

Phytochemical Screening, Determination of Some Chemical Compounds and Antimicrobial Activity of *Cassia Occidentalis* (Senna coffee) (Whole plant) Found in Shelleng Local Government Area, Adamawa State, Nigeria

^{1*}Alexander, Priscilla, ²Titus, Anbomotuntya, ³Hassan Wafi Garba

^{1,3}Department of Pure and Applied Chemistry, Faculty of Science, Adamawa State University, PMB 25, Mubi, Adamawa State, Nigeria

²Primary Health Care Development Authority Shelleng, Adamawa state, Nigeria

*Corresponding Author's E-mail: priscillaalexander21@yahoo.com

Abstract - This study was carried out to determine the phytochemicals, some chemical compounds and antimicrobial activities of *Cassia occidentalis* Roots, leaf, and seed found in Shelleng Local Government Area Adamawa State, Nigeria. The phytochemicals were extracted and analyzed using High Performance Liquid Chromatography (HPLC), the patent minerals were determined using Atomic Absorption Spectroscopy (AAS) and Flame Emission Spectroscopy (FES). The patent vitamins and proximate composition were analyzed using the standard methods. The results of the phytochemical constituents revealed the presences of alkaloids, flavonoids, saponins, terpenes and phenols with phenols having the highest concentration. The result of the proximate analyses showed that the plant has rich and good nutritional values having high carbohydrate contents, fiber and protein. Total ash content, fats and moisture were present in good amount. The result of the Patent vitamins revealed the presences of vitamin A, C, B6, B12 and K in the plant, with high concentration of vitamins A and C recorded in leaf and seed, however, vitamin K was not detected. The result obtained revealed that *Cassia occidentalis* plant is rich in minerals. The following minerals were present K, P, Ca, Zn, Mn and Mg, with K, P and Ca recorded the highest concentration in the leaf, seed and root. Other minerals such as Zn, Na, Fe, Cu, Mg and Mn are present in good amount and contributed to the nutritional values of the plant. The zone of inhibition, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined. The antimicrobial screening indicated that all the extracts exhibited different activity against different microbes. However, the results of this study showed that the extract of *Cassia occidentalis* plant could be potent in inhibition of the studied microorganisms and also the plant has good secondary metabolites. More research effort should be directed onto this species, in order to further validate

many claims on the species. Research to be conducted on *Cassia occidentalis* to ascertain the level of its toxicity.

Keywords: Phytochemical constituents, chemical compounds, antimicrobial activity, *Cassia occidentalis*, Shelleng, Adamawa, Nigeria.

I. INTRODUCTION

Plants are important sources of drugs; especially in traditional medicine. It is a common practice in Nigeria and other parts of the world to use plant in the form of crude extracts, decoction, infusion or tincture to treat common infection and chronic conditions. According to World Health Organization, [1] over 70 % of the world populations rely on medicinal plants for primary health care and there are reports from various researchers on natural substances of plant origin which are biologically active, with desirable antimicrobial and antioxidant properties. Medicinal plants are traditionally used globally for the treatment of many diseases, including but not limited to; asthma, gastrointestinal problems, skin disorders, respiratory and urinary problems, hepatic and cardiovascular disease [2]. Plants are a significant source of various health-beneficial phytochemicals such as flavonoids, phenols, saponins, alkaloids, vitamins, minerals, and carbohydrates^[3]. The plant-based medicines are common as a source of primary healthcare in many parts of the world, especially Africa, Asia, and parts of America [3]. *Cassia occidentalis* is a large genus of flowering plants in the legume family; Fabaceae (Leguminosae). This genus is distributed throughout the tropics comprising more than 300 species [4] *Senna* species are commonly used as ornamentals, famine foods, and many species are used in both traditional and modern medicines. *Cassia occidentalis* commonly known as *Sennacoffee* [5]. It is found in open pastures and fields, cultivated with cereals such as soybean, corn, sorghum, and others [6]. Various parts of *Senna coffee* (seeds, roots, leaves, and stems) are

traditionally claimed to be useful in treating different medical conditions and infections [7].

Senna coffee is a medicinal plant which has been used for its diuretic, laxative, blood purifier, antibacterial, and antifungal properties as well as for the treatment of hemorrhoids, gout rheumatism, diabetes, whooping cough, convulsions, heart disease, and snake bite [8]. The chemical constituent of this plant varies with plant parts. The plant root contains emodin, while the seeds contain chrysarobin and N-methylmorphine [9]. *Cassia occidentalis* has been reported to be poisonous (Adomi, 2008). Despite claims of being poisonous, the seeds are still use in roasted form as a substitute for coffee with little nutritional information [9]. The screening of the actual bioactive elements of plant origin and assessment of elemental composition of the widely used medicinal plant (Senna coffee) is highly essential [10]. In this present study the main focus is to carried out analysis on the proximate composition, determine the patent minerals and vitamins of the study plant (Senna coffee) in other to provide information on the nutritive values of the various parts of the plant and its vitamins contents, in which information are scarce. Senna coffee is a pan tropical weed with a wide range of medicinal and nutritional values [11]. It is commonly found in Adamawa State, where it is use traditionally, its use as anti-biotic, anti-venom, Analgesic and anti-microbial plant. In view of the above there is need to have an overview of the plant especially the scarcity of information on its nutritive values and the patent vitamins and minerals.

II. MATERIALS AND METHODS

All the chemicals, reagent and solvents used in this study were of analar grade and the apparatus and equipment used were of laboratory standard.

2.1 Sample collection and Preparation

Fresh samples of Senna coffee plant was obtained from Awa-Bali area of Shelleng L.G.A. Adamawa State, Nigeria, and was identified by the Botany Department of Adamawa State University Mubi, Nigeria. A voucher specimen was deposited in the herbarium of the department for reference. The collected plant parts were washed in clean water several times and rinsed with distilled water to remove soil, dust or dirt and were air-dried at room temperature 28°C to constant weight, after which it was ground to a uniform powdered using mechanical grinder. The powered samples were kept in an air –tight plastic container for further analysis [12].

2.2 Phytochemical Screening:

The presences of some phytochemical constituents were determined both qualitatively and quantitatively using

standard methods adopted by [13]; [14] with some modification. The proximate analysis was carried out, and the moisture content, total ash, crude protein, fat content, carbohydrate and crude fiber were determined following the Official Methods of the Association of Official Analytical Chemists [13],

2.3 Preparation of Extracts

The ethanol extract was obtained by macerating the powdered sample (100g of each of the dry powdered plant materials) in 700ml of 70% aqueous ethanol. The separated extracts were concentrated using a rotary evaporator under vacuum with the water bath set at 45°C. The separated extract was then filtered through whatman's No. 1 filter paper and stored in an airtight sample bottles which were stored in a desiccators for further analysis [12].

2.4 Antimicrobial Screening

250g of the ground plant materials was cold extracted in ethanol and methanol separately. Another 250g of plant samples were extracted in water for 4 days with occasional shaking. Ethanol and Methanol used were of analytical grade. The separated extract was then filtered through whatman's No. 1 filter paper and the ethanol and methanol filtrate were separately concentrated to dryness in vacuum using a rotary evaporator to remove the ethanol and methanol. The aqueous extract was lyophilized to obtain a dry powder extract. [12]

2.5 Test Microorganisms

The test microorganisms that were used in this study (bacteria: staphylococcus aureus, Escherichia coli, and Aspergillusniger) were obtained from Biological laboratory of Adamawa State University, Mubi. The bacteria isolate was first being sub-cultured in nutrient broth (Oxoid) and incubated at 37°C for 18hrs while the fungal isolates was sub-cultured on a Sabouraud dextrose agar (SDA) (Oxoid) for 72hrs at 25°C. [13].

2.6 Minimum bactericidal Concentration

The MBC of the plant extracts was determined by a modification of the method of spencer (2004). Samples were taken from plates with no visible growth in the MIC assay and sub-cultured on freshly prepared nutrient agar plates and SDA plates, and later be incubated at 37°C for 48hrs and 25°C for up to 72hrs for bacteria and fungi respectively. The MBC was taken as the concentration of the extract that did not show any growth on a new set of agar plates. The MBC was not determined for water extract since the antibacterial activity was low [15].

2.7 Minimum inhibitory concentration (MIC)

The estimation of MIC of the crude extracts was carried out, using the method of (Akinpelu and Kolawole [16]). Two fold dilutions of the crude extract were prepared and 2ml aliquots of different concentrations of the solution was added to 18ml of pre-sterilized molten nutrient agar, SDA for bacteria and fungi respectively at 40°C to give final concentration regimes of 0.050 and 10mg/ml. The medium was then poured into sterile petri dishes and allowed to set. The surface of the medium, was then allowed to dry under laminar flow before streaking with 18hrs old bacterial and fungal cultures. “The plates were later being incubated at 37°C for 24hrs and at 25°C for up to 72hrs for bacteria and fungi respectively, after which they was examine for the presence or absence of growth”. The MIC was taken as the lowest concentration that prevented the growth of the test microorganism [17].

2.8 Determination of Patent Minerals (Essential elements)

The essential elements comprising calcium, magnesium, potassium, iron and manganese were determined according to the method of [13] with slight modification. The ground samples were sieved with a 2 mm rubber sieve and 2 g of each of the plant samples were subjected to dry ashing in a well-cleaned porcelain crucible at 550 °C in a muffle furnace. The

resultant ash was dissolved in 5 ml of HNO₃ /H₂O₂ (1:1) and heated gently on hot plate until brown fumes disappeared. To the remaining material in each crucible, 5 ml of deionized water was added and heated until a colorless solution was obtained. The mineral solution in each crucible was transferred into a 100 ml volumetric flask by filtration through a whatman filter paper and the volume was made to mark with deionized water. This solution was used for elemental analysis by atomic absorption spectrophotometer and Flame photometer [17].

2.9 Determination of Patent vitamins (Water- and Fat-Soluble Vitamins) using High Performance Liquid chromatography (HPLC)

Analysis was performed by injecting 20ul of carefully prepare sample into a Buck scientific (USA) BLC10/11 – model HPLC equipped with UV 325 nm and UV 254nm detectors for fat and water soluble vitamins respectively. A C18, 4.6 x 150mm, 5um column and a mobile phase of 95:5 (methanol: water) was use at a flow rate of 1.00 mL/minute and an ambient operating temperature. A 0.1mg of mix standards was analyze in a similar manner for identification. Peak identification was conducted by comparing the retention times of authentic standards and those obtain from the samples. Concentrations were calculated using a four-point calibration curve. [18].

III. RESULTS AND DISCUSSIONS

Table 1: Qualitative phytochemical constituents

Sample	Tannis	Alkaloids	Flavonoids	Saponins	Terpenes	Steroids	Phenols
Leaf	+++	++	++	++	+	++	+++
Roots	++	++	++	++	+++	+	+++
Seed	+++	+++	+++	+++	+++	+++	+++

Key: += slightly present, ++ = Present, +++ = Highly Present

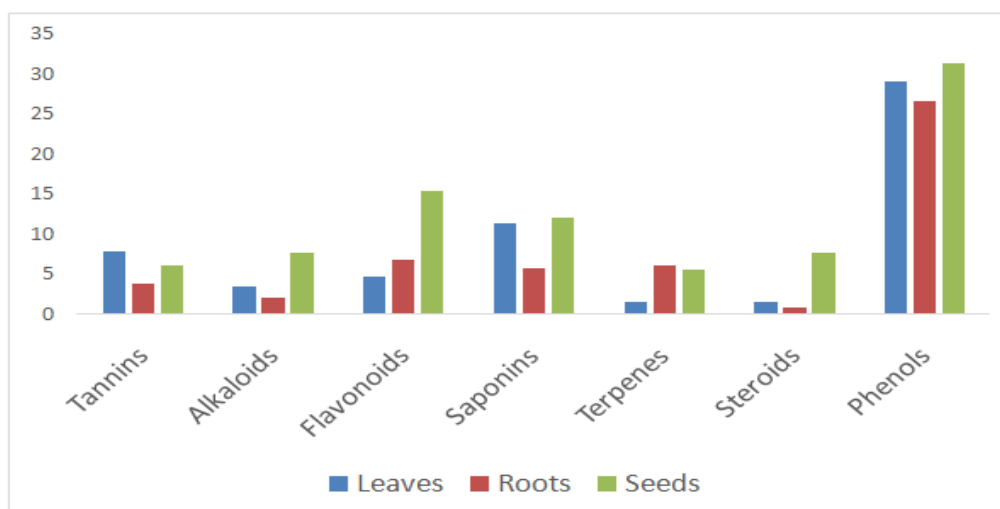


Figure 1: Quantitative Phytochemical Constituents (g/100g) in Leaves, Root and Seeds

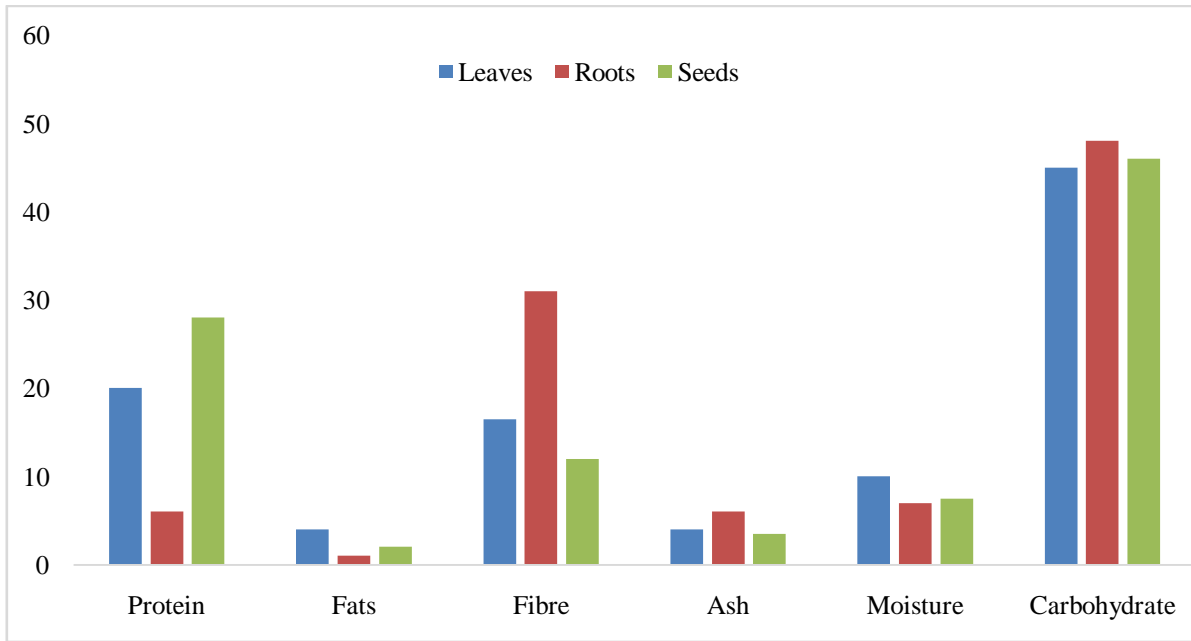


Figure 2: Proximate Composition (%) in *Cassia Occidentalis*

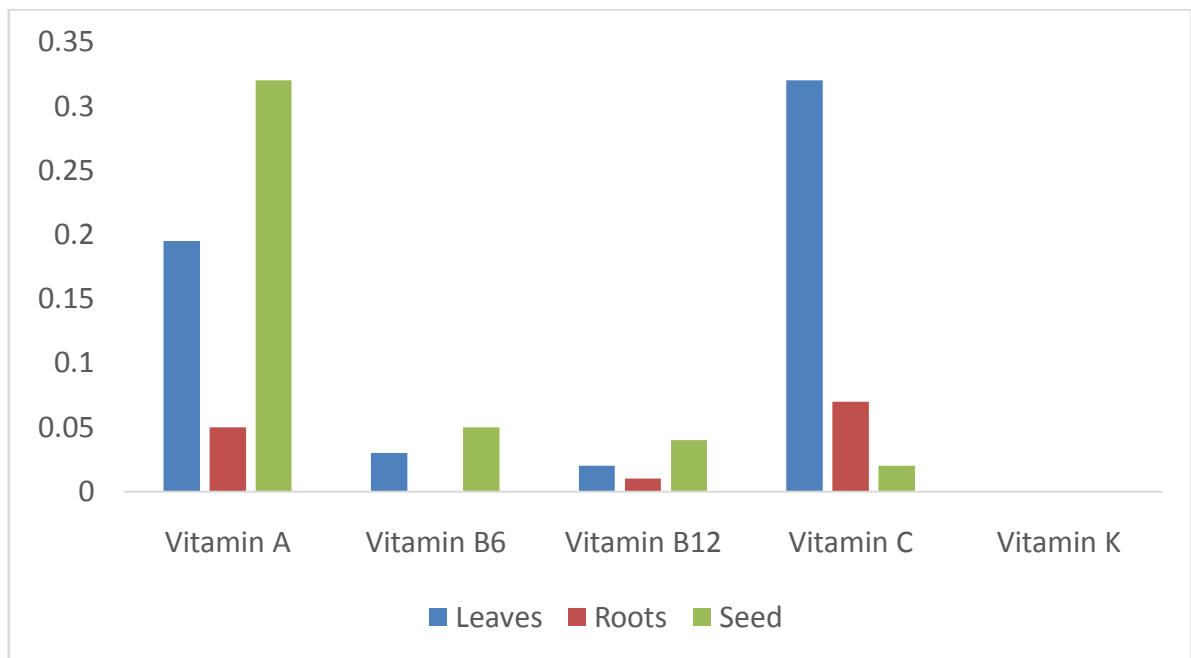


Figure 3: Patent Vitamins contents (mg/g) in leaves, Root, and Seed

Table 2: Antimicrobial Activity using different organisms

Test Organisms	Zone of inhibition (mm)				
	25mg/ml of the Ethanolic extract			Streptomycin 1mg/ml	Ketoconazole 1mg/ml
	Leaf	Root	Seed		
Staph. Aureus	3.5±0.1	4.5±0.01	17±0.05	27±0.03	-
Escherichia coli	2.5±0.04	5.5±0.1	20.5±0.02	22±0.5	-
Aspergillusniger	4±0.01	8±0.02	18±0.01	-	34.5±0.03

Table 3: MIC and MBC Regimes of the ethanolic extracts (mg/ml)

Test Organisms		Ethanolic extracts			Streptomycin	Ketoconazole
		Leaf	Root	Seed		
Staph. Aureus	MIC	5.0	5.0	2.0	0.13	-
	MBC	10	12	5.0	0.175	-
Escherichia Coli	MIC	5.0	5.0	6.0	0.16	-
	MBC	10	14	8.0	0.14	-
Aspergillusniger	MIC	5.0	5.0	5.0	-	0.65

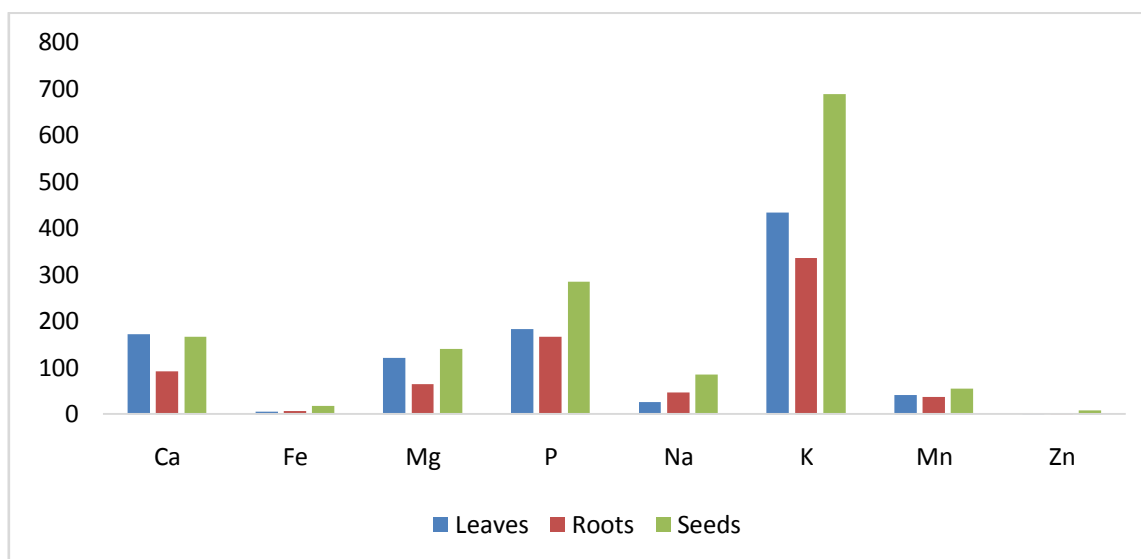


Figure 4: Patent Mineral contents (mg/100g)

Phytochemical Constituents

The result of the phytochemical constituents is presented in figure 1 and table 1. The result of the investigation revealed the presence of some secondary metabolite such as Saponins, Phenols, Flavonoids, Terpenes, Steroids, Alkaloids and Tannins. In general, it can be seen from the result that, Phenol has the highest concentration of phytochemical constituents followed by flavonoids and saponins in both leave, root and seed of *Cassia Occidentalis*. These phytochemicals are in the increasing concentrations as phenols > flavonoid > saponins > tannins > alkaloids > terpenes and > steroid. The presence of these metabolites reveals great potential for the plant as a source of useful phytomedicine. Phenols compounds are widely distributed throughout the plant kingdom and play crucial roles in various plant physiological processes, such as growth, development, defense mechanisms, and response to environmental stressors. [19]. Flavonoids, in particular, have been studied for their ability to inhibit the growth of cancer cells, reduce inflammation, and protect against oxidative stress [20]. Alkaloids help in decreasing blood pressure and balancing of the nervous system in mental patients, alkaloid also possess anti-malaria property and a good source for the treatment of malaria. Many alkaloids have been used in

traditional and modern medicine as painkillers, stimulants, and sedatives, while others have been used as recreational drugs or as poisons.[21]. The presence of tannins indicated that the plant is an astringent, which help in wound healing and as anti-parasitic, while terpenes are useful as anti-tumor and anti-viral agent since some terpenes are known to be cytotoxic to tumor cells [22]. This research work agrees with that of [23]; [24] which indicated the presence of Saponins, Steroids, Flavonoids, Alkaloids and Terpenes.

Proximate Composition

The proximate composition of a sample refers to the percentage of various components such as protein, fat, fiber, ash, moisture, and carbohydrates present in the sample. These components play a crucial role in determining the nutritional value of the sample and can vary significantly depending on the plant species and parts being studied. The results of the proximate composition as presents in figure 2, revealed the concentration of the various proximate compositions in leaves samples to be; protein 20.00%, fat 4.00%, fiber 16.50%, ash 4.00%, moisture 10.00%, and carbohydrates 45.00%. The roots, on the other hand, recorded as, protein 6.00%, fat 1.00%, fiber 31.00%, ash 6.00%, moisture 7.00%, and

carbohydrates 48.00%. Lastly, the seeds contained protein 28.00%, fat 2.00%, fiber 12.00%, ash 3.50%, moisture 7.50%, and carbohydrates 46.00%. The result shows that, carbohydrate content was 45.10, 48.23 and 46.49% in leaf, root and seed respectively, the high carbohydrate content shows that all parts of *cassia occidentalis* plant are good sources of energy. The result of the moisture content shows that the plant parts has little chance of microbial attack and also suggested that the shelf life of this plant will be longer [25]. High fiber content also revealed that the plant will be known to support bowel regularity, maintain normal cholesterol and blood sugar levels, reduce constipation and used for the prevention of heart diseases and some types of cancer [26]. *Cassia occidentalis* can be a source of valuable dietary fiber in human nutrition. The low ash content of the plant shows that the amount of inorganic substance is low and this suggested that the plant may be a poor source of dietary inorganic salts or supplement. Proteins obtained through diet are vital for human nutrition, providing essential amino acids that the body cannot synthesize [27].

Patent Vitamins

Patent Vitamins are organic molecules that are essential to an organism in small quantities for proper metabolic function. Vitamins are organic substances that are generally classified as either fat soluble or water soluble. The results of the patent vitamins as shown in figure 3, indicated the presence of the following vitamins, vitamins A, B₆, B₁₂, C, and K which were found in all the plant parts, with Vitamin A having high concentration in seed and leave, while vitamin C has high concentration in the leave with value of (0.320mg/g) and vitamin K was not detected in all the plant parts. These vitamins are very essential for daily wellbeing of man and animals. The high concentration of vitamin C shows the plant potency to improve body immunity against diseases and healing of wound in the body. The results of this study agree with the work performed by [28]. The results also fall within the permissible limits of WHO [29]. Vitamin A (retinol) is an essential nutrient needed in small amounts by humans for the normal functioning of the visual system; growth and development; and maintenance of epithelial cellular integrity, immune function, and reproduction [30]. Therefore, the result of this study shows that *cassia occidentalis* plant is good sources of vitamins,

Patent Minerals

Patent minerals are those elements on the earth and in foods that our bodies need to develop and function normally [31]. Those essential for health include calcium, phosphorus, potassium, sodium, magnesium, iron, zinc, iodine, chromium, copper, fluoride, molybdenum, manganese, and selenium [31].

The patent mineral contents of the samples are presented in figure 4. From the result of this study it shows that the plant is rich in minerals, such as K, P, Ca and Mg while Mn, Zn, Na, and Fe were also present in good amount. However, potassium was the most abundant mineral element recorded in the seed, leave and root of *cassia occidentalis* plant followed by P and Ca. Potassium is an essential mineral that is needed by all the tissue in the body. Potassium is mainly found in foods as supplements, its main function in the body is to help maintain normal fluid levels inside the body, while sodium maintains normal fluid levels outside the cell. It is associated with chronic diseases, especially cardiovascular diseases, high potassium intake can help to relax blood pressure and excrete sodium [32]. Phosphorus, an essential mineral, is found naturally in food and is available as a dietary supplement, phosphorus is a component of bones, teeth, DNA and RNA it is needed for the growth, maintenance, repair and production of the genetic building blocks of all tissues and cells, and it is also needed to balance the use of vitamins and minerals, including vitamin D, iodine, magnesium and zinc [33]. Mg plays an important role in the structure and function of the human body. Calcium is important for circulation of hormones, muscle and nerve, it helps bones and teeth, and also helps to maintain body strength [34]. Fe, Zn and Mn play a very important role for the improvement of antioxidant in human system [35]. This investigation suggested that *cassia occidentalis* plant has good nutritional values.

Antimicrobial activity

The result of the antimicrobial screening of *cassia occidentalis* was presented in table 2. The result was obtained by using streptomycin and Ketoconazole as the standard, which shows 27±0.03mm diameter on *Staphylococcus Aureus*, 22±0.5mm diameter on *Escherichia coli* and *Aspergillus niger* which shows no activity and the other show no activity completely on the test organism respectively. Antimicrobial activity test revealed that the seed sample possess the highest antimicrobial activity against all the test organisms *E. coli* (20mm), *S. aureus* (17mm) and *A. niger* (18mm), when compared to roots against *E. coli* (8mm), and resistant against *S. aureus* (5mm) and *A. niger* (4mm) and leave against *E. coli* (4mm), and resistant against *S. aureus* (2mm) and *A. niger* (3mm) respectively. The test organisms used in this study are associated with various forms of human infections. From a clinical point of view, for instance *E. coli* causes septicemias and can infect the gall bladder. Meninges, surgical wounds, skin lesions and the lungs, especially in debilitated patient [36].

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the ethanolic extract of the sample

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the ethanolic extracts was determined and the result was presented in table 3. The results which shows leaves sample has the activity of 5.0mg/ml MIC and 10mg/ml MBC on *S.aureus*, 5.0mg/ml MIC and 10mg/ml MBC on *E.coli* and 5.0mg/ml MIC but no activity on MBC on *A.niger* while the root sample has 5.0mg/ml MIC and 12mg/ml MBC on *S.aureus*, then 5.0mg/ml MIC and 14mg/ml MBC on *E.coli* but shows 5.0mg/ml MIC and show no activity in MBC and lastly the seed sample has 2.0mg/ml MIC and 5.0mg/ml MBC on *S.aureus*, 6.0mg/ml of MIC and 8.0mg/ml of MBC on *E.coli* and lastly 5.0mg/ml of MIC and no activity shows in MBC on *A.niger*. Using streptomycin and Ketoconazole as standard which shows 0.13mg/ml, 0.175mg/ml of MIC and MBC on *S.aureus* respectively while 0.16mg/ml and 0.14mg/ml of MIC and MBC respectively on *E.coli* while lastly show completely no activity on *A.niger* and Ketoconazole has no activity in both MIC and MBC of *S.aureus*, *E.coli* and *A.niger* with the exception of MIC in *A.niger* of 0.65mg/ml

The MIC and MBC of the extracts ranged from 14-2 mg/ml, with the seeds sample demonstrating the lowest values (MIC 2mg/ml., MBC 5g/ml each) against *E. coli*, followed by the leave sample (MIC 5mg/ml., MBC 10mg/ml) against all the test organisms. Most of the MIC values were lower than the MBC values indicating that the extracts could be bactericidal in action. Low MIC and MBC values are also an indication of high efficacy [37].

IV. CONCLUSION

The phytochemical screening showed that the leaves, root and seed extracts of the *Cassia occidentalis* are rich sources of secondary metabolites such as alkaloids, saponins, flavonoids, steroids, tannins, terpenes and phenol. The analysis on *cassia occidentalis* revealed that the plant is rich in carbohydrate and can help supply the energy needed for the function of the cell when consumed. The mineral content of the plant parts suggested that the plant can contribute significantly to the nutrient requirements of man owing to the high concentrations of potassium, phosphorous, calcium and iron as it may help build strong bones and teeth; and the antimicrobial screening showed that they exhibit inhibitory activities against the test organisms. The result obtained showed that *cassia occidentalis* could be exploited in the treatment of infectious diseases caused by the studied microorganisms due to the inhibition properties shown by the seed, leaves and the root extracts on the tested organisms. More effort should be geared towards planting this plant in order to exploit its full potential for the betterment of humanity.

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