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Preliminary Phytochemical Screening, Antioxidant and Antimicrobial Activities of Crude Leaf Extracts of *Pteleopsis Habeensis* Aubrev. Ex. Keay (Combretaceae)

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ABSTRACT

Pteleopsis habeensis (Aubrev ex Keay), which belongs to the family Combretaceae, is known as Lalen giwa in Hausa language in northern part of Nigeria of Sub Saharan Africa. The area of distribution of *Pteleopsis habeensis* is restricted to only a few regions: the Bandiagara escarpments in Mali (with the plant population possibly extending into Burkina Faso), the Akosombo and Bui regions in Ghana, and the Yankari Game Reserve and it immediate surroundings in Nigeria. The shrub is use for the treatment of malaria fever, stomach ache, Aphrodisiac and in the destruction of tumours. The leaves of *Pteleopsis habeensis* were collected from faculty of pharmacy medicinal plant garden University of Maiduguri Borno, Nigeria, in March 2020. The plant was authenticated at the department of biological sciences, University of Maiduguri Borno, Nigeria. Phytochemical screening of crude leaf extract of *Pteleopsis habeensis* revealed the presence of alkaloids, flavonoids, tannins, triterpinoids, steroids and cardiac glycosides. The crude leaf Methanol and *n*-Butanol extracts of *Pteleopsis habeensis* exhibited antimicrobial activities against *Escherichia coli, Pseudomonas aeroginosa, Staphylococcus aureus,* Streptococcus spp and the fungus *Candida albican* using disc diffusion method and broth dilution methods. The fungus *Candida albican*, showed the highest sensitivity to methanol extract with zone of inhibition 14 mm at 100mg/ml compared to it sensitivity to *n*-Butanol extract with zone of inhibition 10mm at 100mg/ml. The Minimum Inhibitory concentration is 12.5mg/ml for Methanol extract and 25mg/ml for *n*-Butanol extract. The evaluation of the antioxidant activity of the *n*-Butanol and Methanol crude extracts was carried out in vitro through the radical model DPPH, and the antioxidant capacity of the two extracts was measured based on their Percentage scavenging activity and IC_{50} concentration which corresponds to the concentration of the extracts capable of reducing the initial DPPH absorbance by 50%. The DPPH Assay showed high free radical scavenging activity of the extract that is comparable to Ascorbic acid. The percentage scarvenging activity of Methanol extract was higher than that of the *n*-Butanol extract in the DPPH assay. This study has therefore showed that *Pteleopsis habeensis* crude leaf extracts contains phytochemicals, has antioxidant and antimicrobial activity, hence a potential source of a candidate drug whose bioactive constituents can be isolated for pharmaceutical use.

Key words: Pteleopsis habeensis, Phytochemicals, Antimicrobial activity, Antioxidant activity, DPPH assay, Candida albican

INTRODUCTION

Plants have been used as medicine for as long as humans have been on Earth. Around 3000 BC, the first documented use of plants in medicine was for the treatment of leprosy using oil derived from the *Hydnocarpus gaertn* plant. Egypt was already using other plants, such as castor seed oil (*Ricinus communis* L.) and opium poppies (*Papaver somniferum* L.). Chinese pharmacopeia, written circa 1122 BC, and the Eber Papyrus, written approximately 1500 BC, both document the earliest Egyptian usage of herbs and plants in traditional medicine (Mohammad, 2014). More than 1,400 dispensaries in India still practice the Indian Ayuveda, which was first reported in 1,200 BC with a list of 127 herbs. The use of plants as medicine was documented in the annals of later civilizations in Greece, Rome, Arabia, Europe, Africa, Australia, and America. Our ancestors in Nigeria

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have long used plants to cure a wide range of common illnesses, from pregnancy and childbirth to adulthood (Owunobi, 1989).

The majority of the plants that these ancient societies acknowledged as having therapeutic qualities are still in use today (Soladoye *et al.,* 2012). Claimed that even if the development of synthetic pharmaceuticals has increased, natural plant drug ingredients are still collected in great numbers and have a substantial economic impact worldwide. Among other sources, plants continue to be a reliable source of bioactive chemicals with therapeutic benefits. They have also been the subject of the greatest research and exploitation due to their bioactive medicinal components. The use of plants in alternative medicine has gained international interest in recent years. Traditional medicine is used in Latin American, Asian, and African nations to assist address some of their basic medical needs. Millions of individuals in the United States and Europe are returning to traditional herbal therapy to prevent or treat a variety of illnesses, despite the fact that their health and longevity have significantly improved (WHO, 2006). According to the World Health Organization, up to 80% of people in Africa currently receive their primary medical care through traditional medicine (WHO, 2006). Though few have been documented, many African plants are employed as antibacterial agents in traditional medicine. "The realization that traditional medicine, which has been taken for granted and rejected for decades, has a crucial role to play in making affordable health care delivery system available to the rational development of drugs that are of more specific efficacies and fewer side effects than synthesized drugs," is evident from the startling rate at which traditional medicine is now used by all segments of society, including the rich, the poor, the educated, and the uneducated (Owunobi, 1989; Yakubu and Musa, 2012). Researchers are looking more closely at medicinal plants as a result of traditional medicine's acceptability as an alternative to conventional medical care. As a result, scientists are increasingly more interested in studying therapeutic plants. According to (Sule *et al.*, 2011) large body of evidence has accumulated to demonstrate the promising potential of medicinal plants used in various traditional, complementary and alternate systems of treatment of human diseases. Microbiologists have recently become interested in certain higher plant products and are looking for phytochemicals to use as antimicrobials; these plant products would be safe for human health and biodegradable (Kumar *et al.,* 2008; Wang et al., 2010) in (Sule *et al*., 2011).

Pteleopsis habeensis (Aubrev ex Keay), which belongs to the family Combretaceae, is known as Lalen giwa in Hausa language in northern part of Nigeria of Sub Saharan Africa. The tiny genus *Pteleopsis* contains roughly ten species that are found in three locations throughout equatorial Africa (Mohammed, 2014). The area of distribution of *Pteleopsis habeensis* is restricted to only a few regions: the Bandiagara escarpments in Mali (with the plant population possibly extending into Burkina Faso), the Akosombo and Bui regions in Ghana, and the Yankari Game Reserve and it’s immediate surroundings in Nigeria. It is possibly also present in Benin. (Oyen, 2010).

In Mali *Pteleopsis habeensis* forms low thickets with Combretum micranthum G.Don. In the Yankari Game Reserve in Nigeria it covers several square kilometers, and forms pure stands of coppice-like woodland of numerous thin stems, uniform in height and girth, forming a closed canopy 8–15 m high (Mohammad, 2014). Along watercourses, the plant *Pteleopsis habeensis* grows on sandstone, usually on gravelly slopes. Despite being linked to stream beds, the environment is extremely dry because to the sandy, well-drained soils, and the species is never seen in or close to the streambed itself (Hawthorne, 1998).

Medicinal plant

Many plant species employed in herbalism (herbal medicine) are included in the phrase "medical plant." It is the study of and application of plants for herbal medicine. The uses of medicinal plants include food, medicine, fragrances, and other spiritual pursuits (Mahtab, 2016).

People have been searching for natural remedies for their illnesses since ancient times. Like with animals, the use of therapeutic plants was initially instinctual (Stojanoski, 1999). Everything was based on experience because, at the time, there was insufficient information about the cause of the ailment or about which plant and how to use it as a remedy (Akhileshwar, 2018).

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Traditional medicine

According to the World Health Organization, traditional medicine is the entirety of knowledge, expertise, and methods derived from indigenous cultural theories, beliefs, and experiences, whether or not they can be explained. It is used to maintain health as well as to prevent, diagnose, treat, or improve physical and mental illness (WHO, 2013; Tilburt *et al.,* 2008). Because of its natural origin and fewer adverse effects, traditional herbal medicine and its preparations have been used extensively for thousands of years in both developed and developing nations (Anjoo, 2012). Originally, these remedies were unrefined herbal formulations including tinctures, teas, poultices, powders, and other concoctions (Bulunasa, 2005). The use of plants for healing purposes predates human history and forms the origin of much modern medicine. One issue is that substances that were formerly used in traditional medicine to treat symptoms are now utilized in industrialized nations as part of initiatives for disease prevention or health promotion; as a result, chronic exposure has supplanted acute therapy (Allison *et al.,* 2001). This implies that a claim that a product is safe based on "thousands of years of evidence" might not hold true given how the product is now being utilized. This does not specifically imply that a substance is dangerous; rather, it indicates that safety in the contemporary setting cannot be taken for granted.

About 75–80% of people worldwide still rely on herbal medicine for primary healthcare, primarily in underdeveloped nations. This is mostly due to the widespread perception that, aside from being affordable and readily available locally, herbal medications have no negative side effects. The World Health Organization (WHO) reports that the use of herbal treatments is two to three times more common worldwide than that of conventional medications (Pal *et al.,* 2003).

Phytochemistry

Phytochemicals are chemical substances that are created during plant growth as a result of metabolic reactions (Breslin, 2017). Chemicals produced by plants through primary or secondary metabolism are known as phytochemicals (from the Greek phyto, meaning "plant") (Molyneux, *et al.,* 2007; Harborne *et al.,* 1999). Under stress, human bodies produce more reactive oxygen species (ROS) (such as superoxide anion radicals, hydroxyl radicals, and hydrogen peroxide) but fewer enzymatic antioxidants (such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase) and nonenzymatic antioxidants (such as ascorbic acid (vitamin C), and tocopherol (vitamin E) (Manjula and Ammani, 2012). An imbalance could result from the above causing damage to the body cell (Aruoma, 1998; Bhatia *et al.,* 2003) and other health challenges (Steer, 2002). Using these natural plant antioxidants has greatly improved preventive medicine. Alkaloids, amines, betalains, vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, and other secondary metabolites with strong antioxidant activity are among the many compounds found in plants that scavenge free radicals (Manjula and Ammani, 2012). The majority of phytochemicals are antioxidants, which basically lessen tissue damage brought on by physiological processes. They contribute to plant development or defense against rivals, diseases, or predators and typically exhibit biological activity in the plant host (Harborne, 1973).

Plant phenolics are a significant class of chemicals that function as principal antioxidants or free scavengers. Flavonoids and tannins are examples of phenolic compounds (Potterat, 1977).

Alkaloids are used in medicine for a variety of purposes, including antimalarial, cardiovascular, anti-cancer, and anti-poisoning properties. According to reports, saponins have anticancer qualities (Evans, 2002). Additionally, saponins are triterpene and steroid glycosides with hypertensive and cardiodepressant effects (Olaleye, 2007).

Natural cardioactive medications called cardiac glycosides are used to treat cardiac arrhythmia and congestive heart failure (Singh *et al.,* 2010).

It has been documented that seasonal and geographic fluctuations impact the phytochemical components of plants, which in turn impacts the outcome of antimicrobial activity (Van, 2008; Ncube *et al.,* 2005)

Antioxidant

Antioxidants are defined as "substances capable of delaying, retarding, or preventing oxidation processes" (Hudson, 2012). People use plants to cure and alleviate a variety of illnesses. These days, traditional medicines

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are utilized in place of contemporary medication in a number of nations worldwide (Ramawat, 2008). Researchers are presently looking into the potential of countless medicinal plants with antioxidant activity to combat a wide range of illnesses, including cancer, Alzheimer's disease, atherosclerosis, cerebral cardiovascular events, diabetes, and hypertension, to name a few (Liu, 2013; Devasagayam, 2004). In order to provide lead molecules for modern design and synthesis, plants act as reservoirs for potentially useful chemical compounds that can be employed to make medications (Arun, 2011). Since oxygen is the final electron acceptor in the electron flow system that generates energy in the form of ATP, oxidation—the movement of electrons from one atom to another—represents a crucial aspect of aerobic life and our metabolism (Davies, 1995). But when the electron flow becomes uncoupled (transfer of unpaired single electrons), issues could occur and free radicals could be produced. Examples of oxygen-centered free radicals, known as reactive oxygen species (ROS), include superoxide (O2•-), peroxyl (ROO•), alkoxyl (RO•), hydroxyl (HO•), and nitric oxide (NO•). The hydroxyl (half-life of 10-9 s) and the alkoxyl (half-life of seconds) free radicals are very reactive and rapidly attack the molecules in nearby cells, and probably the damage caused by them is unavoidable and is dealt with by repair processes. On the other hand, the superoxide anion, lipid hydroperoxides, and nitric oxide are less reactive. In addition to these ROS radicals, in living organisms there are other ROS nonradicals, such as the singlet oxygen (O_2), hydrogen peroxide (H_2O_2), and hypochlorous acid (HOCl). Humans have evolved with antioxidant systems to protect against free radicals. These systems contain both endogenous (made by the body) and exogenous (obtained from the diet) antioxidants (Pier-Giorgio, 2000). The first include (a) enzymatic defenses, such as Se-glutathione peroxidase, catalase, and superoxide dismutase, which metabolize superoxide, hydrogen peroxide, and lipid peroxides, thus preventing most of the formation of the toxic HO•, and (b) nonenzymatic defenses, such as glutathione, histidine-peptides, the iron-binding proteins transferrin and ferritin, dihydrolipoic acid, reduced Coenzyme Q10, melatonin, urate, and plasma protein thiols, with the last two accounting for the major contribution to the radical-trapping capacity of plasma. Since the diverse defenses target distinct species at distinct cellular compartments, they are complementary to one another. However, some ROS still manage to get out and inflict harm in spite of these defense antioxidants, which can either scavenge free radicals and chain propagation or suppress free radical generation and chain initiation. Therefore, repair antioxidants that may fix damage and are based on proteases, lipases, transferases, and DNA repair enzymes also contribute to the body's antioxidant system (Warma, 1995). Dietary antioxidants are required to reduce the cumulative effects of oxidative damage over the course of a person's life because of the inadequacy of our endogenous defense systems and the existence of certain physiopathological conditions (cigarette smoke, air pollutants, UV radiation, high polyunsaturated fatty acid diet, inflammation, ischemia/reperfusion, etc.) in which ROS are produced in excess and at the wrong time and place. Vitamins C, E, A, and carotenoids are well-known antioxidants that come from food and have been thoroughly researched (Sies, 1997). In addition to these antioxidant vitamins, additional compounds found in plants may be responsible for some of the health advantages of eating fruits and vegetables. Plant polyphenols have been shown to be a significant class of defensive antioxidants over the last ten years. These substances, which include phenols, phenolic acids, flavonoids, tannins, and lignans, are present in almost all plant diets, frequently in high concentrations (Pier-Giorgio, 2000)

Antimicrobial

An antimicrobial is a substance that either eradicates or inhibits the growth of bacteria. The most frequent causes of infections are a range of bacterial etiologic agents, including Salmonella species, *Staphylococcus aureus*, and pathogenic *Escherichia coli*. Drug resistance to human pathogenic microorganisms has been widely reported worldwide in recent years (Piddock, 1989; Singh, 1992; Mulligen, 1993). Because of the ongoing usage of antibiotics, microorganisms have developed resistance. Plants are evaluated for their antibacterial activity in a number of steps. In order to prevent needless waste of time and resources, choosing plants for antimicrobial purposes is essential (Van, 2008).

Most antimicrobial screening studies on Nigerian plants tested the leaves, although a small number also examined the root, stem bark, fruit, and/or seed of the chosen plant or plants. In antimicrobial research, the extraction procedure is crucial since it greatly influences the study's outcome (Nasir *et al.*, 2015; Ncube *et al.*, 2005)

Several antimicrobial test techniques were used in the review of more than 400 papers on the antibacterial examination of Nigerian medicinal plants. The two most popular techniques for examining the antimicrobial

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activity of Nigerian medicinal plants are the minimum inhibitory concentration (MIC) and Agar well diffusion (AWD) assays. These techniques were employed from 1971 to 2016. A quantitative technique for determining antimicrobial activity, the MIC assay works on the basis of exposing a test organism to a range of test substance dilutions (Van, 2008; Nasir *et al.,* 2015; Balouiri *et al.,* 2016). The minimum inhibitory concentration (MIC) of an antimicrobial drug that, in known circumstances, inhibits the visible development of a microorganism (Nasir *et al.,* 2015; Balouiri *et al.,* 2016). Assays that use MIC methods, like broth dilution, agar dilution, and micro (microtitre plates) and macro test tubes, are commonly used and recognized standards for determining an organism's susceptibility to inhibitors (Van, 2008). The majority of articles on Nigerian plants—58.1% for extract and 1.9% for essential oil—support this. Perhaps because it shows the concentration of the plant extract that has the greatest microbiostatic effect on the test organisms, the AWD is a commonly employed assay technique (Nasir *et al.,* 2015; Balouiri *et al.,* 2016).

Taxonomical Description of the plant

Scientific classification of the plant

Kingdom - Plantae

Phylum - Tracheophyta

Class - Magnoliopsida

Order - Myrtales

Family- Combretaceae

Genus - *Pteleopsis*

Specie- *habeensis*

Botanical name - *Pteleopsis haheensis*

Aubrev. ex. Keay (Hawthorne, 1998)

Local names: Nigeria (Hausa): Lállèn giíwaá

Mali (Dogon): Guan, (Manding-maninka): kolobe (Burkill, 1985).

Synonym: *Terminalia habeensis* (Aubrev. ex. Keay) (Gere, 2012)

The family Combreataceae

Members of the combretaceae family are significant evergreen trees that are found throughout Africa. They are also widely used in traditional medicine in Sudan and Africa to cure a variety of illnesses (Ikram *et al.,* 2015).

Chemisty of the family Combretaceae

A wealth of different chemicals with possible medicinal uses can be found in the kingdom of plants (Ghani, 1986). Nigeria is home to a large number of Combretaceae species, which are used in traditional medicine to treat respiratory conditions like TB, hemoptysis, pneumonia, pulmonary disorders, cough, hay fever, asthma, and catarrh (Mann *et al.,* 2007) and other human diseases (Mann *et al.,* 2009).

Here in Nigeria leaf, stem bark and root essential oils were obtained by hydro-distillation. 88% leaf oil comprised of eleven compounds, with abundance of z-9octadecenoic acid (Adamu *et al.,* 2013), n-hexadecanoic acid (Ahmad *et al.,* 2013), n-octadecanoic acid (Barku *et al.,* 2013), methyl-7E-7-octadecenoate (Akanbi *et al.,* 2012), and methylhexadecanoate (Joulain *et al.,* 1998). Thirteen compounds amount to 80% of the stem bark oil, its significant compounds being z-9-octadecenoic acid (Moronkola *et al.,* 2014), n-hexadecanoic acid (Akanbi *et*

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al., 2012), methyl-9z-octadecenoate (Barku *et al.*, 2013), methylhexadecanoate (Joulain *et al.*, 1998) and eicosane (Mann *et al.*, 2009). Fourteen compounds make-up 91% of root oil, dominated by methyl-7E-7-octadecenoate (Moronkola *et al.*, 2014), n-hexadecanoic acid (Mann *et al.*, 2009), methyl linoleate (Barku *et al*., 2013), z-9-octadecenoic acid (Moronkola *et al.*, 2014) and methylhexadecanoate (Joulain *et al.*, 1998). Leaf, stem bark and root oils are characterized by the following classes of compounds respectively (%): fatty acids (Moronkola *et al.*, 2014); esters (Mann *et al.*, 2009; Barku *et al.*, 2013); hydrocarbons (Moronkola *et al.*,2014; Akanbi *et al.*, 201); leaf and root oils contain terpenoids (Akanbi *et al.*, 2012); dl-arabinose (sugar) is in stem bark oil. Methylhexadecanoate and hexadecanoic acid are common to the three oils. They can serve as chemotaxonomic markers characteristic for this species.

Figure I. *Pteleopsis habeensis* plant from medicinal plant garden Faculty of Pharmacy, University of Maiduguri.

Botanical description of the plant

Pteleopsis habeensis Aubrev. ex Keay is a tiny, twisted tree or straggling shrub with a dense, rounded crown. The branchlets are slightly hairy and the bark is grey-brown, fibrous, and flaking off even in young branches. The inner bark is pale brown. Leaves subopposite, petiole 1-4mm long, hairy; blade ovate, c. 3-6cm x 1.5-2.5cm, base rounded or obtuse, apex obtuse or obtusely acuminate, purplish at the start of the dry season, hairy when young, later glabrous or slightly pubescent, pinnately veined with 5-7 pairs of only slightly prominent secondary veins merging near the margin, small veins reticulate. The pseudo-umbel inflorescence is 1.5–2 cm in diameter. White flowers. Fruit is quite thin and around 10 mm long. The tiny genus *Pteleopsis* has roughly ten species, all of which are found in tropical Africa (Brink *et al*. 2012)

Ethnomedicinal uses of the plant

In the northern region of Nigeria, sub-Saharan Africa, a plant called *Pteleopsis habeensis* (Aubrev. ex Keay), which is a member of the combretaceae family, is referred to as lallen giwa in Hausa. There are roughly ten species in the tiny genus *P. habeensis*, and they are found in three different locations throughout equatorial Africa. This significant medicinal plant is indigenous to Ghana, Mali, and Nigeria (Mohammed, 2014). The plant is used as an aphrodisiac, to relieve stomachaches, to treat malaria, and to destroy tumors (Abdullahi *et al*., 2003; Aliyu *et al*., 2017).

Previous studies on the plant

The plant is used to treat a variety of ailments. In the northern region of Nigeria, *Pteleopsis habeensis* is frequently used to treat a variety of illnesses. The presence of cardiac glycosides, alkaloids, flavonoids, tannins, saponins, steroids, and triterpenes was revealed by phytochemical screening of the crude methanolic extract of *Pteleopsis habeensis* leaves (Muhammad, 2014). Another study's phytochemical results showed that the crude

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methanol extract of *P. habeensis* contains terpenoids, alkaloids, flavonoids, tannins, anthraquinones, saponins, steroids, and cardiac glycosides (Aliyu *et al*., 2017). The antimicrobial screening's findings demonstrated the antibacterial properties of *Pteleopsis habeensis* crude methanol stem bark extract against both gram-positive and gram-negative bacteria (Muhammad, 2014). Muhammad claimed that the same study had no antifungal impact on the examined fungus, *Candida albicans*. However, it was shown that *Escherichia coli* were somewhat more susceptible to the crude extract (Muhammad, 2014).

The DPPH assay showed high free radical scavenging activity of the extract that is comparable to ascorbic acid (Aliyu *et al*., 2017). These phytoconstituents are known to have demonstrated antioxidant and antibacterial properties earlier (Cowan, 1999). Such phenolic chemicals indicate the plant's potential for therapeutic use (Aliyu *et al*., 2017).

According to their research, bioactive phytochemicals are present, and all Pteleopsis species exhibit antioxidant and antibacterial properties (Baba-Moussa *et al*., 1999). The plant's antimalarial, anticancer, and antidiarrheal properties may be attributed to these compounds, which also support the plant's historic medicinal use. The components of the *Pteleopsis habeensis* extracts were identified and separated by thin layer chromatography (Aliyu *et al*., 2017).

MATERIALS AND METHODS

Materials

They include: Beaker, Cotton wool, Filter paper, Conical flask, Glass funnel, Spatula, Test tubes, Petri dish, Stainless steel tray, Rotatory evaluator, Syringes, Weighing balance,
Pestle and mortar, Hand glove, Autoclave, Wire loop, Incubator, Meter rule, and UV Spectrophotometer.

Reagents/ Solutions/Solvents

Dragendroff's reagent, Molish's reagent, Chloroform, Butanol, Methanol, N-Hexane, Acetic anhydride, Concentrated Sulphuric acid, Concentrated Hydrochloric acid, Sodium hydroxide, Distilled water, Ferric chloride, Lead sub-acetate, Ammonia, Mayer's reagent, Glacial acetic acid, Fehling's solution, Water bath, Wagner's reagent, and Diphenylpicrylhydrazyl (DPPH).

Pathogens

The bacteria used include: Escherichia coli, Pseudomonas aeroginosa, Staphylococcus aureus and Streptococcus pyogenes. The fungus used was Candida albican

Methodology

Plant collection, Identification and Preparation of Plant Samples

The leaf of the plant *Pteleopsis habeensis* was collected from Faculty of Pharmacy medicinal plant garden University of Maiduguri and surrounding area of Borno State and identified by a Taxonomist, Professor S. Sanusi, Biological science department, University of Maiduguri, Borno State. Plant part collected was shade dried at room temperature for two weeks then the dried leaf was grounded using wooden pestle and mortar to fine powder and then stored properly for further use.

Extraction of plant material

A 500g of the leaf of *Pteleopsis habeensis* was extracted successively using maceration method with *n*-Hexane followed by chloroform then *n*-Butanol and Methanol respectively in a glass jar each for 3 days (72 hours), at room temperature with gradual agitation. The extracts were filtered and evaporated to dryness on a stainless steel tray at room temperature to obtain the crude extract which was stored properly for further use.

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Preliminary Phytochemical Screening

Little quantity of the extracts were subjected to preliminary phytochemical screening using standard methods, as outlined below:

Test for Saponins

Frothing Test: About 0.5 g of the extract was shaken with water in a test tube followed by warming on a water bath. Frothing which persist on warming was taken as an evidence for the presence of saponins (Sofowora, 1993).

Fehling Test: To small portion of the extract in a test tube, 5 ml of an equal mixture of Fehling solution A and B was added and boiled on a water bath; brick red precipitate which indicate presence of saponin glycosides (Evans, 2002).

Test for Steroid/ Triterpene

Lieberman-Buchard Test: a small portion of the extract was dissolved in chloroform. Equal volume of acetic anhydride was added, followed by concentrated sulphuric acid down the side of the test tube. The mixture was observed for the presence of a brown ring at the interphase which indicates the presence of steroid/triterpene (Evans, 2002).

Salkowski Test: a small quantity of the extract was dissolved in 1 ml chloroform and to it 1 ml of concentrated sulphuric acid was added down the side of the test tube. Formation of red coloration at the interphase was taken as an indication for the presence of steroid (Sofowora, 1993).

Test for Flavonoids

Ferric chloride Test: 0.5 g of the extract was stirred with 10 ml of distilled water and filtered. Two drops of 1% ferric chloride was added to 2 ml of the filtrate. Formation of a blue-black or green or blue-green precipitates indicates the presence of flavonoid (Evans, 2002)

Sodium hydroxide Test: Few quantity of the extract was dissolved in water and filtered; 2 ml of 10% aqueous sodium hydroxide solution was then added. The solution was observed for the presence of yellow color, a change in color from yellow to colorless on addition of dilute hydrochloric acid indicates the presence of flavonoids (Evans, 2002).

Test for Tannins

Ferric chloride Test: 0.5 g of the extract was stirred with 10 ml distilled water and filtered. Two drops of 1% ferric chloride solution was added to 2 ml of the filtrate. Formation of a blue-black (hydrolysable/gallitannins) or green or blue-green (condensed/cathehic tannins) precipitate indicates the presence of tannins (Evans, 2002).

Lead sub-acetate Test: to a small quantity of the extracts, three drops of lead sub-acetate solution were added. The solution was observed for the presence of green precipitate which indicates the presence of tannins (Evans, 2002).

Test for Alkaloids

About 0.5 g of the extract was stirred with 5 ml of 1% aqueous hydrochloric acid on a water bath and filtered. Ammonia solution was added to the filtrate until it is basic, then chloroform was added shake gently to allow separation. The chloroform layer was collected, then dilute HCl was added shake gently to separation and the aqueous layer was divided into three. To the first portion, few drops of freshly prepared Dragendorff's reagent was added and observed for formation of orange to brownish precipitates. To the second, one drop of Mayer's reagent was added and observed for formation of white to yellowish or cream color precipitates. To the third,

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1ml of Wagner's reagent was added to give a brown or reddish or reddish-brown precipitates, in the presence of alkaloids (Evans, 2002).

Test for Cardiac Glycosides

Keller-Killiani Test: About 0.5 g of the extracts was dissolved in 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This will then be under layered with 1 ml of concentrated sulphuric acid. A brown ring obtained at the interphase indicates the presence of deoxy sugar characteristic of cardenolides (Evans, 2002).

Evaluation of Antioxidant Activity

2, 2-Diphenyl-1-picrylhydrazyl radical (DPPH) (sigma-Aldrich) was used as free radical agent while the standard drug used for comparison was ascorbic acid (Vitamin C). The solvent used for dissolving the extracts, DPPH and Vitamin C with Methanol (sigma-Aldrich)

1mg each of the two extracts and the Vitamin C was dissolved in 10ml of Methanol in individual test tubes. Using serial dilution technique, concentrations of 100ug/ml, 50ug/ml, 25ug/ml, 12.50ug/ml, 6.25ug/ml and 3.125ug/ml, each of the two extracts and the Vitamin C were prepared in individual test tubes. Using the same Methanol mentioned above, 0.001% of DPPH solution was prepared. To the prepared concentrations of the extracts and Vitamin C, 1ml of the 0.001% DPPH solution was added each and mixed thoroughly.

The above prepared samples were incubated in a dark place at room temperature for about 30 minutes. The absorbance was taken at 517nm using a UV Spectrophotometer. Similarly, the absorbance of 0.001% DPPH solution was taken at the same wavelength.

Results were expressed in % as below:

$$\% \text{ Antioxidant Activity (\%AA)} = \frac{\text{DPPH-Extract/STD}}{\text{DPPH}} \times 100$$

Antimicrobial Studies of the leave extracts of *Pteleopsis habeensis*

Test Bacteria and fungi

For the antibacterial activity study, a total of four bacteria were used; three Gram negative and two Gram positive bacteria. The Gram negative bacteria used include*: Escherichia coli* and *Pseudomonas aeroginosa,* while the Gram positive bacteria used were: *Staphylococcus aureus* and *Streptococcus pyogenes.* For the antifungal activity *Candida albican* was used.

These organisms were obtained from the Department of Medical Microbiology, University of Maiduguri Teaching Hospital, Maiduguri, Nigeria.

Sterilization of the Materials

The media were sterilized in a portable autoclave at 121°C for 15 minutes while the pipettes and other glass wares were sterilized by dry heat in a hot air oven at 200°C for 15 minutes. The circular discs were sterilized in the petri dish using hot air oven at 200°C for 15 minutes.

Antibacterial Sensitivity Tests

Two methods were used for the antibacterial sensitivity test, these are: disc diffusion and broth dilution methods.

Disc Diffusion Method

The agar disc diffusion method by (Xu and Lee 2001; Mahasneh, 2002) was used to determine the growth inhibition of the test organisms by the plant extract. Sterilized filter paper discs (6 mm in diameter) were

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impregnated in appropriate concentrations of the extract (100, 50, 25, and 12.5 mg/ml). The discs (made from Whatman No. 1 filter paper) were allowed to absorb the extracts as described by Mahasneh, 2002. The agar plates were aseptically inoculated with broth cultures for the test microorganisms using sterile pipettes and the plates allowed to dry. The discs containing the plant extract were transferred using flamed but cooled forceps onto the surface of the agar plates. They were sufficiently spaced to prevent the resulting zones of clearing from overlapping. The plates were then incubated for 24 h at 37°C. After 24 h, the zone of inhibition around each disc was measured and recorded in mm using a transparent meter rule.

Broth dilution tests.

This procedure involved preparing serial dilutions of sample extracts (100, 50, 25 and 12.5 mg/mL) in a liquid growth medium dispensed in test tubes (Jorgensen, 2007). The plant extract-containing tubes of different concentrations were inoculated with a standardized bacterial suspension of $1–5\times10^5$CFU/ml. Following overnight incubation at 35°C, the tubes were examined for visible bacterial growth as evidenced by turbidity. The lowest concentration of antibiotic that prevented growth represented the minimal inhibitory concentration (MIC)

Antifungal Assay

Similarly the Antifungal activity of the leaf extracts was carried out using the agar disc diffusion method and broth dilution test as described above using nutrient agar. The plates were incubated at room temperature for 48 hours and inhibition of growth was noted. The minimum inhibitory concentrations (MICs) and minimum fungicidal concentrations (MBC) were recorded after 48hours.

RESULTS, DISCUSSION AND CONCLUSION

Result

Plant extraction

The extractive values for the crude *n*-Hexane, Chloroform, *n*-Butanol and Methanol leaf extracts of *Pteleopsis habeensis* from 500g plant material each were found to be 1%w/w (5.0g), 1.34% w/w (6.7g), 1.28% w/w (6.4g) and 1.24%w/w (6.2g) respectively (Table 1)

Table 1: Yield of crude *n*-Hexane, Chloroform, *n*-Butanol and Methanol leaf extract of *Pteleopis habeensis*

S/N	SAMPLE	YIELD (g)	PERCENTAGE (%)
1.	*n*-Hexane	5.0	1.00
2.	Chloroform	6.7	1.34
3.	*n*-Butanol	6.4	1.28
4.	Methanol	6.2	1.24

Preliminary Phytochemical Screening of *n*-Butanol and Methanol crude extracts

The results of Phytoconstituents from the *n*-Butanol and Methanol leaf extract of *Pteleopsis habeensis* reveal the presence of alkaloids, cardiac glycoside, flavonoids saponins, steroid/triterpenes and tannins (Table 2).

Table 2: Preliminary Phytochemical screening of crude *n*-Butanol and Methanol leaf extracts of *Pteleopsis habeensis*

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S/No.	Phytochemicals	Specific test	Observation	*n*-Butanol	Methanol
1.	Alkaloids	Dragendroff's test Mayer's test	Formation of orange to brownish precipitates. Formation of white to yellowish or cream color precipitates.	- -	+ +
2.	Cardiac glycosides	Keller- killiani test	Formation of brown ring at the interphase.	+	+
3.	Flavonoids	Ferric Chloride test Sodium Hydroxide test	Formation of a blue-black or green or blue-green precipitates. Formation yellow color, a change in color from yellow to colorless.	- -	+ -
4.	Saponins	Frothing test Fehling Test	Frothing which persist on warming. Formation of brick red precipitate.	- +	+ +
5.	Steroid/ Triterpenes	Lieberman- Buchard test Salkowski test	Formation of brown ring at the interphase. Formation of red coloration at the interphase.	+ +	+ +
6.	Tannins	Lead sub-acetate test Ferric chloride test	Formation of green precipitate. Formation of a blue-black or green or blue-green precipitates.	- -	+ +

Keys: +=PRESENT, -=ABSENT

Antioxidants Activity

Absorbance reading

The result of mean absorbance readings of crude *n*-Butanol and Methanol leaf extract of *Pteleopsis habeensis* using Ascorbic acid as control at 517nm showing scavenging effects of the crude *n*-Butanol and Methanol *Pteleopsis habeensis* leaf extracts at different concentrations (Table 3)

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Table 3: Mean absorbances readings of crude *n*-Butanol and Methanol leaf extract of *Pteleopsis habeensis*.

Conc. (µg/ml)	*n*-Butanol	Methanol	Ascorbic Acid
100^{abc}	0.636±0.000	0.224±0.105	0.006±0.000
50^{bc}	1.139±0.000	0.891±0.045	1.101±0.001
25^{bc}	1.017±0.000	1.363±0,001	1.075±0.100
12.5^{abc}	1.107±0.000	1.464±0.000	1.371±0.003
6.25^{abc}	1.493±0.000	1.398±0.003	1.321±0.000
3.125^{ac}	1.809±0.000	1.219±0.120	1.444±0.001

DPPH Value = 1.954

Values are expressed as Mean ± SEM (n= 3), p-value <0.05 (Anova)

Keys: SEM (standard error of mean),

Multiple comparisons (Anova): a,b,c = significance between groups, p<0.05

a= between "Ascorbic acid and *n*-Butanol"

b= between "Ascorbic acid and Methanol"

c= between "*n*-Butanol and Methanol"

Percentage scavenging activity

Percentage scavenging effects of crude *n*-Butanol and Methanol leaf extracts of *Pteleopsis habeensis* extracts and Ascorbic acid at different concentrations (Table 4)

Table 4: Percentage scavenging activity

Conc. (µg/ml)	*n*-Butanol (%)	Methanol (%)	Ascorbic Acid (%)
100	67.50	88.56	99.69
50	41.85	54.49	43.77
25	48.08	30.38	45.10
12.5	43.44	25.24	29.98
6.25	23.74	28.60	32.53
3.125	7.59	37.74	26.25
Ic_{50} (µg/ml)	57.17	42.22	38.17

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MINIMUM INHIBITORY CONCERNTRATION

Minimum Inhibitory Concerntration of crude *n*-Butanol leaf extract of *Pteleopsis habeensis*

The results of minimum inhibitory concentration of crude *n*-Butanol leaf extract of *Pteleopsis habeensis* (Table 5).

Table 5: Minimum inhibitory concentration crude *n*-Butanol leaf extract of *Pteleopsis habeensis*

	CONCENTRATION			
ORGANISMS	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml
Staphylococcus aureus	+	+	+	+
Streptococcus pyogenes	+	-	+	+
Escherichia coli	+	+	+	-
Pseudomonas aeruginosa	+	+	+	+
Candida albican	-	-	+	-

Keys; Turbid Growth= +, No Growth= -

Minimum Inhibitory Concerntration of crude Methanol leaf extract of *Pteleopsis habeensis*

The results of minimum inhibitory concentration of crude Methanol leaf extract of *Pteleopsis habeensis* (Table 6).

Table 6: Minimum inhibitory concentration of crude Methanol leaf extract of *Pteleopsis habeensis*

	CONCENTRATION			
ORGANISMS	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml
Staphylococcus aureus	+	+	+	+
Streptococcus pyogenes	+	+	+	+
Escherichia coli	+	-	+	-
Pseudomonas aeruginosa	+	+	+	+
Candida albican	+	-	+	+

Keys; Turbid Growth= +, No Growth= -

ZONES OF INHIBITION

Zones of inhibition of the crude *n*-Butanol leaf extract of *Pteleopsis habeensis*

The results of the zones of inhibition (mm) of the crude *n*-Butanol leaf extract of *Pteleopsis habeensis* (Table 7).

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Table 7: Zones of inhibition (mm) of the crude *n*-Butanol leaf extract of *Pteleopsis habeensis*

concentration (mg/ml)	**Mean diameter of zone of inhibition (mm)**				
	Staphylococcus aureus	*Streptococcus pyogenes*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Candida albican*
100	10	NZI	NZI	11	10
50	9	NZI	NZI	11	8
25	NZI	NZI	NZI	8	8
12.5	NZI	NZI	NZI	8	NZI
Ciprofloxacin	30	31	28	15	N/A
Fluconazole	N/A	N/A	N/A	N/A	34

Zones of inhibition of the crude Methanol leaf extract of *Pteleopsis habeensis*

The results of the zones of inhibition (mm) of the crude Methanol leaf extract of *Pteleopsis habeensis* (Table 8).

Table 8: Zones of inhibition (mm) of the crude Methanol leaf extract of *Pteleopsis habeensis*

concentration (mg/ml)	**Mean diameter of zone of inhibition (mm)**				
	Staphylococcus aureus	*Streptococcus pyogenes*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Candida albican*
100	11	11	11	9	14
50	10	9	9	10	12
25	10	11	9	11	9
12.5	NZI	9	8	8	8
Ciprofloxacin	34	22	22	NZI	N/A
Fluconazole	N/A	N/A	N/A	N/A	34

KEY:

Control= ciprofloxacin (2.0 mg/ml), Fluconazole (2.0 mg/ml)

NZI= No Zone of Inhibition

N/A=Not Applicable

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DISCUSSION

Phytochemical screening of the leave extract of *Pteleopsis habeensis* reveals the presence of saponins, flavonoids, steroids and triterpenes, tannins, cardiac glycosides and alkaloids (Table 2). Which is in conformity with the studies of (Mohammed, 2014) and (Aliyu *et al.*, 2018). From Table 2, the Methanol extract contains more active phytochemicals compared to the *n*-Butanol extract. This indicates that more polar solvent (Methanol) have more extractive effects than the less polar solvent (*n*-Butanol). This buttress the report of (Bimakr, 2010), thus the extracting solvent significantly affect the measured total phytochemical content as a result of solvent polarity which is an important parameter that affects the yield of a plant material.

Antioxidant Activities of the Crude Extracts. The DPPH antioxidant assay is based on the principle that any substance capable of donating an atom of hydrogen or an electron is an antioxidant or antiradical species and its potency is demonstrated as DPPH Colour is transformed from purple to yellow in the test sample owing to the formation of neutral DPPH-H molecule upon the uptake of a hydrogen atom from antioxidant species (Guerrini et al., 2009)

The evaluation of the antioxidant activity of the *n*-Butanol and Methanol crude extracts was carried out in vitro through the radical model DPPH, and the antioxidant capacity of the two extracts was measured based on their Percentage scavenging efficiency and IC_{50} concentration which corresponds to the concentration of the extracts capable of reducing the initial DPPH absorbance by 50%. The percentage scarvenging activity of Methanol extract was higher than that of the *n*-Butanol extract in the DPPH assay at 100µg/ml (Table 4). However the two extracts showed lower activity than the standard drug (Ascorbic acid) with percentage antioxidant activity of 99.69% at 100µg/ml. The IC_{50} of *n*-Butanol extract (57.54µg/ml) and Methanol extract (42.22 µg/ml) were higher than that of the Ascorbic acid (38.17 µg/ml). It was observed after statistical analysis (Table 3), no significant difference was seen between 50 µg/ml concentration of *n*-Butanol extract and 50 µg/ml Ascorbic acid, as well as between 25 ug/ml of *n*-Butanol and 25 ug/ml Ascorbic acid. Statistical analysis also showed no significant difference between 3.125 µg/ml of both Methanol extract and standard (Ascorbic acid).

Antibacterial studies reveal that the extract of the plant exhibited activity against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, and *Pseudomonas aeruginosa*. The studies show a dose dependent activity at the dose of 100, 50, 25 and 12.5 mg/ml respectively. More activity was observed with the Methanol extract than with the *n*-Butanol extract. The *n*-Butanol extract shows no zone of inhibition to *Escherichia coli*, and Streptococcus spp. This could be as a result of the more bioactive components that are present in the Methanol extract.

The results presented in Tables 5 and Table 6 showed that crude leaf Methanol and Butanol extracts of *Pteleopsis habeensis* were found to inhibit the growth of the bacteria including the fungus. The fungus *Candida albican*, showed the highest sensitivity to Methanol extract with zone of inhibition 14 mm at 100mg/ml compared to it sensitivity to *n*-Butanol extract with zone of inhibition 10mm at 100mg/ml. The Minimum Inhibitory concentration is 12.5mg/ml for Methanol extract and 25mg/ml for *n*-Butanol extract.

CONCLUSION

The results obtained from this study shows that the crude leaf extracts of *Pteleopsis habeensis* contained saponins, flavonoids, steroids and triterpenes, tannins, cardiac glycosides and alkaloid. The inhibitory role observed on the various microorganisms by the crude extracts is an indication that it contains antimicrobial activities. It can be concluded that the crude leaf extract of *Pteleopsis habeensis* contains bioactive compounds and exhibited profound antioxidant activity that is comparable to Ascorbic acid.

RECOMMENDATION

1. Further work should be carried out in order to isolate the principal bioactive components responsible for the observed antimicrobial and antioxidant activity.

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2**.** Government and Non-Governmental Organization should encourage the cultivation of plant with medicinal properties.

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Appendix I

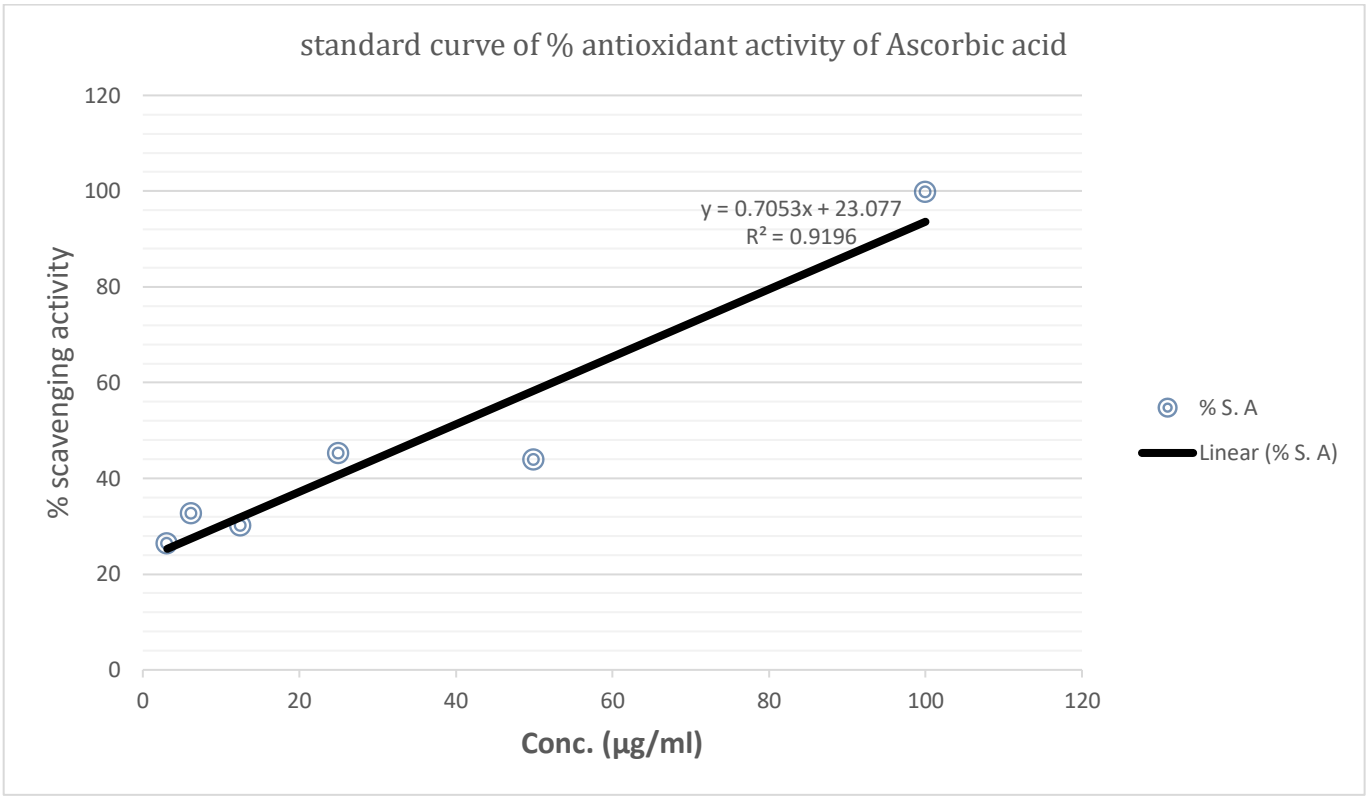

Figure 2. Calibration curve for Ascorbic acid IC_{50}

Appendix II

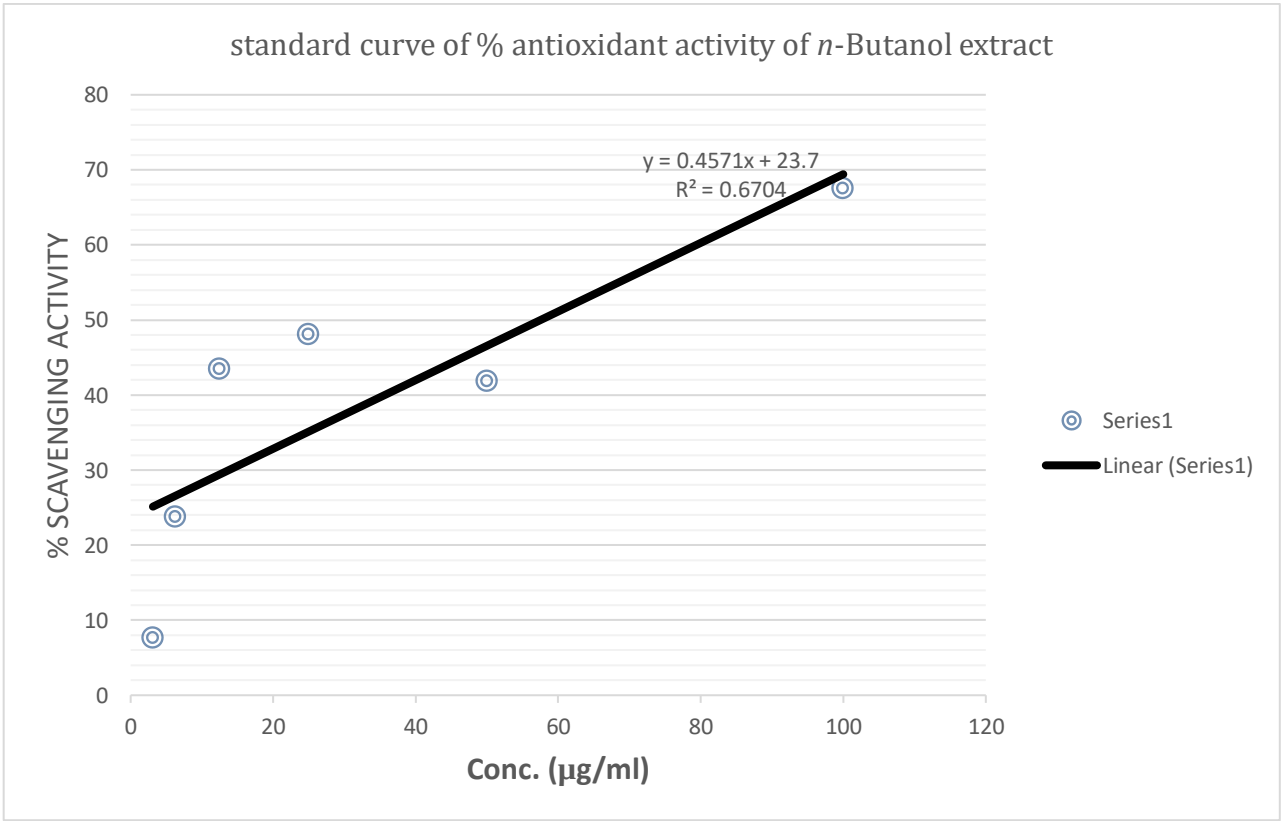

Figure 3. Calibration curve for *n*-Butanol extract IC_{50}

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Appendix III

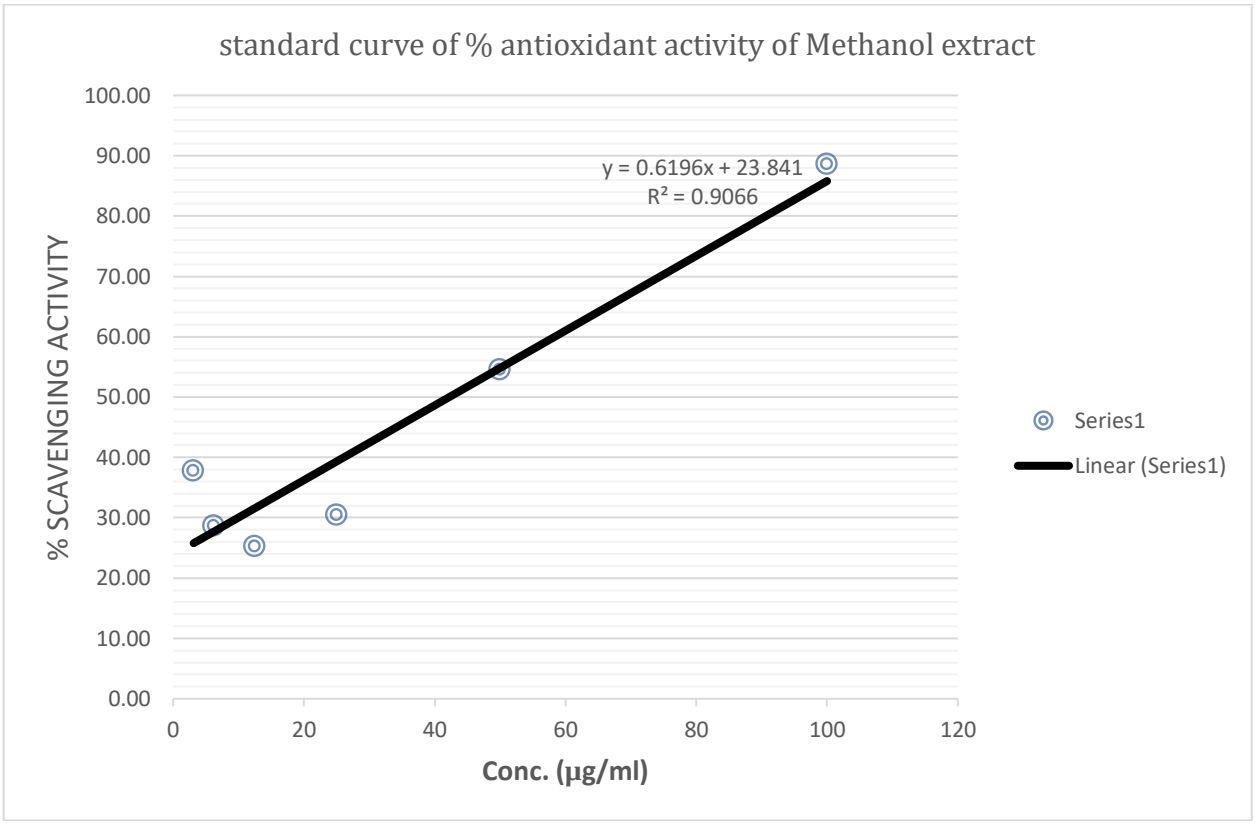

Figure 4. Calibration curve for Methanol extract IC_{50}

Appendix IV

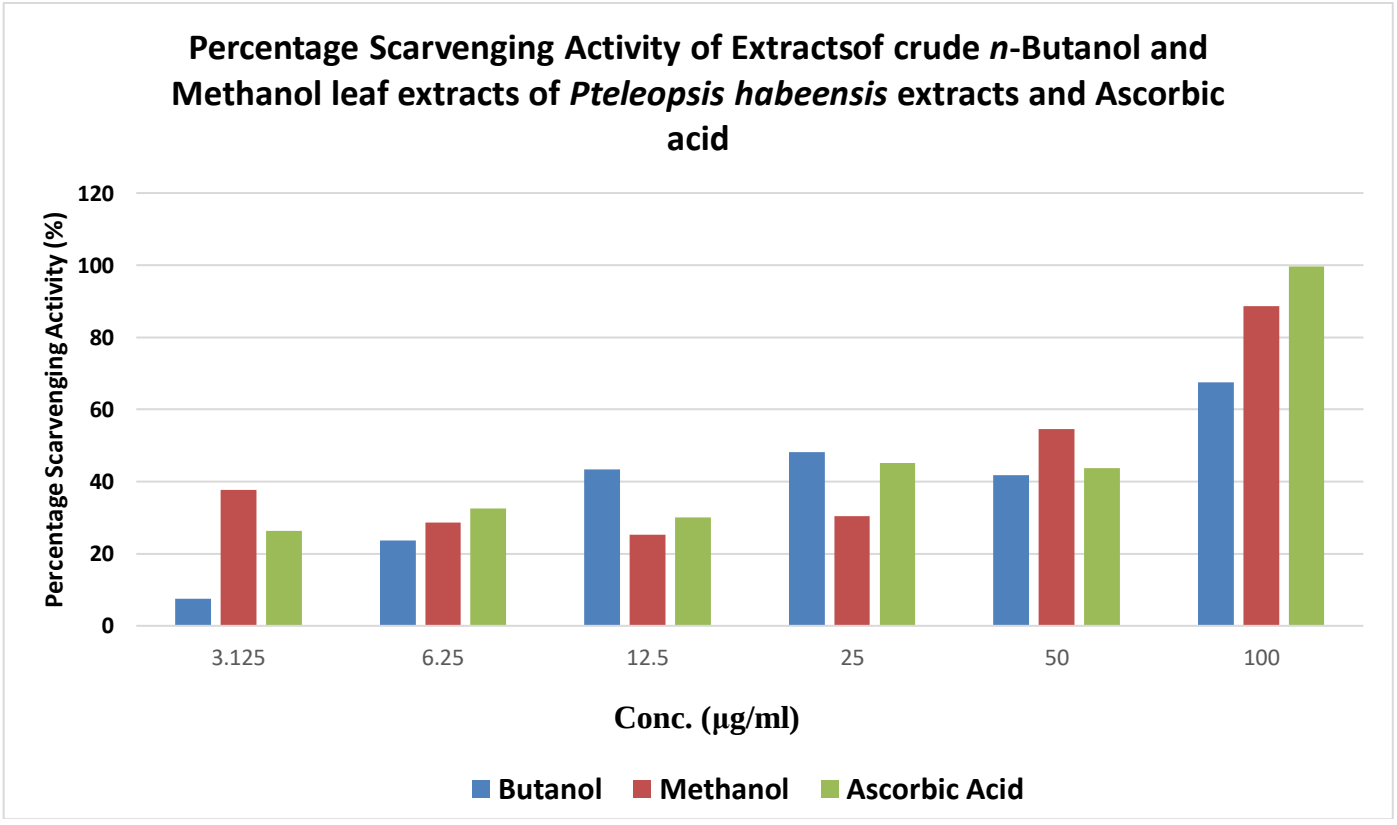

Figure 5: Percentages of antioxidant activities against concentration.