY AND ANTIMICROBIAL PROPERTIES OF *AVOCADO SEED EXTRACT* AND *EUCLEA RACEMOSA SCHIMPERI* IN MANAGEMENT OF TOOTH DECAY.

BY

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February, 2024

DECLARATION

I **NANDUTU REBECCA** declare that this research dissertation is my original work and has not been submitted anywhere for the award of a degree, where other people's work has been used, this has properly been acknowledged and cited according to the university policy.

Signature..... Date..28/08/2024.....

Approval by the supervisor

Signature..... Date.....28/08/2024

Dr. ANDIMA MOSES

DEDICATION

I dedicate this research report to my beloved father Mr. Wanambwa James and mother Mrs. Mutuwa Loy for their continued support throughout my school and university education and bringing me up morally. I owe them a lot.

I also dedicate it to my beloved sons Mwaule Daniel and Kissa Simeon.Not forgetting my beloved sisters and brothers Muboki John Samson, Namutosi Naume, Naluboka Sarah, Mutonyi Mary, Walimbwa Joseph, Mukimba Joyce and Nabafu Ruth.

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I would to take this opportunity to thank my supervisor Dr. Andima Moses for his guidance, time and support towards this research. I also thank my in-law Nabukonde Esther for supporting my university education financially. In the same line, I also thank my beloved husband Masuba Pius for his guidance, emotional and financial support towards my education. I also thank the entire Chemistry Department staff from the head, Dr. Kamoga Omar, Dr. Oriko Richard, Mr. Musagala Peter, Dr. Egor Moses, Mr. Olowo, and all the other chemistry lecturers for their mentorship, encouragement, and support towards the study. I would also like to appreciate my friends Chekwemoi Abigeal, Nangoli Cranima, Lumbuku Laban and so many others who have been so influential in my education achievement at Campus. I finally take this chance to thank the Almighty God for the wisdom, good health, provision, care, love and for always protecting me.

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DEFINATIONS AND ABBREVIATIONS

AR - avocado

ER - euclea racemose

ABSTRACT

Plant extracts are actively being used worldwide due to the presence of biologically active constituents that aid against various diseases owing to their antimicrobial and antibacterial potential. This research work was carried out to determine the phytochemical constituents and antimicrobial activity of extracts of *E. racemose* and *avocado seed* in the management of tooth decay. Anti-microbial studies were carried out by disc diffusion method. Samples from methanol extract were tested against three different bacterial strains comprising one species from Gram-negative bacteria i.e., *Staphylococcus aureus* and two species from Gram-positive bacteria i.e *Escherichia coli*, *Pseudomonas.* The results of the qualitative phytochemical analysis showed that methanolic extract of root bark of *E. racemose and avocado seed* consist of alkaloids, flavonoids, terpenoids, tannins, and saponins. The total phenol, alkaloid, tannins and flavonoid content of the extracts showed that the methanolic extract of avocado seed had a significantly higher total alkaloid (1.706mg/ml) and flavonoid content (0.261mg/ml) than the extracts of E. racemose which had total alkaloid and flavonoid content as 1.602mg/ml and 0.249mg/ml respectively. The extracts had almost the same quantity of total tannins. Phenols were no detected in the extracts. The antimicrobial activity revealed that the avocado seed has highest antibacterial activity against E. coli compared to the extract of root bark of *E. racemose.* The aim of this study was to assess the potential of these plants as a reliable source of antimicrobials that may be used for the treatment of various infectious diseases in the present situation.

The study provides evidence that these plants can act as a reliable source of antimicrobial agent and may be used against several infectious diseases particularly in managing tooth decay.

Keywords: Antimicrobial activity, phytochemical property, HPLC metabolomic analysis.

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1.0 CHAPTER 1 INTRODUCTION

1.1 Background

Increased consumption of sugars and starches, inadequate tooth brushing and inappropriate oral hygiene have led to dental caries. This has resulted into teeth being affected by bacterial that makes plaque (a clear sticky film that coats the teeth) and cavities (permanently damaged area in the hard surface of the teeth that develop into tiny openings or hole) causing tooth decay.

Tooth decay is the condition characterized by tooth ache, tooth sensitivity grey, brown or black sports appearing on the teeth and unpleasant taste in the mouth. The WHO Global Oral Health Status Report (2022) estimated that oral diseases affect close to 3.5 billion people worldwide, with 3 out of 4 people affected living in middle income counties. Globally, an estimated 2 billion people suffer from caries of permanent teeth and 514 million children suffer from caries of primary teeth.

What is tooth decay? Tooth decay is damage to a tooth's surface, or enamel. It happens when bacteria in the mouth makes acids that attack the enamel. Tooth decay can lead to cavities which are holes in ones teeth .If tooth decay is not treated it can cause pain ,infections and even tooth loss(Wilson, Wilson et al. 2021). Dental caries result from the interaction between the tooth structure, the dental plaque on the tooth surface, and fermentable carbohydrates. Salivary and genetic factors also influence the development of dental caries(Lamont and Egland 2015).

The biofilm bacteria metabolize fermentable carbohydrates (glucose, fructose, sucrose, and maltose) from the diet, producing organic acids, mainly lactic acid. These end products of bacterial metabolism decrease the pH and demineralize the dental structure's outer layer. After the sugars are cleared by swallowing and saliva dissolution, the acids are neutralized by the buffer capacity of the saliva, and the biofilm pH returns to neutrality. The biofilm is now saturated by calcium, phosphate, and fluoride ions,

stopping demineralization and favoring the remineralization of the dental surface(Siddiqui and Saba 2020).This process happens every time sugars are ingested, explaining why individuals with a reduced salivary flow are at increased risk of dental caries. However, if acidic conditions prevail due to frequent sugar consumption, there is a shift in the biofilm microorganisms to more acidogenic and cariogenic bacteria. The rate of mineral loss is higher than the deposition of minerals, resulting in the first clinical sign of the disease, the 'white spot,' also known as an incipient lesion(Cury and Tenuta 2009).

It is worth noting that white spots are reversible carious lesions that can be remineralized by non-invasive procedures like fluoride application and behavioral changes, such as improving dental hygiene and decreasing sugar intake. Hence, early referral to a dentist is vital if these lesions are discovered during a clinical examination. By contrast, if incipient lesions are not managed, they will gradually progress to microcavities in the enamel, collapsing and leaving a macroscopic cavity(Nyvad, Fejerskov et al. 2008).

Prevalence of the main oral diseases continues globally with growing urbanization and changes in living conditions. This primarily due to inadequate exposure to fluoride(in the water supply and oral hygiene products such as toothpaste), availability of affordability of food with high sugar content and poor access to oral health care services(Whelton, Spencer et al. 2019).

A world health organization report shows that almost half of the world’s population (45%, 3.5 billion people) suffer from oral diseases.

The WHO Global Oral Health Status Report (2022) estimated that oral diseases affect close to 3.5 billion people worldwide, with 3 out of 4 people affected living in middle income countries(Chávez, Kossioni et al. 2022). Globally, an estimated 2 billion people suffer from caries of permanent teeth and 514 million children suffer from caries of primary teeth.

Risk factors for oral infections

Tobacco use

Alcohol consumption

Unhealthy diet high in free sugars(Sheiham and Watt 2000).

Figure 1: damaged tooth.

1.2 Problem statement

The burden of tooth decay is on increase day by day. Inability to treat this tooth decay has led to increased cases of oral diseases. The (WHO) Global Oral Health Status Report (2022) estimated that oral diseases affect close to 3.5 billion people worldwide, with 3 out of 4 people affected living in middle income countries. Globally, an estimated 2 billion people suffer from caries of permanent teeth and 514 million children suffer from caries of primary teeth.

Most common treatment involve removal of the tooth and this comes with psychologically distress, socially damaging and functionally limiting. Traditionally, many people continue to use traditional plants for health purposes even when there is no information about the plant's phytochemistry and compounds responsible for their activity, thus there is need to justify the scientific nature of use of these plants. This study is aimed at evaluate the photochemistry and antimicrobial properties of avocado seed extract and E. racemosa

Due to the increased consumption of sugars and starches, inadequate tooth brushing and inappropriate oral hygiene have led to dental caries. This has resulted into teeth being affected by bacterial that makes plaque (a clear sticky film that coats the teeth) and cavities (permanently damaged area in the hard surface of the teeth that develop into tiny openings or hole) causing tooth decay.

Tooth decay is the condition characterized by tooth ache, tooth sensitivity grey, brown or black sports appearing on the teeth and unpleasant taste in the mouth. The WHO Global Oral Health Status Report (2022) estimated that oral diseases affect close to 3.5 billion people worldwide, with 3 out of 4 people affected living in middle income counties. Globally, an estimated 2 billion people suffer from caries of permanent teeth and 514 million children suffer from caries of primary teeth.

1.4 General objective

To assess the potential of the root bark of *E. racemosa* and *Avocado seed* in the management of tooth decay

1.5 Specific objectives

1. To determine the phytochemical composition of root bark of *E. racemosa* and *Avocado seed.*

2. To determine the antimicrobial properties of the extracts.

1.6 Research questions

1. What are the antibacterial properties of *avocado seed* extract and *E. racemose schimperi* against common oral bacteria?
2. What is the scientific basis of the use of medicinal plants in the treatment of common oral bacteria?
3. What is the minimum inhibitory concentration required to inhibit the growth of bacteria?

1.7 Scope of the study

This study is experimental research carried out to find out the scientific basis of avocado seed extract and the root bark of Euclea racemosa schimperi in the treatment of tooth decay and thus synthesis an effective and less costly mouth

This experimental study was carried out in Busitema university Nagongera campus chemistry laboratory.

1.7.1 Time scope

This study is going to be carried out in three months' time starting from February up to April 2024.

1.72 significance of the study

This study is aimed at reducing the effects of removing teeth as a result of tooth decay since locally available materials are being used to synthesize a mouth wash.

This research is going to contribute to the growing body of knowledge on natural remedies for treating tooth decay. The findings of this study may lead to the development of effective treatments for tooth decay that are safe and affordable. Additionally, the identification of the phytochemical compounds in avocado seed extract may pave the way for the development of new antibacterial agents for oral health care.

2.0 CHAPTER TWO: LITERATURE REVIEW

2.1 *Percea americana.*

2.1.1 Antibacterial properties

Avocado seed contains elevated levels of phenolic compounds and exhibits antioxidant properties. Researchers investigated the effect of *Avocado Seed* Flour (ASF) on the lipid levels in mice on a hyperlipidemic diet (Asyifah, Lu et al. 2014).

The total phenolic content in the methanolic extract was 292.00 ± 9.81 mg gallic acid equivalents/ g seed dry weight and the antioxidant activity resulted in 173.3 μmol Trolox equivalents/g (Pahua-Ramos, Ortiz-Moreno et al. 2012). Different parts of avocado pear were used **in traditional medications** for various purposes including as an **antimicrobia.** The seed of avocado is one of the under-utilized non-edible parts of the fruit, which are usually discarded as residues.

Conducting research on non-edible parts of fruits is an emerging trend, which may prove to be very profitable in the near future. Mostly, because it involves an important reduction in the production of wastes and the fact that the non-edible parts of many fruits like avocado have high levels of valuable bioactive compounds, particularly natural antioxidants(Bahru, Tadele et al. 2019).

According to several studies, the hypolipidemic effects of the avocado seed focused on methanolic extracts and aqueous extracts. Uses of avocado pear seed include use in the management of hypertension, diabetes, cancer and inflammation(Jimenez, Garcia et al. 2021). Several beneficial medicinal properties of compounds present in the avocado seed have been reported, which are related to the elevated levels of phenolic compounds (64% in seed, 23% in peel, and 13% in pulp)(Bahru, Tadele et al. 2019). Thus, this review article was aimed at reviewing the proximate, functional, anti-nutrients and antimicrobial properties of *avocado seed* to aware basis for its possible dietary use and justification for its **ethno-medicinal use**.

Avocado seed is known to have a hypoglycemic effect and can be used as **a traditional medicine** to treat **toothache,** chronic gastritic, hypertension, and diabetes mellitus(Boadu, Singh et al. 2019).

Asri D (2014) conducted a study of antibacterial effect of ethanol extract of the avocado seed (Persea americana Mill.) as an alternative irrigation against E. faecalis show that at a concentration of 10% avocado seed extract showed antibacterial activity with inhibition zone 2.32 ± 0, 12 mm.

E. racemosa schimperi

Different extracts from the root of *E. racemosa* demonstrated various degrees of activities against standard bacteria of both gram-positive and gram-negative strains(Bahru, Tadele et al. 2019). , all concentrations from all tested extracts showed antibacterial activity compared to the negative control (DMSO) which had inhibition zone of 6 mm (size of formed well).

The methanol extracts of E. racemosa exhibited a maximum zone of inhibition against gram-positive bacteria followed by chloroform and acetone extracts(Gebremariam, Abula et al. 2015). Streptococcus pyogenes was most susceptible gram-positive bacteria followed by Streptococcus pneumonia and Staphylococcus aureus; and from the gram-negative bacteria, Salmonella typhi was the most susceptible followed by Pseudomonas aeruginosa, Klebsiella pneumonia, and Escherichia coli.

The antibacterial activity of most extracts was statistically significant ($P \leq 0.05$) compared to the negative control (DMSO) and had more or less equivalent potency to that of ciprofloxacin, a standard drug used as positive control in this study(Elisha, Botha et al. 2017). Thus, the present study shows that the different extracts of E. racemosa possess significant antibacterial activity and could serve as a possible justification for the traditional use of the plant against different infectious disorders. Some of the phytochemical groups that were detected in the root extracts of the plant could be responsible for the displayed antibacterial activities.

2.2 Ethno-pharmacological uses of medicinal plants (particularly *avocado seed* extract and root bark of *Euclea racemosa schimperi)*

E. racemosa schimperi , an ever green shrub widely distributed in eastern and southern Africa, has been used since early times in Eastern Africa to treat various diseases including cancer(Wube, Streit et al. 2005).

E. racemosa sub sp. schimperi (DC.) Dandy (Ebenaceae) is traditionally used in the treatment of the wound, teeth infections, eye disorders, head ache, pain, spasm, and also in smoking milk products.

In Ethiopia, the roots of E.racemosa are used to treat warts of the rectum, the root/stem part of is used to treat cancer, locally used as a toothbrush and to repel evil eye 6(Gebremariam, Abula et al. 2015). Furthermore, the leaves macerate is used to treat gonorrhea, eczema and constipation.

In Uganda, the root bark is chewed for toothache and cold decoction is drunk for malaria.

In Eastern Tanzania, the root decoction is used against cancer, abdominal pain and convulsive dysmenorrhea.(Lye, Bukenya-Ziraba et al. 2008)

In Kenya , the stem, leaves, root bark are used to treat diabetes.

2.3 Classification of avocado fruit(Jaramillo-Acevedo, Choque-Valderrama et al. 2020).

Domain	Eukarye
Kingdom	Plantea
Subkingdom	Tracheobionta
Division	Magnoliophta
Class	Magnoliopsida
Subclass	Magnollidae

Order	Laurales
Family	Lauraceae
Genus	Persea
Species	Persea Americana

3.0 CHAPTER THERE: METHODS AND MATERIALS

3.1 Collection of plant materials

The avocado seeds were obtained from Nagongera vegetable market.

Roots of E. racemosa were obtained from its natural habitat at Nagongera campus field.

3.2 Avocado Seed Extraction

Avocado seed (Persea americana Mill.) 1 kg was washed, cut into small pieces and dried in the chemistry laboratory for 4 days.

After that, blended and sifted in order to obtain 40 grams of fine powder. Then fine powder soaked in 200ml of methanol for 30 minutes.

Thereafter stirred, closed with aluminum foil and then kept in the fume hood for 24hours.

The mixture was then filtered using a filter paper.

The filtrate was put in the rotary evaporator to obtain the concentrate.

The concentrate was then dissolved in 50ml of acetone and kept in the fume hood for 3 days to allow the solvent to evaporate.

3.2.1 E. racemosa schimperi

Extraction

The collected roots of *E. racemose* were washed with tap water until the sand and mud were removed from the parts, dried, root bark removed, and powdered using the grinder.

100grams of the powder were obtained.

The powder (100g) was soaked in 200ml of methanol for 30 minutes.

Thereafter, stirred, closed with aluminum foil then kept in the fume hood for 24hours.

Each time, the solution was filtered, concentrated under reduced pressure using a rotary evaporator. The crude extract was dissolved in 10ml of methanol and kept in the fume hood for 3days to allow the methanol to evaporate.

Figure 2: grinded avocado seed and root bark of E. racemosa

3.3 PHYTOCHEMICAL SCREENING TESTS

The crude extracts of the root bark of *E. racemosa* and *Avocado seed* was subjected to various phytochemical tests to identify the various active compounds. The tests were as follows:

3.3.1 Test for Flavonoids.

Lead-acetate test: 2 ml of the crude extract was mixed with few drops of basic lead acetate solution. Formation of reddish-brown precipitate indicated the presence of flavonoids.

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

3.3.2 Test for Alkaloids.

Wagner's test

1 cm^3 of HCl was added to 3 cm^3 of the crude extract in a test tube. The mixture was heated for 20 minutes, cooled and filtered. 2 drops of Wagner's reagent were added to the filtrate and observed for reddish brown precipitate.

3.3.3 Test for phenols

2 ml of the extract was mixed with ferric chloride solution. Formation of blue color showed the absence of phenols.

3.3.4 Test for Tannins.

Ferric chloride test: 2 ml of each extract of was added to few drops of $FeCl_3$ solution. Formation of black precipitate indicated the presence of tannins.

3.3.5 Test for saponins.

5g of each extract of was placed in a test tube, added 10ml of water and shaken vigorously for 3 minutes. Formation of frothing confirmed presence of saponins.

3.4 Quantification of the phytochemicals

3.4.1 The total flavonoid content:

The aluminum chloride spectrophotometric assay was used to estimate total flavonoid content in an extract. In this method, the sample contained 1 ml of a methanol solution of the extract in the concentration of 1 mg/ml and 1 ml of 2% $AlCl_3$ solution is dissolved in methanol. The samples are incubated for 1 h at 25 °C(Gebremariam, Abula et al. 2015). The absorbance is determined using spectrophotometer at λ_{max} 420 nm. The samples were prepared in triplicate for each analysis so that a mean value of absorbance can be obtained. A calibration line was constracted. Based on the measured absorbance, the concentration of flavonoids was read (mg/ml) on the calibration line. Thereafter, total flavonoid content in a plant extract was expressed in terms of quercetin equivalent (mg of QU/g of extract).

3.4.2 Quantification of total phenol content

Folin-Ciocalteu method was used to estimate the total phenol content in a plant extract. In this method, a methanolic solution of the extract (1 mg/ml) was added to 2.5 ml of 10% Folin-Ciocalteu reagent dissolved in water and 2.5 ml 7.5% Na_2CO_3 or $NaHCO_3$(Sagar, Aneesha et al. 2018). Blank was similarly prepared which contained 0.5 ml methanol, 2.5 ml 10% Folin-Ciocalteu reagent dissolved in water and 2.5 ml of 7.5% of NaHCO3. Thereafter, the samples were incubated in a thermostat at 45 °C for 45 min. The absorbance was determined using a spectrophotometer at λmax 760 nm. The samples were prepared in triplicate for each analysis so that a mean value of absorbance can be obtained. The same procedures were repeated for the standard solution of gallic acid and the calibration line is constructed. Based on the measured absorbance, the concentration of phenolics is read (mg/ml) from the calibration line. Thereafter, the total phenol content in a plant extract is expressed in terms of gallic acid equivalent (mg of GA/g of extract.

3.4.3 Quantification of total tannin content

To quantify the tannin content, the Folin-Ciocalteu method was used.100 µL of 10 mg/mL extracts was added to a clean test tube containing 7.5 mL of distilled water(Ghali, Vaudry et al. 2015). The Folin-Ciocalteu reagent (0.5 mL) is added to the mixture and vortexed thoroughly. 10 mL of a 35% solution of sodium carbonate (Na_2CO_3) was added to the mixture(Gebremariam, Abula et al. 2015). The mixture in the tube was transferred to a 10 mL volumetric flask and the volume of the mixture was made up to 10 mL with distilled water. The mixture was shaken and kept at ambient temperature for 30 min in the dark. Gallic acid was used as a standard and reference standard solutions (1.0–0.625 mg/mL) were prepared. The absorbance for the solutions was measured at 650 nm against a blank reagent blank. Tannin content was expressed as milligram gallic acid equivalence/gram of extract (mg GAE/g). All the measurements were evaluated in triplicate.

3.4.4 Quantification of total alkaloid content:

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(Adeosun, Oni et al. 2016). 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 is 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 were evaluated in triplicate.

3.5 Test Bacteria

The test bacteria were obtained from the microbiology laboratory of Busitema university Mbale campus and this was Escherichia coli (E. coli). Staphylococcus aureus and pseudomonas. E. coli s a gram negative anaerobic, rod-shaped coliform bacterium of genus Escherichia.

3.5.1 Culturing

Preparation of media (MacCoNKEY agar)

52g of the agar was dissolved in 1 liter of distilled water. The mixture was boiled to dissolve completely. The mixture was poured in durene bottle and sterilized by autoclaving at a temperature of 121^{o}C for 15 minutes

(Autoclaving was done to prevent other microbes from entering the media).

The media was poured into sterilized petri dishes, left to settle for 24hours.Inoculation was done using an inoculating loop. The petri dishes were sealed using parafilm to prevent other microbes from entering the media and the dishes were kept in an incubator for 48hours

Inoculation.

MacCoNKEY agar was melted and then cooled to about 40 °C before pouring them into sterilized petri dishes. The petri dishes were left to cool for 4hours. Bacteria strains were spread over the layer of MacCoNKEY agar with the help of a sterilized inoculating loop. About 2 mg of the respective extract was dissolved in 1ml of dimethyl sulphoxide (DMSO). Discs of 6mm diameter were soaked in each respective extract. The discs were removed and transferred to the prepared plates containing bacteria. The prepared plates were incubated for 24 h at 37 °C. These plates were then observed for zones of inhibition.

3.5.2 Determination of minimum inhibitory concentration

Determination of minimum inhibitory concentration (MIC) were done using two different dilutions from each plant extract.4mg/1ml and 8mg/1ml of the plant extracts were prepared DMSO. The discs were soaked in the above solutions for 20 minutes. The plates were divided into six equal portions, two portions contained the concentration of 4mg/1ml, another two portions contained the concentration of 8mg/1ml and the last two portions contained discs that were soaked in DMSO (control) and then incubated at 37 °C for 24 h. MIC was determined as the lowest concentration of an extract that inhibited growth in the media.

Antibacterial Activity Screening:

The antibacterial activity screening of extracts was performed against three bacteria species of both gram positive and gram-negative strains.

4.0 CHAPTER FOUR: RESULTS AND DISCUSSION

4.1 Phytochemical analysis

Phytochemical screening was used to evaluate the constituents of the plant extracts, and their predomination, along with the search for bioactive constituents that may be helpful in the production of therapeutic drugs. In this study, the qualitative phytochemical analysis of methanol extracts of E. *racemose* and *persea americana* was carried out as shown in Table 2**.** The phytochemical screening results showed the possible presence of flavonoids, alkaloids, phenols, saponins, tannins, and terpenoids in *E. racemose* root bark and the *avocado seed.* The therapeutic potential of E. *racemose and* avocado seed might be due to the presence of these phytochemicals. Terpenoids show multiple pharmacological activities i.e., working as active agents against inflammation, cancer, viruses, and bacteria along with hindering cholesterol synthesis. Flavonoids are known to have antioxidant effects, inhibiting the initiation, promotion, and progression of tumors. Tannins possess antiviral, antibacterial, and antitumor activity.

Table 1: PRELIMINARY PHYTOCHEMICAL SCREENING RESULTS OF *E. RACEMOSA* AND *PERSEA AMERICANA MILL.*

Phytochemical group	E. racemosa	Persea Americana
Alkaloids	+	+
Flavanoids	+	+
Tannins	+	+
Terpenoids	+	+
Phenols	-	+
Saponins	+	+

For + Indicates Present

– Indicates Present

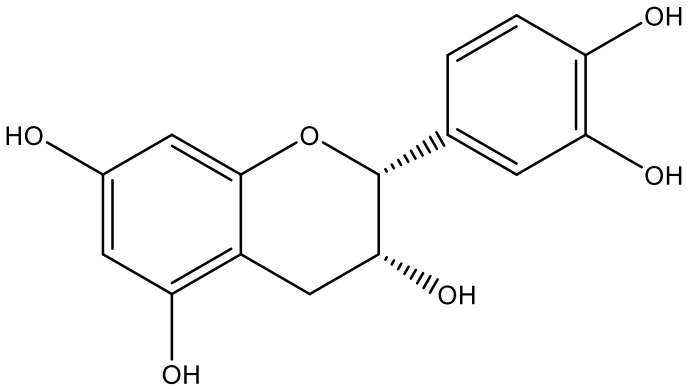
Some of the phytochemical groups that have been detected in the extracts may contribute to the antibacterial activities of the extract.

Alkaloids

Some of the first natural products to be isolated from medicinal plants were alkaloids. The **alkaloids** are a structurally diverse group of natural products containing **Nitrogen.** The nitrogenous portions of the alkaloids(Sarkar, Roy et al.) are derived from amino acids such as ornithine, lysine, tyrosine or tryptophan. Alkaloid play a variety of therapeutical uses on a wide range of infections.

Flavonoids

Flavonoids are phytochemical compounds present in many plant, fruits, leaves, seeds cereals flowers and vegetables with potential applications in medicinal chemistry(Brodowska 2017). They are secondary metabolites which mainly consist of a benzopyrone ring bearing a phenolic or poly-phenolic groups. The presence of bioactive phytochemical constituents present in the plants give them their medicinal value and biological activities(Ivancheva, Nikolova et al. 2006). Some of the chemical structures of only isolated and characterised compound in E. racemose was Myricetin-3-o-orabinopyranoside.



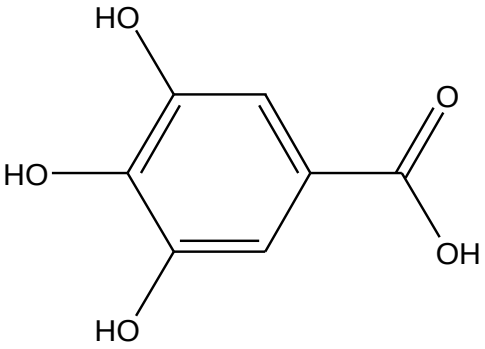
Myricetin-3-o-arabinopyranoside

Phenols

Phenolic compound are secondary metabolites ,which are produced in the shikimic acid of plants and pentose phosphate through phenylpropanoid metabolism.They contain benzene rings, with one or more hydroxyl substituents(Santos-Sánchez, Salas-Coronado et al. 2019).

Tannins

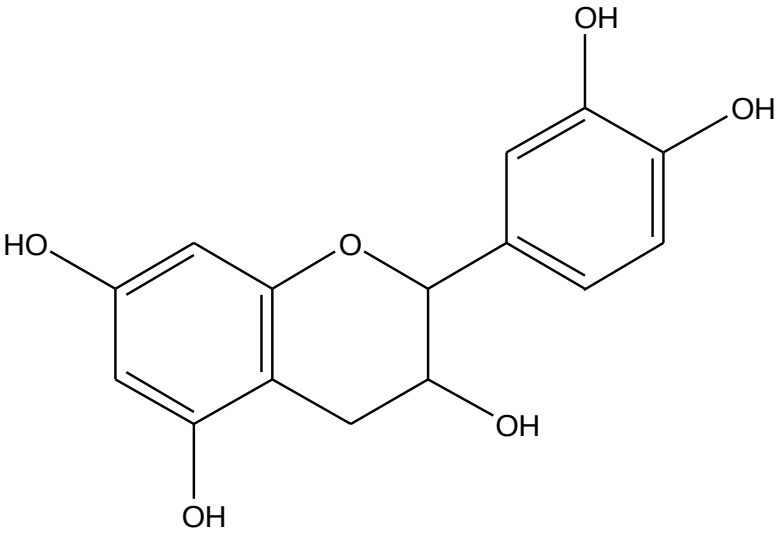
Tannins are water soluble phenolic compounds with a molecular weight between 500-3000 Daltons and may be chemically classified into two groups: hydrolysable and condensed tannins(Chung, Wei et al. 1998). Hydrolyzed tannins are connected by ester-carboxyl linkages which undergo hydrolysis under acidic and basic conditions.



gallic acid

Condensed tannins contain units of flavan-3-ol (catechin) or flavan-3,4-diol.These have complex structures and are resistant to hydrolysis however, the can be soluble in aqueous organic solvents because of their structure.

Complication of tannins with proteins give them an important role in controlling bacteria, fungi and insects.



catechin

Terpenoids

Terpenoids and **steroids** are assembled in nature from is**oprenoid (*C_5* units)** derived from **isopentenyl (**3-methylbut-3-en-1 -yl) pyrophosphate. These ***C_5*** units are linked together in a head-to-tail manner. They have a characteristic branched chain structure.

4.2 Calibration curves

Table 2: Calibration curve for flavonoids

Standards	Concentration (mg/mole)	Absorbance
S/D1	0.200	1.663
S/D2	0.400	1.984
S/D3	0.600	2.067
S/D4	0.800	2.415
S/D5	1.000	2.921
ER1	0.427	1.955
ER2	0.131	1.519
ER3	0.189	1.605
AV1	0.257	1.705
AV2	0.293	1.758
AV3	0.228	1.662

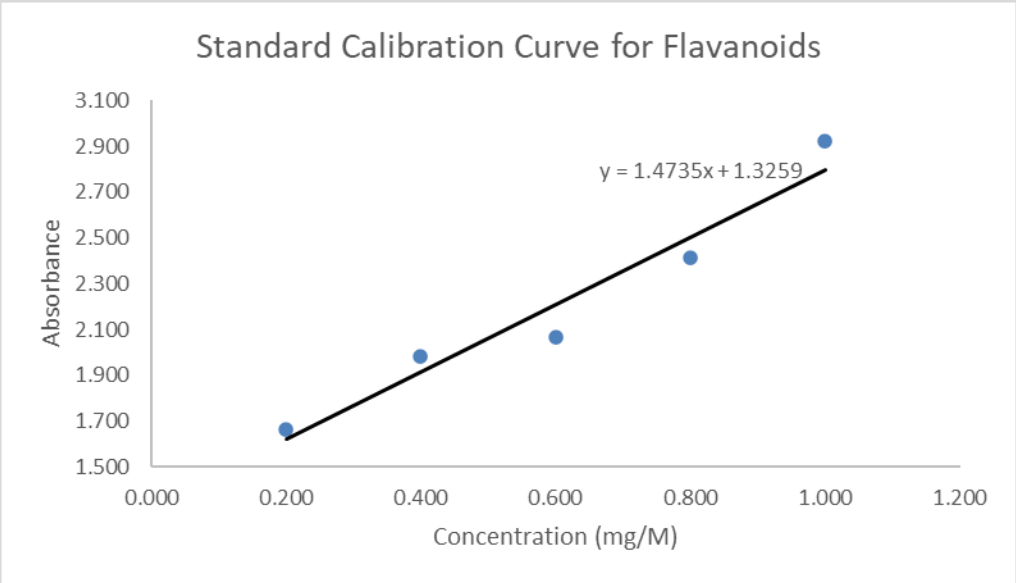


Table 3: Calibration curve for phenols.

standards	Concentration(mg/mole)	Absorbance
S/D1	0.2	2.277
S/D2	0.4	2.583
S/D3	0.6	2.626
S/D4	0.8	2.717
S/D5	1.0	2.921
ER1	2.798	1.99
ER2	2.617	1.861
ER3	3.394	2.413
AV1	2.733	1.943
AV2	2.738	1.947
AV3	2.916	2.073

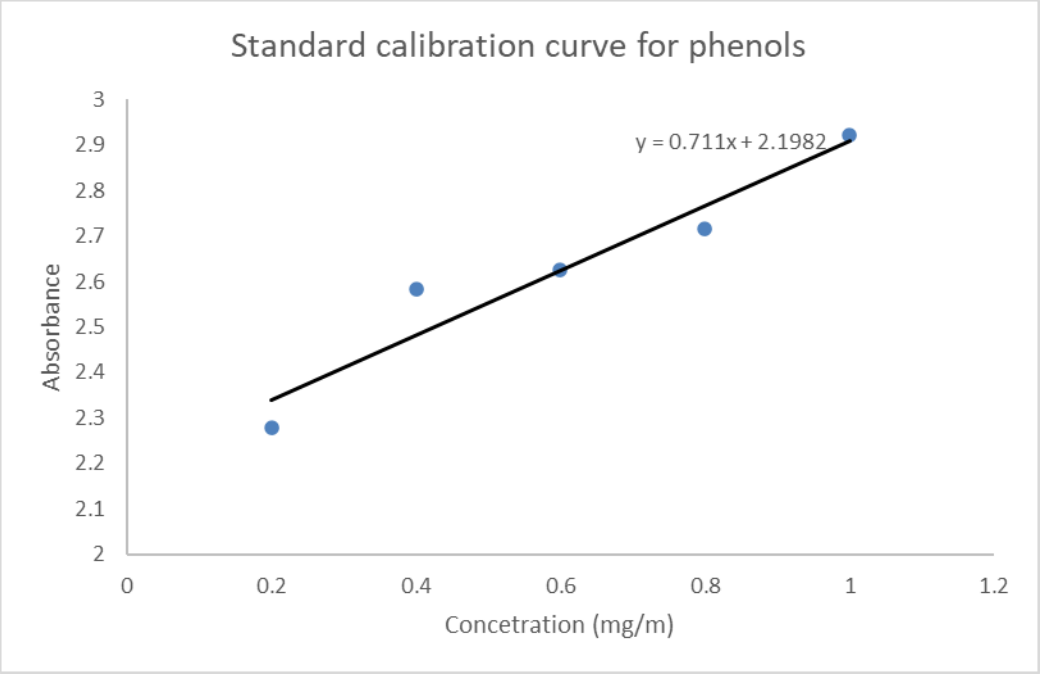


Table 4: Calibration curve for Tannins

Standards	concentration(mg/mole)	Absorbance
S/D1	0.2	1.589
S/D2	0.4	1.883
S/D3	0.6	2.367
S/D4	0.8	2.682
S/D5	1	2.983
ER1	0.597	2.297
ER2	0.919	2.874
ER3	0.774	2.614
AV1	0.712	2.502
AV2	0.806	2.672
AV3	0.762	2.592

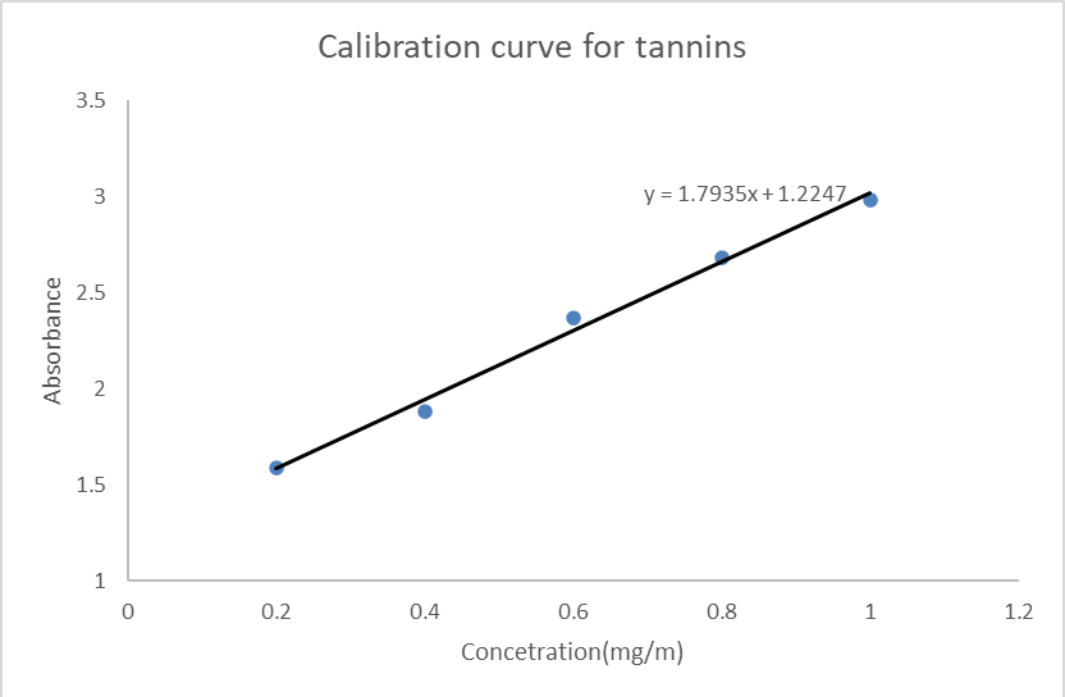


Table 5: Calibration curve for Alkaloids

Standards	concentration(mg/Mole)	Absorbance
S/D1	0.2	1.152
S/D2	0.4	1.364
S/D3	0.6	1.585
S/D4	0.8	1.749
S/D5	1	2.026
ER1	1.586	2.627
ER2	1.471	2.504
ER3	1.747	2.799
AV1	0.759	1.745
AV2	0.929	1.927
AV3	0.477	1.445

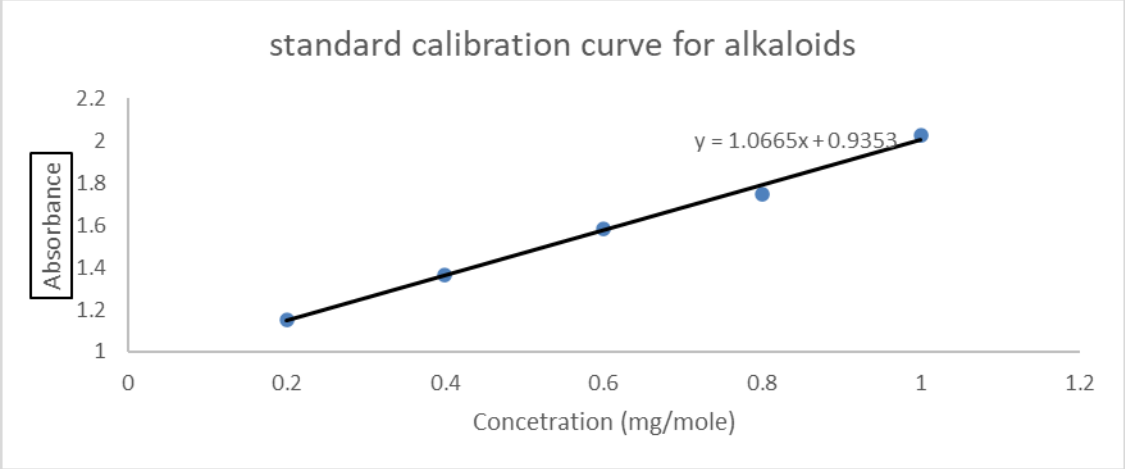


Table 6 Composition of phytochemical groups.

Phytochemical group	*E. racemosa*	*Avocado seed* extract
Flavonoids	0.249	0.261
Phenols	-0.155	-0.296
Tannins	0.764	0.761
Alkaloids	1.602	1.706

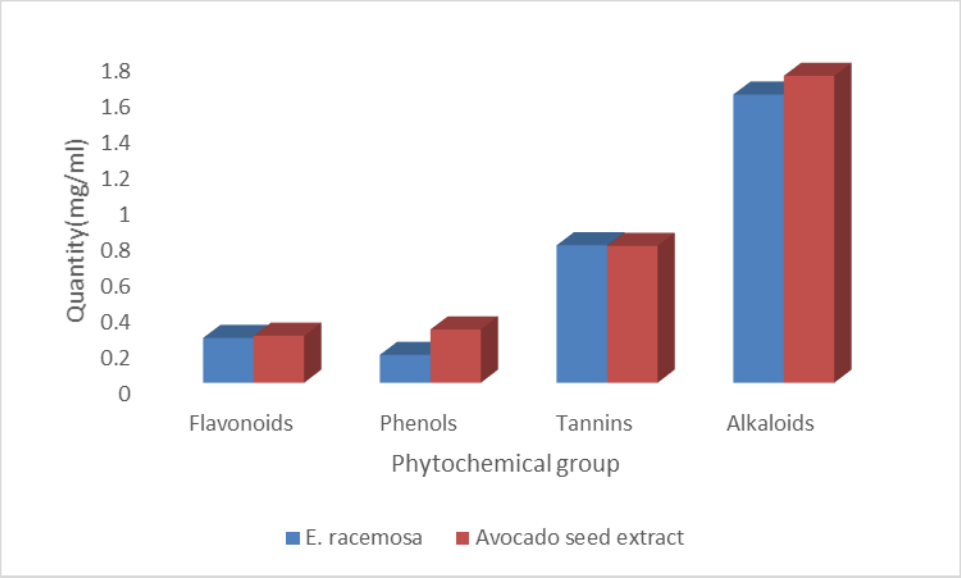


Figure 3: a bar graph showing composition of phytochemicals in the root bark of E. racemosa and Avocado seed.

The bar graphs in figure 3 above shows that both crude extracts contained a high quantity of alkaloids compared to other phytochemical groups.

Total phenols, Tannins, Alkaloids and flavonoid content present in natural products are the significant criteria for evaluating the extract quantitatively as well as its biological strength as they play an essential role in overall physiological processes. In this study, the quantity of total phytochemicals in the samples was calculated using the formula: $T = \frac{cV}{m}$

where T= total phytochemical content mg GAE/g dry extract, c= concentration of garlic acid obtained from calibration curve in mg/mL, V=volume of extract in ml, m=mass of extract in grams determined.

4.2.1 Antimicrobial activity

Both methanol extracts of *persea Americana* and *E.racemosa* showed inhibitory zones against one bacteria strain and that was E. coli and there was no growth of bacteria in media with staphylococcus and pseudomonas.

Figure 4:Inhibition zone for E. racemosa

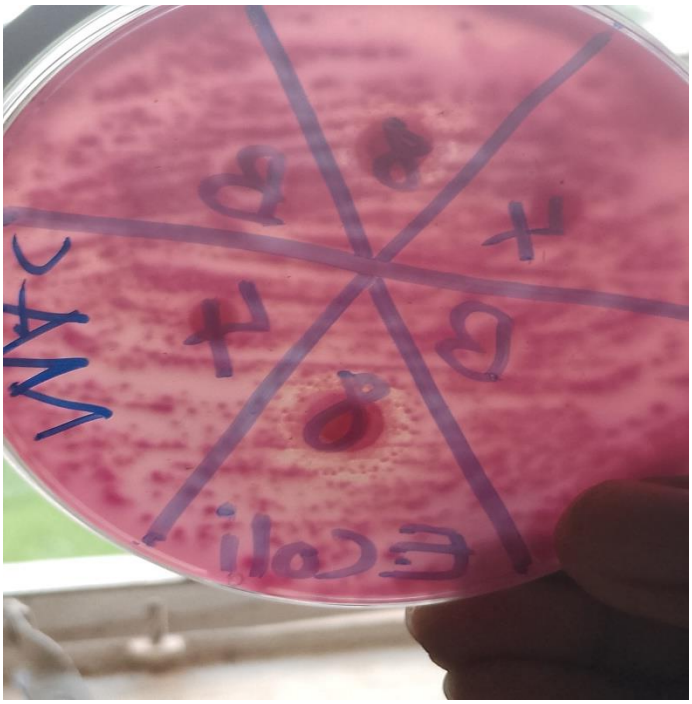


Figure 5 :Inhibition zone for Avocado seed extract

4.2.2 MIC

Determination of minimum inhibitory concentration (MIC) are defined as the lowest concentration of an antimicrobial that will inhibit the growth of a microorganism after overnight incubation. MIC of the methanol extracts from roots of *E. racemose* and persea Americana against E coli, Pseudomonas and Staphylococcus were determined as shown below.

Zone of inhibition =Final diameter –Initial diameter *100

Final diameter

Table 7 Minimum inhibitory concentrations (mics) of methanol extracts of *E. racemose root* extract and *avocado seed* extract at different dilutions against E. coli, staphylococcus, and pseudomonas aureus.

	ZONE OF INHIBITION (%)	
Bacteria strain	*E. racemose*	*Avocado seed*
E. coli	25	57

Staphylococcus aureus	0	0
Pseudomonas	0	0

CHAPTER FIVE: CONCLUSIONS AND RECOMMANDATIONS

5.1 CONCLUSIONS

Increase in the resistance of microbes against available synthetic drugs has shifted the focus to treating the microbial infections using plant extracts. The fact that bacteria has shown advanced resistance to various drugs, a variety of drugs have been identified from natural sources to treat bacterial infections.

The purpose of this research was to determine the phytochemistry and antimicrobial properties of avocado seed and root bark of E racemose in the management of tooth decay. The phytochemical screening results showed the presence of flavonoids, alkaloids, tannins, saponins, phenols and terpenoids. Phytochemicals are natural products that are organic in nature and are synthesized by living systems. The presence of these phytochemicals is responsible for the therapeutical uses of these plants in the treatment of bacterial infections. The quantification results showed that both the avocado seed and root bark of E. racemose had high quantity of alkaloids (1.706mg/ml and 1.602mg/ml) respectively compared to other phytochemicals. Both plant extracts showed bacteria inhibition against E. coli with the highest percentage of inhibition being exhibited by avocado seed extract.

5.2 RECOMMENDATIONS

According to this research, I recommend that the *avocado seed* and root bark of *E. racemosa* be used in the management of tooth decay.

Further research should be carried out to investigate the toxicity of the avocado seed use on human health.

Communities should be educated especially in areas with limited dental care services on the benefits of using these natural remedies in dental care. I also recommend that a herbal toothpaste be synthesized from these plants because of their antimicrobial properties.

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