PHYTOCHEMICAL SCREENING AND *IN VITRO* ANTIOXIDANT ACTIVITY OF METHANOL EXTRACTS OF *CHASMANTHERA DEPENDENS* ROOT

BY

ODOKOR CHOICE IROGHAMA

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DEPARTMENT OF MEDICAL BIOCHEMISTRY

SCHOOL OF BASIC MEDICAL SCIENCES

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IN FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCES DEGREE IN MEDICAL BIOCHEMISTRY

SUPERVISED BY:

MRS. O. IKPONMWOSA- EWEKA

JANUARY, 2023.

CERTIFICATION

We the undersigned, hereby certify that ODOKOR CHOICE IROGHAMA carried out this work in the department of Medical Biochemistry, University of Benin, Benin City and we approve same as adequate in scope and quality for the award of Bachelor of Science degree in Medical Biochemistry.

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MRS.O. IKPONMWOSA-EWEKA **DATE**

(PROJECT SUPERVISOR)

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PROF AKHERE O. OMONKHUA **DATE**

(HEAD OF DEPARTMENT)

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EXTERNAL EXAMINER **DATE**

DEDICATION

This work is dedicated to Almighty God for His unending love and faithfulness and to my mother Mrs Odokor Cordilla who is laboriously giving her today for my tomorrow and my brothers.

ACKNOWLEDGEMENT

I want to thank God Almighty for his sufficient grace and strength during the course of this project. I also want to sincerely appreciate my project supervisor Mrs. Ikponmwosa- Eweka for her guidance and encouragement and my ever loving and doting mother Mrs Odokor Cordilla and brothers for their unwavering support and being there for me financially, morally and their unending love and prayer, indeed I am grateful to them.

I will also like to acknowledge all the lecturers in the Department of Medical Biochemistry for their contributions to my academic pursuit especially Dr. Kelly Oriakhi, and Mr. Osagie A. Ododo, for their unequivocal support. I am grateful to my colleagues not to forget my friends Ayo-Akopre success, Deborah Ivanna Osegbe and Ofiagada Samuel who contributed one way or the other to the success of this work.

Thanks to you all and God bless you all for your help and encouragements during the course of this project work.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Chasmanthera dependens is commonly used in Africa traditional system for the management of several pathologies. This research was designed to asses the phytochemical and antioxidant activity of the methanol extract of Chasmanthera dependens roots. The result of the qualitative phytochemical screening revealed the presence of flavonoid, tannins, Terpeniods, reducing sugars, saponins and proanthovyanindins in the extract. The quantitative phytochemical screening further confirmed the concentration of flavonoid(8.61±0.74) tannins(87.05±1.27), proanthoncyanidins (45.1±1.5) and phenols(199.1±1.9). In vitro antioxidant properties of the extract has antioxidant properties as revealed by it's ferric acid antioxidant power (FRAP), and reducing potential. The phytochemicals in the extract was further identified and quantified by HPLC screening. The HPLC fingerprinting revealed a reported activity of Phytochemical with rich medicinal value. The study also shows that Chasmanthera dependens scavenged DPPH (16.59) reducing power increases in absorbance as the concentration of the plant increased. Conclusively this study provide more information on the medicinal use of Chasmanthera dependens and its good antioxidant properties.

CHAPTER ONE

1.0 INTRODUCTION

In the history of humanity, plants have always been present as a source of health. The knowledge of the various healing properties of plants has been transmitted in an empirical way. However, over time, man has been interested in knowing where the properties of plants come from. In the process of knowledge generation, man has developed many methodologies to know the structures of organic compounds responsible for the healing properties of plants. This is the birth of phytochemistry that is defined as the science responsible for the study of the compounds contained in plants. In this field, various techniques have been developed, ranging from the preparation of the plant tissue sample to sophisticated techniques for the elucidation of organic structures.

Medicinal herbs or plant are used in the therapeutic treatment and cure of illnesses. The use of medicinal plant is not only for the treatment of diseases but also for maintaining good health and conditions. Many countries in the world depends on herbal medicine for primary care. The reason for this is because of their cultural acceptability, strong healing powers, easily available, safe, lesser side effect and better compatibility with the human body (Kaur and Arora, 2009). The word “herb” is comes from the Latin term “herba” and an old French word “herbe.” Herb now makes reference to any selected plant, such as a fruit, seed, stem, bark, flower, leaf,

stigma, or root, and a non-woody plant. Previously, the term “herb” only referred to non-woody plants, like those derived from trees and shrubs. Such medicinal herbs are also used as food, a flavonoid, medicine, or perfume, as well as in some religious rituals. Plants have long been used in the medicinal purposes, dating back to the prehistoric time frame. While the pharmaceutical industry is thought of as a modern phenomenon, drug discovery and development has always been with us since the early days of human civilization. These folks medicine were mainly of plant orgin with the addition of minerals and animal substances. Thou these remedies were discovered and developed separately in different civilization they often make use of the same plant and herbs for similar illnesses. Herbs were described in ancient Unani manuscripts, Egyptian papyrus, as well as Chinese writings. There is evidence that Unani Hakims, Indian Vaids, but also European and Mediterranean cultures have used herbs as medicine for over 4000 years. Herbs have been used in healing rituals by native cultures such as Rome, Egypt, Iran, Africa, and America, whereas others evolved traditional medical systems including such Unani, Ayurveda, and Chinese Medicine wherein herbal therapies were being used systematically. Traditional medical systems are still widely practiced on a variety of fronts. Population growth, insufficient drug supply, prohibitive treatment costs, adverse effects from several synthetic drugs, and the development of resistance to presently were using drugs for communicable

diseases have all contributed to an increased emphasis on prevention. India has been recognized to become a rich repository of medicinal plants among ancient civilizations.

Nature has been an important source of medicine and has helped mankind in the maintenance of health since ancient time. The plant kingdom is a wealth house of potential drugs and at the present year there has been an increasing consciousness about the magnitude of medicinal plants (Pawar *et al*., 2011). In Europe, around 1300 medicinal plants are used, with 90% of them coming from natural sources. Approximately 50,000 - 80,000 flowering plants are utilized for medical purposes, according to the international union for conservation of nature and the world wildlife fund (Abiola *et al*., 2020). Medical plants have emerged as important aspects of African civilization throughout the millennia, and they are now generally acknowledged as symbols of continent's rich cultural and scientific past. the pharmaceutical sector has rekindled interest in herbal heath care formulations, herbal-based cosmetics. As a result, medicinal plants in Afi have economic value in addition to their medical and cultural purposes. The global d national markets for medical herbs have been expanding, and the selling of medicinal plants products has resulted in major economic gains.

1.1 Justification of study

Most developed and developing countries are in great demands of herbal medicines as a source of primary health care because of their medicinal attributed, lesser cost, high safety margins and wide spectrum treatment.

Chasmanthera dependens has been reported to possess various beneficial effects. Most of this effects seen may be attributed partly to the phytochemicals constituents of the plant. Having travelled through the era of herbal medicine. Conventional drugs as met serious setback due to resistance,even the most successful antimalarial drugs.thus the need for herbal medicine cannot be over emphasis.

1.2 AIMS AND OBJECTIVES

The aim of this study is to evaluate the phytochemical constituents (qualitative and quantitative), and the antioxidant activity of the methanol extract of *Chasmanthera dependens*

The specific objectives include;

> Extraction of the phytochemical constituents of *Chasmanthera dependens* using methanol as solvent.

> Phytochemical constituents (qualitative and quantitative) of the extract to determine the active components and their amounts in the extract.

> Assessment of *in vitro* antioxidant activity of the extract.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Chasmanthera dependens

Chasmanthera dependens is a genus of flowering plant belonging to the menispermaceae family. It's natural habitat is in Tropical Africa (Adjanohoun *et al*., 1991). The plant has a rough stem and Isa woody climber. The immature branchlets are small, hairy and closely packed. The leaves are oval or rounded, measuring 3_10 cm in length and width. The leaves are also alternating, simple, with no stipules and petioles that are 7_14 cm long. On both side, the leaf surface is papery and sparely hairy (Hamill *et a*l., 2003). The stalks are green and up to 30 cm long, with a heart shaped base and a short drawn-out apex. The plant produces several floweing, slender stalks covered in soft hair. The fruit is made up of three ellipsoid and unequal -sided druplet, eachwith one seed. The seeds are ovoid and curved, about 1_2 cm in length (Gemedo-Dalle *et al.*, 2005). It thrives in rocky terrains, especially in wild forest borders and savanna. In west and central Africa, it is grown as a medicinal herb. From Sudan in the north to Ghana on the west coast , plant can be found the way down to Ethiopia and south Africa (Mosango, 2008). *Chasmanthera dependens* can be found from sierra Leone to Eritrea and

Somalia in the east, and from Tanzania to Angola, Zambia and Zimbabwe. It is commonly planted in home Gardens especially in Ghana.

Chasmanthera dependens can be found in forest edges, savanna, and secondary forest, frequently among rocks but it can also be found in deep and moist evergreen forest, semi-deciduous forest, and riverine forest at elevations of up to 1500 meters. It prefers moist, well-drained soil with plenty of sunlight. Seed and wildings are used to propagate chasmy dependens. For six months, the seeds remain dormant. *Chasmanthera dependens* is grown as a medicinal plant in west Africa, mostly in household gardens. It can be found growing in cocoa plantation and thought to lower production. It's also home to fruit-eating medfly (Ceratitis sp.)

Fig 1: *Chasmanthera dependens* leaves (Gemedo-Dalle *et al*., 2005)

Chasmanthera dependens is found in wild or private gardens. The leaves are pulverized, and the leaf juice is utilized right once or saved to treat sparins and bruises later. Fresh or roasted stem can be used. It's also possiy to dry it and store it

for later use. The plant is called 'aguru' in Igbo(South East Nigeria), 'atoo' in Yoruba(South West Nigeria).

2.2 Taxonomy

Kingdom: plantae

Clade: Tracheophytes

Clade: Angiosperms

Clade:. Eudicots

Order: Ranunculales

Family: Menispermaceae

Genus: Chasmathera

Species: dependens

Botanical names: Chasmathera dependens

2.3 Medicinal Uses

Fever, cough, jaundice, cholera, gastrointestinal illnesses, rheumatism, veneral diseases and snake bite are among the common folk medical indications of

Menispermaceae medicine over the world. *Chasmanthera dependens* is used to treat veneral disease, as well as sprained joint, bruises and as a general tonic for physical and mental ailment (Barbosa- Filho *et al*., 2000). Leaf and stem sap are used locally in West Africa to heal sprains and bruises, as a bandage for fractures and as an embrocation to relieve pain and stiffness when mixed with Shea butter. Chewing the bark is used to treat venereal discharges and as a general tonic for physical and nervous weakness in inflammatory and tiring disorder (Okoli *et al*., 2003).

In Nigeria , stem maceration is consumed along with the stem and roots of variety of different plants to treat convulsions. The stem is roasted and eaten in Kenya to cure baby convulsions. The plant is used to treat dementia, sneakbites and epilepsy in Uganda. Malaria is cured by drinking a decoction of freshly crushed roots mixed with Vernonia sp. roots. Children drink a deconction of pounded roots mixed with Tagetes sp. leaves to treat cough (Adekunle and Okole, 2002). Leaf of sap is used to stop bleeding of wound in DR Congo. Ethanol extracts and crude water extract of the root have been used substantially as antifungal agents against Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Candida albicans, Microsporum audonii, Trichoderma viride and Trichophyton mentagrophytes.

2.4 Phytochemical

Phytochemicals are bioactive component of plant which is beneficial to the health of humans. Plant synthesizes by hundreds of chemical compounds for the defense against insect, fungi, diseases and herbivorous mammals , these chemical compounds are phytochemicals (Mathai *et al*., 2000.). Some plant cells produce important secondary metabolites of the interactions of the plant with the environment (protection against predators, pathogens or environmental stress) or some related to the reproductive mechanism of the plant (attraction of insects for the promotion of pollination). The discipline whose main objective is the study of the chemical constituents of plants is Phytochemistry. The study of such compounds includes: their chemical structures, metabolism (biosynthesis and degradation), natural distribution, biological function, extraction and qualitative-quantitative evaluation.

Phytochemicals are defined as bioactive nutrient plant chemicals in fruits, vegetables, grains, and other plant foods that may provide desirable health benefits beyond basic nutrition to reduce the risk of major chronic diseases (Liu, 2004). Many phytochemicals act as antioxidants, but they have several other functions, such as mimicking hormones, altering absorption of cholesterol, inhibiting inflammatory responses, and blocking the actions of certain enzymes. Numerous

phytochemical have been discovered in plant and they have established biological active potentials which can be classified into two groups, primary and secondary.

Primary Metabolites

These are the chemical compounds produced during the growth and development, processes. They are also involved in the primary metabolic processes of respiration and photosynthesis. The primary metabolites are formed in the growth phase. They maintain the physiological functions of the body and are known as central metabolites. They are the intermediate products of anabolic metabolism, which are used by the cells for the formation of essential macromolecules.

Amino acids, vitamins sugars.lipids,chlorophyll , organic acids, are some of the primary metabolites produced industrially. Alcohol is the major primary metabolite produced on a large scale, industrially.

Secondary Metabolites

These compounds are produced by the organisms that are not required for primary metabolic processes. However, they can be important ecologically or otherwise. Secondary metabolites are considered to be the end products of primary metabolites because they are derived by the pathways in which the primary metabolites involve..

secondary metabolites such as phenolic compounds, alkaloids, flavonoids, terpenoids, tannins, saponins, cardiac glycosides, essential oils, etc. which are important in plant defense against herbivory and adaption to environmental stress (Saxena *et al*., 2015). Streptomyces and other related actinomycetes are sources of novel secondary metabolites.

2.4.1 FLAVONOID

Flavonoids, a group of natural substances with variable phenolic structures, found in fruits, vegetables, grains, bark, roots, stems, flowers, tea and wine. Flavonoids are now considered as an indispensable component in a variety of nutraceutical, pharmaceutical, medicinal and cosmetic applications. This is attributed to their anti-oxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties coupled with their capacity to modulate key cellular enzyme function. These compounds are present in plant tissue as red, blue, and purple anthocyanin pigments which help the plant in reproduction by recruiting pollinators and seed dispersers (Winkel *et al*., 2001). Flavonoids have several subgroups, which include chalcones, flavones, flavonols and isoflavones. These subgroups have unique major sources. For example, onions and tea are major dietary sources of flavonols and flavones. Plants produce approximately 12,000 different alkaloids, which can be classify into groups according to their carbon skeletal structures (Ziegler and

Peter, 2008) Alkaloids exhibit a wide range of pharmacological effects including anti-oxidant (Danikiewicz *et al*., 2014) antibacterial activity (Karou and Savadogo, 2014).

2.4.2 TANIIN

Tannins are complex chemical substances derived from phenolic acids (sometimes called tannic acid). They are classified as phenolic compounds, which are found in many species of plants, from all climates and all parts of the globe. They are large molecules that bind readily with proteins, cellulose, starches, and minerals. These resulting substances are insoluble and resistant to decomposition. Tannins occur in many species of coniferous trees as well as a number of flowering plant families. These tannins can leach out of the plants. The water in the soil becomes rich with tannins and seeps into the ground water or drains into lakes and streams. These waters become brown in color and look like tea.

The antioxidant properties of tannins are widely utilized in thefood and medical fields. In recent years, many studies havebeen conducted to identify the relevant antioxidant activity oftannins. Owing to its antioxidant capacity, such as preventingcardiovascular disease, cancer or osteoporosis, tannins haveattracted much attention (squilaro *et al.,* 2018 and lall *et al*., 2015).

2.4.3 SAPONINS

Saponins, also selectively referred to as triterpene glycosides, are bitter-tasting usually toxic plant-derived organic chemicals that have a foamy quality when agitated in water. They are widely distributed but found particularly in soapwort (genus Saponaria), a flowering plant, and the soapbark tree (Quillaja saponaria). They are used in soaps, medicinals, fire extinguishers, speciously as dietary supplements, for synthesis of steroids, and in carbonated beverages (the head on a mug of root beer). They are used in soaps, medicinals, fire extinguishers, speciously as dietary supplements, for synthesis of steroids, and in carbonated beverages (the head on a mug of root beer). Saponins are both water and fat soluble, which gives them their useful soap properties. Some examples of these chemicals are glycyrrhizin, licorice flavoring; and quillaia (alt. quillaja), a bark extract used in beverages.Saponins have exhibited a plethora of pharmacological activities, including antiviral, anti-inflammatory, anticancer, antifungal, antimicrobial, antioxidant, and immunomodulatory effects.

2.4.4 ALKALOIDS

Alkaloids are a huge group of naturally occurring organic compounds which contain nitrogen atom or atoms (amino or amido in some cases) in their structures.. From among many classes of naturally occurring organic compounds such as

carbohydrates, lipids, proteins, amino acids, anthocyanins, flavonoids, and steroids, the one that seems to be quite special is alkaloids. This is due to the fact that They are derived from amino acids and can be synthetized as secondary metabolites by plants and some animals. These compounds play an important role in living organisms. Alkaloids occurred to be extremely important for human beings for ages, besides they are secondary metabolites, what could suggest that they are useless. Alkaloids showed strong biological effects on animal and human organisms in very small doses. Alkaloids are present not only in human daily food and drinks but also as stimulant drugs. They showed anti-inflammatory, anticancer, analgesics, local anesthetic and pain relief, neuropharmacologic, antimicrobial, antifungal, and many other activities. Alkaloids are useful as diet ingredients, supplements, and pharmaceuticals, in medicine and in other applications in human life. Alkaloids are also important compounds in organic synthesis for searching new semisynthetic and synthetic compounds with possibly better biological activity than parent compounds. Plants produce approximately 12,000 different alkaloids, which can be classify into groups according to their carbon skeletal structures (Ziegler and Peter, 2008) Alkaloids exhibit a wide range of pharmacological effects including anti-oxidant (Danikiewicz *et al*., 2014) antibacterial activity (Karou and Savadogo, 2014).

Alkaloids showed quite diverse medicinal properties. Many of them possess local anesthetic properties, but their practical use is limited for clinical purpose. Morphine is one of the most known alkaloids which had been used and still is for medical purposes. This alkaloid is a powerful narcotic which is used for the relief of pain, but its usefulness is limited because of addictive properties (Chisholm H *et al.,* 2015). Tubocurarine is an alkaloid, is an ingredient of poison curare, and is used in surgery as muscle relaxant. Quinine is a powerful antimalarial agent and more often is replaced by synthetic drugs, which are more effective and less toxic.

2.4.5 PHENOL

Phenols are compounds possessing one or more aromatic rings with one or more hydroxyl groups. They are broadly distributed in the plant kingdom and are the most abundant secondary metabolites of plants. Plant phenols are generally involved in defense against ultraviolet radiation or aggression by pathogens, parasites and predators, as well as contributing to plants' colors. They are ubiquitous in all plant organs and are therefore an integral part of the human diet.

Phenols are widespread constituents of plant foods (fruits, vegetables, cereals, olive, legumes, chocolate, etc.) and beverages (tea, coffee, beer, wine, etc.), and partially responsible for the overall organoleptic properties of plant foods. For example, phenols contribute to the bitterness and astringency of fruit and fruit

juices, because of the interaction between phenols, mainly procyanidin, and the glycoprotein in saliva. In relation to their chemical structure, these compounds contain at least one phenol group.This phenol is composed of an aromatic ring with one or more hydroxyl groups. Although phenolic compounds can be present in their free form in plants, they are generally present bound to sugars or proteins

2.5 ANTIOXIDANT POTENTIAL OF MEDICINAL PLANT

Medicinal plants are rich source of antioxidants which have the potential to counteract oxidative stress or DNA and protein damage in tissues and prevent the living organisms from chronic diseases such as cancer, cardiovascular diseases, diabetes, and aging.

Antioxidants significantly delay or prevent oxidation of oxidizable substrates when present at lower concentrations than the substrate .Antioxidants can be synthesized in vivo (e.g., reduced glutathione (GSH), superoxide dismutase (SOD), etc.) or taken as dietary antioxidants . Plants have long been a source of exogenous (i.e., dietary)

It is believed that two-thirds of the world's plant species have medicinal importance, and almost all of these have excellent antioxidant potential

Rapid production of free radicals may cause alteration in the structure and function of cell constituents and membranes and can results in human neurologic and other disorders such as cancer, diabetes, inflammatory disease, asthma, cardiovascular, neurodegenerative diseases, and premature aging (Mclarty, 1997; Young and Wood, 2001; Yang *et al*., 2001; Sun *et al*., 2002; Bimal *et al*., 2011). Therefore, the prevention of the above conditions requires the presence of antioxidants or the free radical scavenging molecules in the body. There are plenty of antioxidant substances present in plants (fruits, vegetables, medicinal herbs, etc.) and the free radical scavenging molecules present in them are in the form of phenolic compounds (e.g. phenolic acids, flavonoids, quinones, coumarins, lignans, tannins), nitrogen compounds (alkaloids, amines), vitamins, terpenoids (including carotenoids), and some other endogenous metabolites, (Zheng and Wang 2001; Cai *et al*., 2003; Govindarajan *et al*., 200

The most commonly used methods for measuring antioxidant activity are those which involve the generation of free radicals which are then neutralized by antioxidant compounds. DPPH is a well-known radical and a trap ("scavenger") for their radicals (Husain *et al.,* 1987, Visioli *et al*., 2000; Parr *et al*., 2004; Solai *et al*., 2010).

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 REAGENT

- Distilled water
- Methanol
- Naphthol
- Sodium hydroxide
- Hydrogen chloride
- Aluminium chloride
- Quercetin
- Molybdate
- Sulphuric acid
- 2, 2, diphenyl-1-picrylhydrazyl (DPPH)
- Tripyridyltriazine (TPTZ)
- Ascorbic acid

- Gallic acid
- Dragendorff's reagent
- Lead acetate
- Folin-ciocalteau
- Potassium acetate
- Catechin
- Folin-denis reagent
- Potassium ferrycyanide
- Trichloro acetatic acid
- Potassium hydroxide
- Ethanol
- Acetonitrile
- Sulphate anhydrous
- Ferric chloride
- Hydrochloric acid

3.2. APPARATUS

- Test tubes
- Test tubes racks
- Beaker (50ml ,100ml and 200ml)
- Pipette
- Automatic pipette (5µl, 10µl, 50µl and 100µl)
- Micro pipette
- Measuring cylinder
- Masking tape
- Funnels
- Sieve
- Whatman filter paper
- Electronic sensitive weighing balance
- Electronic compact weighing balance
- Foil paper
- Glass stirrer
- Spatula
- Concentration jars
- Universal bottles

- Latex examination gloves
- Thermostatic Water bath
- Spectrophotometer
- Incubator

3.3 METHOD

3.3.1 Collection of plants

The roots of *Chasmanthera dependens* were collected from Oba Isin village, Kwara State, Nigeria. The plants were identified and authenticated by Dr H.A. Akinobosun in the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Edo State, Nigeria. The specimen was deposited at the University of Benin Herbarium with voucher UBHC387.The plant parts were sliced to reduce their size, air-dried at room temperature and pulverized using a laboratory mill. The coarse powder was then sieved, using a 2 mm mesh sieve, to obtain smooth samples.

3.3.2 Preparation of extract

Precisely 3 kg of each of the pulverised plants was steeped in methanol for three days. The contents were stirred many times a day and filtered with Whatman filter

paper at the end of the third day. The filtrate was evaporated to dryness with a rotary evaporator before being freeze-dried with a freeze-dryer. The extracts were weighed, placed in an airtight container, and refrigerated at 4°C until use.

3.4 QUALITATIVE PHYTOCHEMICAL SCREENING OF CRUDE METHANOL EXTRACT OF CHASMANTHERA DEPENDENS

The methnol extract of *Chasmanthera dependens* were screened qualitatively for the presence of carbohydrates, alkaloids, tannins, flavonoids, saponins and cardiac glycosides using standard protocols (Sofowara, 1982; Trease and Evans, 1989; Harborne et al., 1998).

3.4.1 Test for alkaloids

Two millilitres (2 ml) of the filtrate were mixed with 2 drops of Dragendorff's reagent. The appearance of a rusty-brown precipitate evidenced a positive outcome.

3.4.2 Test for flavonoids (lead acetate test)

A few drops of lead acetate solution were added to 2 mL filtrate. Yellow precipitate formation showed the presence of flavonoids.

3.4.3 Test for tannins

One millilitre (1 mL) of extracts was pipetted into a test tube and boiled for five minutes. Then, droplets of a 15% ferric chloride solution were applied. The blue-black colouration indicated the existence of tannins.

3.4.4 Test for reducing sugars (Fehling's Test)

To 2 ml of filtrate was added 2 drops of Benedict's reagent (a mixture of equal volumes of Fehling's solutions A and B). The resulting solution was heated over a boiling water bath for 3 minutes. Formation of a orange or brick red precipitate shows presence of reducing sugar.

3.4.5 Test for tarpenoid (Salkowski Test)

The filtrate (5 ml) was mixed with 2 ml of chloroform and concentrated H2SO4 was carefully added drop wisely to form a layer. A positive result gives a reddish brown coloration at the interface.

3.4.6 Test for saponins

The ability of saponins to cause foaming in an aqueous solution was employed as a saponin screening test. The addition of 1 mL of extract to 5 mL of distilled water

was conducted. The resulting solution was forcefully stirred and inspected for the presence of foam, indicating saponins.

3.4.7 Test for carbohydrates (Molisch's test)

Two drops of alcoholic naphthol with a concentration of 1% were added to 2 mL of filtrate, followed by 2 mL of sulphuric acid at an angle. Positive results were shown by creating a violet ring at the interface of two liquid layers.

3.4.8 Test for phenols (ferric chloride test)

Three to four drops of ferric chloride solution were added to the extracts. The appearance of a blue-black colour suggested the existence of phenols.

3.5 QUANTIFICATION OF PHYTOCHEMICAL

3.5.1 Determination of Total Phenol

The total phenol content of the extracts was quantified by the Folin-Ciocalteau method, as described by Cicco et al. (2009).

Principle

This assay is based on the reduction of the Folin-Ciocalteau reagent (phosphosmolydate and phosphotungstate) by the phenolic compounds. The reduced Folin-Ciocalteau reagent is blue and thus detectable with a

spectrophotometer at 500 -760 nm. The intensity of the colour is directly proportional to the concentration of phenolics in the samples.

Procedure

A solution of the extract (0.5 mL) with a 1000 µg/mL concentration was added to 4.5 mL of deionized distilled water and 0.5 mL of Folin Ciocalteau's reagent (previously diluted with water 1:10, v/v), which was then added to the extract solution. After mixing the tubes, they were maintained at room temperature for 5 min, followed by adding 5 mL of 7 % sodium carbonate and 2 mL of deionized distilled water. After mixing the samples, the samples were incubated for 90 min at room temperature. A spectrophotometer measured the absorbance at 750 nm. The total phenolic content was expressed as milligrams of gallic acid equivalents (GAE) per gram of extract (mg GAE/g extract). The standard curve was prepared with gallic acid in six concentrations (10, 25, 50, 75, 100 and 150 mg /L).

3.5.2 Determination of Total Flavonoid

The total flavonoid content was estimated using the approach described by Ebrahimzadeh et al. (2008).

Principle

This is based on the reaction between flavonoids and aluminium chloride, which results in combination with maximum absorbance at 420 nm. The intensity of the colour is directly proportional to the sample's flavonoid concentration.

Procedure

Briefly, 0.5 mL of extract sample (1 mg/mL) was combined with 1.5 mL of methanol, after which 0.1 mL of 10% aluminium chloride was added, followed by 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. At room temperature, the mixture was incubated for 30 minutes. The absorbance was measured with a spectrophotometer at 415 nm. The results are reported as mg of quercetin equivalents (QE) per gramme of extract (mg QE/g extract). Six concentrations of quercetin (10, 25, 50, 75, 100, and 150 mg/L) were used to create the standard curve.

3.5.3 Determination of Tannin Content

The total tannin content was determined using a modified version of the Folin-Denis method (Polshettiwar et al., 2007).

Principle

This is based on the observation of a blue colour produced by the reduction of phosphomolybdic and phosphotungstic acid in an alkaline solution by tannin-like substances. The colour intensity is directly related to the sample's tannin concentration and is measured at 725 nm.

Procedure

Briefly, 0.5 mL of the extract (1 mg/mL) was combined with 1.25 mL of Folin Denis reagent and 2.5 mL of sodium carbonate at 10% concentration. After 30 min of room-temperature incubation, the absorbance at 760 nm was measured using a spectrophotometer. The results were reported as milligrammes of an equivalent tannic per gramme (mg TA/g extract). For the standard curve, tannic acid was prepared in six concentrations (10, 25, 50, 75, 100, and 150 mg/L).

3.5.4 Determination of Proanthocyanidin

The technique described by Sun et al. (1998) was utilized to determine proanthocyanidin.

Principle

In the butanol-HCL reagent, condensed tannins are depolymerized. At 500 nm, the colour intensity is directly proportional to the concentration of proanthocyanidins in the sample.

Procedure

0.5 mL of an extract preparation containing 1.0 mg/mL was combined with 1.5 mL of a vanillin-methanol solution containing 4% vanillin and 0.75 mL of strong hydrochloric acid. After leaving the mixture undisturbed for 15 min, the absorbance was measured at 500 nm. Results were reported in milligrammes of catechin per gramme of extract (mg CE/g extract). Catechin was prepared in six concentrations (10, 25, 50, 75, 100, and 150 mg/L) for the standard curve.

3.5.5 Diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging ability assay

The DPPH radical scavenging activity of the extracts was determined utilizing a modified version of the method described by Jain et al. (2008).

Principle

This is based on the compound's antioxidant capacity to decrease DPPH by donating hydrogen, resulting in a change in colour from deep violet to golden

yellow. The colour intensity is directly related to the sample's DPPH activity and is measured at 517 nm.

Procedure

Precisely 0.1 mL of a 0.1 mM DPPH solution in methanol was mixed with 3.0 mL of extracts in methanol containing 0.01-0.2 mg of extract per mL. The reaction mixture was combined correctly and left in the dark for 30 min. A spectrophotometer was utilised to determine the absorbance of the combination at 517 nm. Ascorbic acid was used as a standard.

DPPH radical scavenging activity (%)

$$= [(A0-A1)/ (A1)] \times 100,$$

Where A0 was the absorbance of DPPH radical + methanol; A1 was the absorbance of DPPH radical + sample extract or standard.

3.5.6 Ferric ion Reducing Antioxidant Power (FRAP) Assay

The ferric-reducing antioxidant power (FRAP) test was adapted from Benzie and Strain (1996)

Principle

It is based on the extract's ability to convert the complex of ferric tripyridyltriazine (Fe (III)-TPTZ) to ferrous tripyridyltriazine (Fe (II)-TPTZ) at low pH. Fe (II)-TPTZ's brilliant blue colour is spectrophotometrically measured at 593 nm.

Procedure

Precisely 1.5 mL of freshly prepared FRAP solution (25 millilitres of 300 millimolar acetate buffer pH 3.6, 2.5 mL of 10 mM 2,4,6-tripyridyls-triazine (TPTZ) in 40 millilitres of hydrochloric acid, and 2.5 mL of 20 mM ferric chloride solution) was added to 1 millilitre of extracts at a concentration of 1.0 mg per mL. After 30 min of incubation at 37 ° C, the absorbance of the reaction mixtures was measured at 593 nm. The calibration curve was produced using FeSO4, with ascorbic acid as the positive control. The FRAP values (in mg Fe (II) per g extract) and extract values were extrapolated from the standard curve.

3.5.7 Reducing power assay

The reducing power of the extracts was determined using the technique described by Lai et al. (2001).

Principle

This approach is based on the principle of increasing the reaction mixtures' absorbance. At 700 nm, the antioxidative chemical forms a coloured complex with potassium ferricyanide, trichloroacetic acid, and ferric chloride. An increase in absorbance shows that the antioxidant activity has increased.

Procedure

Precisely 1 mL of extracts at varying concentrations (0.1-1.0 mg per mL) in water was mixed with 2.5 mL of 0.2 molar phosphate buffer, pH 6.6, and 2.5 mL of 1% potassium ferricyanide. After 20 minutes at 50°C, 2.5 mL of 10% trichloroacetic acid was added to stop the reaction. The absorbance at 700 nm was measured spectrophotometrically after adding 2.5 mL of purified water and 0.5 mL of 0.1 % FeCl3. Increased absorbance measurements are related to a rise in reducing power. Ascorbic acid was a positive control used.

3.5.8 Total antioxidant capacity

The total antioxidant capacity of the extracts was determined using the phosphomolybdenum technique of Prieto et al. (1999).

Principle

The method relies on the extract converting molybdenum (VI) to molybdenum (V) and producing a green phosphate/molybdenum (V) complex at a pH of 5.

Procedure

An aliquot of 0.1 mL of the sample solution was mixed with 1 mL of the reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were sealed and incubated at 95 °C for 90 min in a water bath. After the samples cooled to room temperature, the absorbance at 765 nm of the combination was measured and compared to a blank. A typical blank was made by incubating 1 mL of the reagent solution with the correct amount of solvent under the same conditions as the sample. Ascorbic acid was used as reference material. The antioxidant capacity of the samples was determined in relation to ascorbic acid and expressed as mg ascorbic acid equivalent (AAE) per gramme of extract.

3.5.9 HPLC Analysis

Using a Buck 930 HPLC system (Waldbronn, Germany) equipped with a G1311C quaternary pump, a G1329B autosampler (0.1–100 L), a G1316A column oven (273–333 K), and a G1315D-DAD detector (190–950 nm), the monosaccharides

tagged with PMP were analysed. As the analytical column, a Shodex SHUGAR KS-802 column (4.6 mm, 150 mm, 5 m; Agilent) was utilised. The injection volume at 80°C was 20 L, and the eluant flow rate was 0.6 mL/min. The mobile phase A consisted of 100% acetonitrile, while the mobile phase B consisted of a mixture of distilled water and acetonitrile (90:10, v/v) in the presence of 0.045% KH2 PO4-0.05% triethylamine buffer (pH 7.5); gradient elution was performed at 94-94-88-88% B with linear decreases at 0-4-5-20 minutes. At a wavelength of 245 nm, UV detection was conducted. The phytochemicals were identified by comparing the area and mass of an internal standard to the area of the discovered phytochemicals. The concentration of different phytochemicals in micrograms per gramme.

CHAPTER FOUR

4.0 QUALITATIVE PHYTOCHEMICAL

Table 1: Qualitative phytochemical analysis of methanol extract of *C. dependens* (roots)

Phytochemicals	*C. dependens* (roots)
Alkaloids	+
Flavonoids	+
Tannins	+
Saponins	+
Terpenoids	+
Phenols	+
Reducing sugar	+
Carbohydrate	+

Table1: '+' = indicates presence of constituent

Table 2: Quantitative Phytochemical constituent of methanol extract of *C. dependens* (root)

Total Phenol (mg GAE/g extract)	199.1±1.9
Total Flavonoid (mg QE/g extract)	8.61±0.74
Total Tannin (mg TAE/g extract)	87.05±1.27
Total Proanthocyanidins (mg CE/g extract)	45.1±1.5

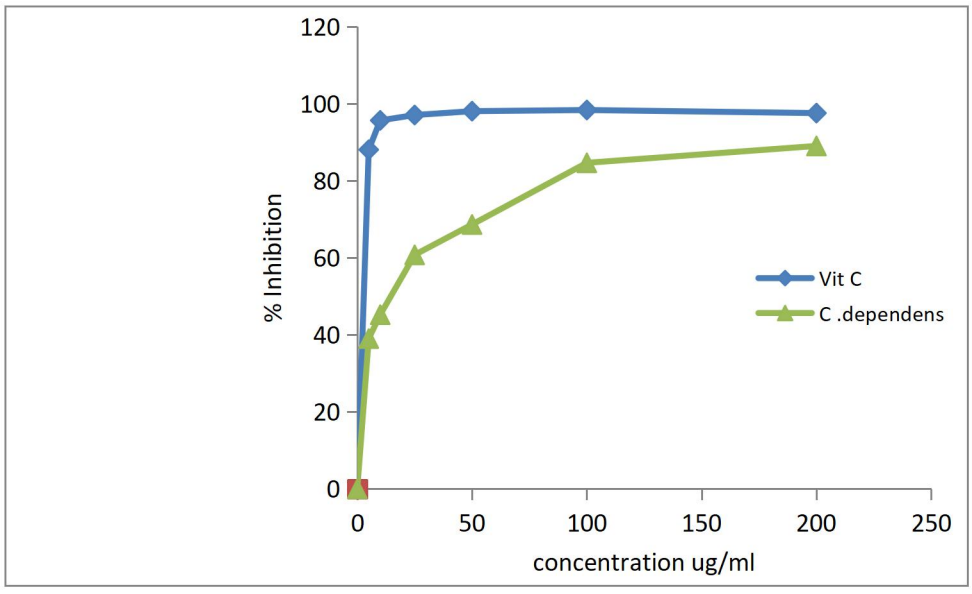


Figure 2: DPPH's radical scavenging activity of methanol extracts of *C. dependens.* Values are expressed as mean± SEM, *n* = 3/group

Table 3: DPPH IC_{50} values of extract of *C. dependens*

Sample	IC_{50} (µg/ml)
Ascorbic acid	0.02
C. dependens	16.59[a]

Data represent mean ± SEM of triplicate analysis. Different lowercase letters within the column indicate significant differences at $p \leq 0.05$.

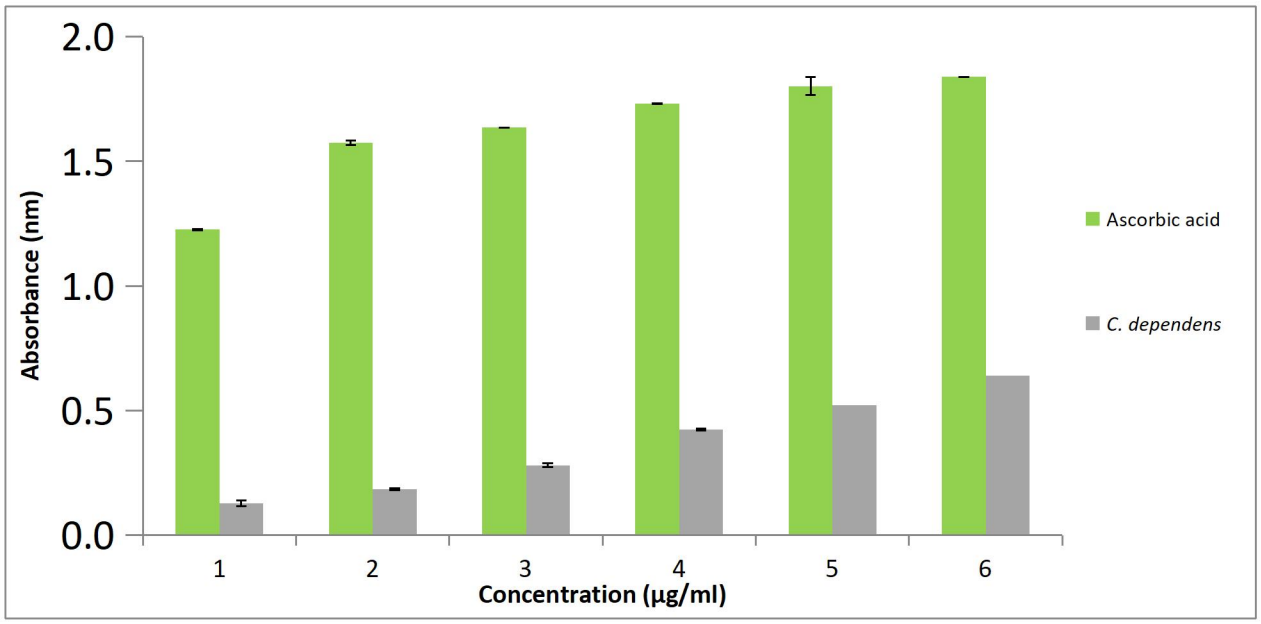


Figure 3: Reducing Potential Activity of *C. dependens.* Values are expressed as mean ± SEM, n = 3/group

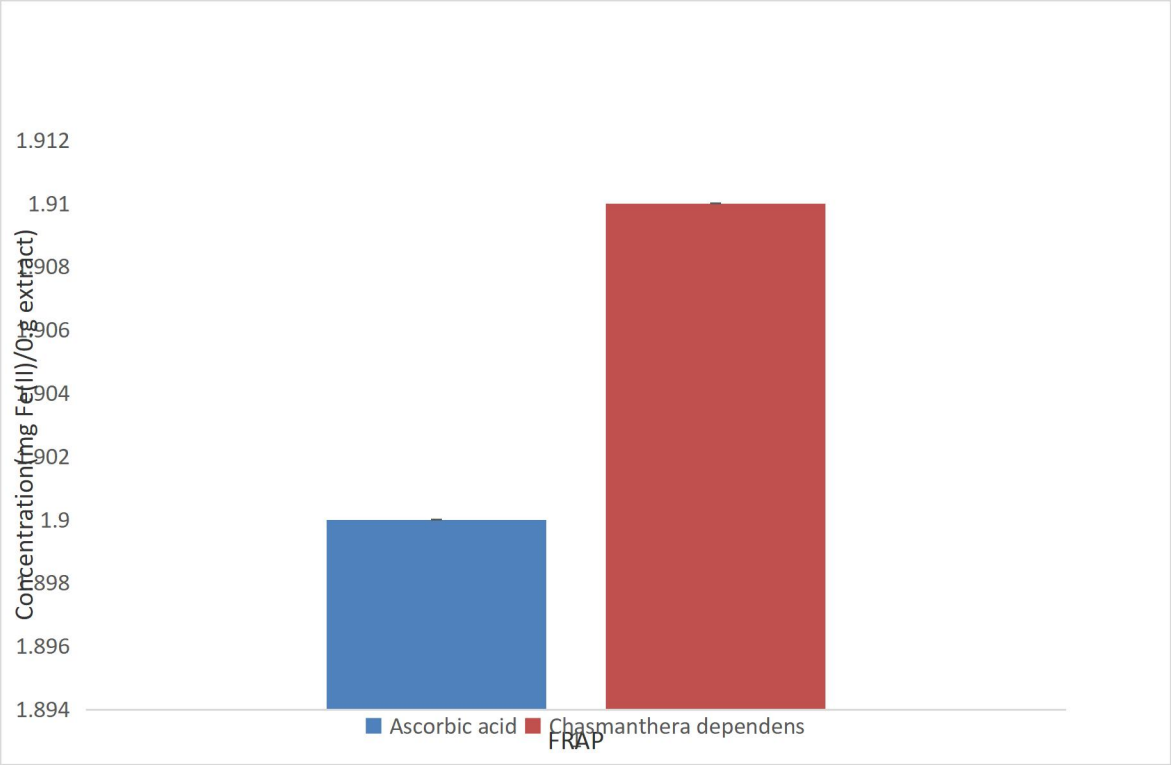
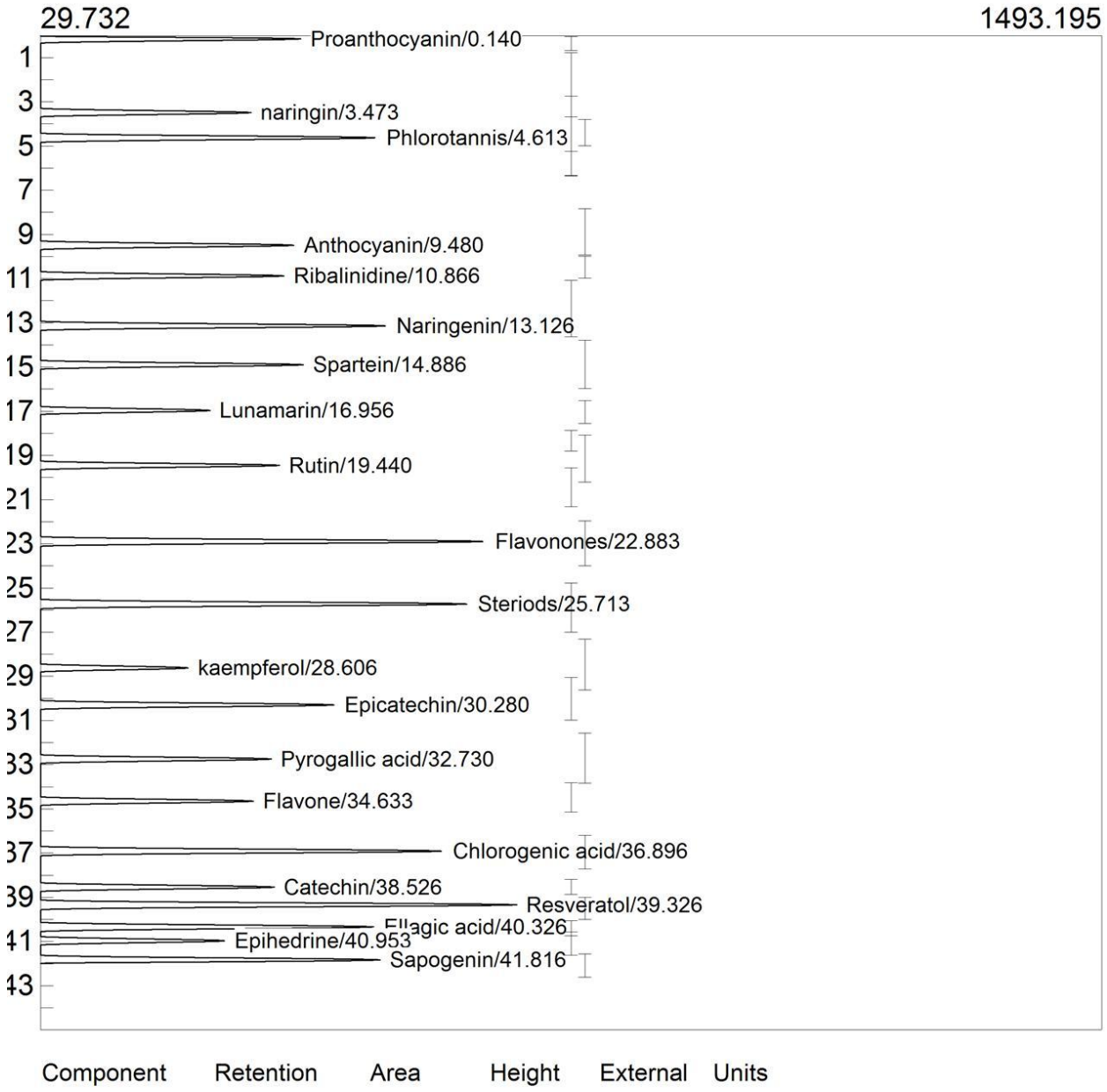


Figure 4 : Ferric acid reducing antioxidant potential (FRAP) of methanol extracts of *C. dependens.* Values are expressed as mean± SEM (n = 3). Different lowercase letters represent significant difference between means at $p < 0.05$.

Lab name: Springboard Lab Awka
Client: Omorede
Method: Syringe Injection
Column: RESTEK 15METER MXT-1
Carrier: Methanol
Data file: Omorede C.D phytochemical analysis 02.CHR ()
Sample: Phytochemistry
Comments: TYPE YOUR COMMENTS HERE



Type of phytochemicals	Phytochemical	Concentration (µg/ml)	Reported Activity
Flavonoids	Proanthocyanin	5.74	Antioxidant (Lai et al., 2018)
	Anthocyanin	6.25	Anti-diabetic, anticancer, antimicrobial (Song et al., 2017)
	Flavonones	14.12	Antioxidant, antihyperlipidermic and anti-inflammatory properties (Pache et al., 2016)
	Naringin	5.09	Anti-inflammatory and anticancer activities (Chen et al.,2016)
	Flavone	5.09	Antioxidant, antimicrobial and anti-inflammatory (Catarino et al., 2015)
		5.68	Antimicrobial,

	Rutin		gastroprotective and anti-allergic agent (Negahdari et al., 20210)
	Kaempferol	2.04	Anticancer, anti-inflammatory activities (Calderon-Montano et al 2011)
	Epicatechin	8.32	Anticancer and anti-diabetic
	Naringenin	2.74	Antioxidant, anticancer, anti-inflammatory and prevention of cardiovascular diseases (Yao et al., 2004)
	Catechin	0.97	Antioxidant, antiviral, anti-allergic and anticancer properties (Spizzirri et al., 2009)
Tananins	phlorotanins	3.94	Antihypertensive, radioprotective and anti-allergic activities (Wijesekara et al., 2010)

Saponins	Sapogenin	9.66	Hypocholesterolemic (Xu et al., 2021)
Alkaloids	Ribalinidine	4.01	A ntioxidant (rahmani et al., 2010)
	Lunamarin	4.92	Anticancer, anti-amoebic, anti-estrogenic and immunomodulatory activity (Ojukwu et al., 2021)
	Spartein	8.97	Anticonvulsant drug (Villalpando-Vargas and Medina-Ceja, 2016)
	Steriods	13.60	Antitumor and anti-inflammatory (patel and savjani,2015)
	epihedrine	4.59	Antimicrobial, CNS stimulant (tulgaar et al 2018)

Other phenols	Resveratol	6.65	Antioxidant potential (Salehi et al., 2018)
	Ellagic acid	3-60	Anticancer and anti-inflammatory (Rios et al 2018)
	Pyrogallic acid	5.95	Antibacterial and antioxidant (Lima et al., 2016)
	Chlorogenic acid	11.80	Antioxidant and DNA damage protective activities (Li et al 2021)

CHAPTER FIVE

5.0 DISCUSSION AND CONCULSION

5.1 Discussion

Plant secondary metabolites such as polyphenols are widely distributed in the plant kingdom and have been reported to protect the cell constituents against destructive oxidative damage associated with various degenerative diseases (Olorunnisola *et al*., 2012). Their biological activities are believed to be due to their redox properties which play important roles in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Olorunnisola *et al*., 2012; Seladji *et al*., 2014). Free radicals and reactive oxygen species are constantly formed in the human body during norm cellular metabolism for instance during energy production in the mitochondria electron transport chain, arachidonic acid metabolism, Ovulation, fertilization and in xenobiotic metabolism (Hallwell and Guheridge, 2007). Organisms are blessed endogenous antioxidant defense systems which are capable of countering against this adverse reactions of free radicals (Sivakrishnam and Kottao-Muthus, 2013). Much attention is being focused on the use of anitoxidant to inhibit and protect damage due to free radicals and reactive oxygen species.

Many plants of which *Chasmanthera dependens* is inclusive are recognized as sources of natural antioxidant like phenolic acids, flavonoids, tanins and some other metabolites. Studies have shown that many of these antioxidant compounds possesss anit-therosderotic, anti-inflammatory, antitumor, anti- mutagenic, antibacterial,anit- carcinogenic and antiviral activities (Sala et al., 2005; Rice-Evans, 1995). The use of DPPH radical method is widely used to evaluate the free radicals scavenging ability of samples (Lee *et al*., 2003). The DPPH method is sensitive, rapid, reproducible and requires simple conventional laboratory equipment for accessing antioxidant activity of sample (Du *et al*., 2009) The reduction of DPPH radicals by antioxidant can be measured at 517nm.

The IC50 value of this amount of anitoxidant required to reduce the DDPH radical concentration by 50%. The IC50 value is inversely proportional to the anitoxidant activity (Chandra *et al.*, 2011). From the result a low IC50 was obtained for the methanol extract of *Chasmanthera dependens*, thus suggesting that *Chasmanthera dependens* may possess strong anitoxidant capacity, hence a good source of natural antioxidant. Tanins helping healing (Okwu and Okwu, 2004; Oliver, 2000). Flavonoids reduces the risk of heart disease. Saponins also promotes healing of wound (Okwu and Okwu, 2004). Alkaloids are medicinally useful, possessing analgesic, antiplasmodic and bactericidal effects.

The qualitative analysis of methanol extract of *Chasmanthera dependens* root showed high content of Phenol as well as Saponins, Terpeniods and Alkaloids

Quantitative analysis revealed that Phenol showed the highest concentration in methanol extract of *Chasmanthera dependens* and flavonoids had the least concentration.

In the study there was progressive increase in the reductive potential as the concentration was increased the reducing power of compound measure as a significant indicator to it's potential anitoxidant activity because the presence of reluctant such as antioxidants substances in the sample causes the reduction of ferric cyanidine to the ferric form (Rahman *et al*., 2013). It provide insight into the reductive and hence antioxidant property.

The FRAP test is based on the ability of antioxidants present in the test extracts to reduce $Fe^{3+}$to Fe^{2+}. From my result (fig 3), methanol extract showed high degree of ferric reducing antioxidant power (FRAP).

5.2 Conclusion

Qualitative analysis of chasmanthera dependens showed that the methanol extract of *Chasmanthera dependens* contained alkoloids, saponins, tanins, Terpeniods as well as reducing sugar. A synergistic relationship among the Phytochemical is

assumed to be responsible for the antioxidant property and overall beneficial effect derived from *Chasmanthera dependens*.

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