

Comparative Phyto-Constituents Analysis from the Root Bark and Root Core Extractives of *Cassia ferruginea* (Schrad D. C) Plant

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Abstract: In an attempt to investigate the phyto-constituents present in the root bark and root core of *Cassia ferruginea* (Schrad D.C) plant due to acclaimed medicinal wide range uses of the plant especially for mental illness, sexual dysfunction and microbial infections, both the root bark and root core of *Cassia ferruginea* (Schrad D.C) plant were collected, processed and extracted using 95% ethanol. Phytochemical analysis method was used to determine the various constituents present qualitatively. The study revealed the presence of Alkaloids, Tannin, Saponins, Phenols, Flavonoid, and Cardiac glycosides in both root bark and the root core sample, in addition to Protein, Quinone, and Coumarins in the root bark except for steroid which was only present in the root core of *Cassia ferruginea* (Schrad D.C) plant. The study has shown the nature distribution of phyto-constituents in plant at different parts, no matter how close the parts are. As such all the parts of plant are necessary to be considered useful as its contained different components.

Keywords: *Cassia ferruginea* (Schrad D.C) plant, Phyto-Constituents and Phytochemical Analysis

INTRODUCTION

Plant materials are central to tradio-medicinal practices and have remained useful sources of new drugs. Although, orthodox medical practice is generally acceptable, alternative health care is still relied on all over the world [1, 2]. In the developing countries of the world, traditional herbal medicine is often used side by side with western medicine.

Phytochemicals are a large group of plant-derived compounds hypothesized to be responsible for much of the disease protection conferred from diets high in fruits, vegetables, beans, cereals, and plant-based beverages such as tea and wine [3]. Indeed, once consumed and absorbed, flavonoids act favorably in the body, through actions such as inhibiting xanthine oxidase and arachidonic acid metabolism [4]. More research is needed to fully explain the actions of phytochemical compounds in the human body [5]. Therefore, a recommended intake for phytochemicals does not currently exist. Today, many health authorities such as the American Cancer Society and the American Heart Association recommend consuming a diet high in fruits and vegetables to ensure that an individual ingests an adequate amount of phytochemical compounds [6, 7]. The long-term effects of pharmacological doses of

phytochemicals on human health are not well understood and therefore supplementation is not recommended.

Cassia ferruginea (Schrad D.C) [11] belong to the family *fabacea (leguminosae)* is one of the largest families of flowering plants with 18,000 species classified ground 650 genera [12]. *Cassia ferruginea* (Schrad D.C) is a deciduous tree with a wide, flat-topped crown; it can grow 8-15metrees tall. The short, cracked bole can be 50-70cm in diameter. The tree is sometimes harvested from the wild for local use as a medicine and source of wood. Highly ornamental when in bloom, it is used in landscaping. Several isolated compounds include physcion, emodin, rhein, chrysophanol, aloe emodin and kaemferol [13] are common to cassia species. Chemical studies showed that the genus cassia contains some alkaloid, tannins, carbohydrate, steroid and cardiac glycoside [14]. Since there have been several reports on the Phytochemicalsof their leaf for various cassia species, but reports on the Phytochemical constituent of the roots especially *Cassia ferruginea* (Schrad D.C) have been scarce. This paper was therefore, design to investigate phytochemical constituents present in both the root bark and root core of the plant.

Table 1: Potential of Food for Health Benefits from Some Phytochemical Compounds

Food	Phytochemical	Possible Benefit
Soy Beans, Soy Milk, and Tofu	Isoflavones (Genistein and Daidzein)	A reduction in blood pressure and increased vessel dilation [8]
Strawberries, Red Wine, Blueberries	Anthocyanins	Improvement of vision, inhibition of nitric oxide production, induction of apoptosis, decreased platelet aggregation, and neuroprotective effects [8]
Red Wine, Grape Juice, Grape Extracts, Cocoa	Proanthocyanidins and flavan-3-ols	Inhibition of LDL oxidation, inhibition of cellular oxygenases, and inhibition of proinflammatory responses in the arterial wall [8]
Garlic, onions, leeks, olives, Scallions	Sulfides, thiols	Decrease in LDL cholesterol [9]
Carrots, tomatoes, and tomato products, and various types of fruits and vegetables	Carotenoids such as lycopene, beta-carotenes	Neutralization of free radicals that cause cell damage [9]
Broccoli and other cruciferous vegetables such as kale, horseradish	Isothiocyanates (sulforaphane)	Neutralization of free radicals that cause cell damage [9] and protection against some cancers [10]

Taxonomy Classification of *Cassia ferruginea* (Schrad D.C) plant

Kingdom -	<i>Plantae</i>
Phylum -	<i>Tracheophyta</i>
Class -	<i>Magnoliopsida</i>
Order -	<i>fabales</i>
Family -	<i>faaceae</i> (leguminosae)
Sub-family-	<i>Caesalpinioideae</i>
Tribe -	<i>Cassieae</i>
Subtribe -	<i>Casssinea</i>
Genus -	<i>Cassia</i>
Species -	<i>Cassiaferruginea</i>



Fig-1: Showing *Cassia ferruginea* Plant Pictorially

Classes of Phytochemicals Glycosides

Glycosides are colorless, crystalline carbon, hydrogen and oxygen-containing (some contain nitrogen and sulfur) water-soluble phyto-constituents, found in the cell sap. Chemically, glycosides contain a carbohydrate (glucose) and a non-carbohydrate part (aglycone or genin) [15, 16].

Flavonoids

Flavonoids re important group of polyphenols widely distributed among the plant flora. Structurally, they are made of more than one benzene ring in its structure (a range of C15 aromatic compounds) and numerous reports support their use as antioxidants or free radical scavengers [15].

Phenolics

They are classified into (i) phenolic acids and (ii) flavonoid polyphenolics (flavonones, flavones, xanthenes and catechins) and (iii) non-flavonoid polyphenolics. Caffeic acid is regarded as the most common of phenolic compounds distributed in the plant flora followed by chlorogenic acid known to cause allergic dermatitis among humans [15].

Saponins

Saponins are regarded as high molecular weight compounds in which, asugar molecule is combined with triterpene or steroid aglycone. There are two major groups of saponins and these include: steroid saponins and Triterpene saponins. Saponins are soluble in water and insoluble in ether, and like glycosides on hydrolysis, they give aglycones. Saponins are extremely poisonous, as they cause heamolysis of blood and are known to cause cattle poisoning [15].

Tannins

Tannins are soluble in water and alcohol and are found in the root, bark, stem and outer layers of plant tissue. Tannins have a characteristic feature to tan, i.e. to convert things into leather. They are acidic in reaction and the acidic reaction is attributed to the presence of phenolics or carboxylic group [15].

Terpenes

They are flammable unsaturated hydrocarbons, existing in liquid form commonly found in essential oils, resins or oleoresins (Firn, 2010). Commonly important monoterpenes include terpinen-4-ol, thujone, camphor, eugenol and menthol. *Diterpenes (C20)* are classically considered to be resins and taxol, the anticancer agent, is the common example. The *triterpenes (C30)* include steroids, sterols, and cardiac glycosides with anti-inflammatory, sedative, insecticidal or cytotoxic activity. Common triterpenes: *amyriins*, *ursolic acid* and *oleanic acid sesquiterpene (C15)* like monoterpenes, are major components of many essential oils [17]. The sesquiterpene acts as irritants when applied externally and when consumed internally their action resembles that of gastrointestinal tract irritant.

Anthraquinones

These are derivatives of phenolic and glycosidic compounds. They are solely derived from anthracene giving variable oxidized derivatives such as anthrones and anthranols [18]. Other derivatives such as chrysophanol, aloe-emodin, rhein, salinosporamide, luteolin and emodin have in common a double hydroxylation at positions C-1 and C-8.

Essential oils

They mostly contribute to the odoriferous constituents or 'essences' of the aromatic plants that are used abundantly in enhancing the aroma of some spices [17]. Essential oils have been associated with different plant parts including leaves, stems, flowers, roots or rhizomes. Chemically, a single volatile oil comprises of more than 200 different chemical components, and

mostly the trace constituents are solely responsible for attributing its characteristic flavour and odour.

Steroids

Plant steroids (or steroid glycosides) also referred to as 'cardiac glycosides' are one of the most naturally occurring plant phyto-constituents that have found therapeutic applications as arrow poisons or cardiac drugs (Firn, 2010). Steroids (anabolic steroids) have been observed to promote nitrogen retention in osteoporosis and in animals with wasting illness [18, 19].

Alkaloids

Alkaloids are a structurally diverse group of over 12,000 cyclic nitrogen-containing compounds that are found in over 20% of plant species [20]. Although no single classification exists, alkaloids are often distinguished on the basis of a structural similarity (e.g. indole alkaloids) or a common precursor (e.g. benzyloisoquinoline, tropane, pyrrolizidine, or purine alkaloids). These are the largest group of secondary chemical constituents made largely of ammonia compounds comprising basically of nitrogen bases synthesized from amino acid building blocks with various radicals replacing one or more of the hydrogen atoms in the peptide ring, most containing oxygen. The degree of basicity varies considerably, depending on the structure of the molecule, and presence and location of the functional groups [21,]. They react with acids to form crystalline salts without the production of water [15].

Table 2: Structural Features and Activities of Various Phytochemicals from Plants. [22]

Phytochemicals	Structural features	Example(s)	Activities
Phenols and Polyphenols	C ₃ side chain, - OH groups, phenol ring	Catechol, Epicatechin, Cinnamic acid	Antimicrobial, Anthelmintic, Antidiarrhoeal
Quinones	Aromatic rings, two ketone substitutions	Hypericin	Antimicrobial
Flavones		Abyssinone	
Flavonoids	Phenolic structure, one carbonyl group Hydroxylated phenols, C ₆ -C ₃ unit linked to an aromatic ring Flavones + 3-hydroxyl group	Chrysin, Quercetin, Rutin	Antimicrobial Antidiarrhoeal
Flavonols		Totanol	
Tannins	Polymeric phenols (Mol. Wt. 500-3000)	Ellagitannin	Antimicrobial, Anthelmintic, Antidiarrhoeal
Coumarins	Phenols made of fused benzene and α - pyrone rings	Warfarin	Antimicrobial
Terpenoids and essential oils	Acetate units + fatty acids, extensive branching and cyclized	Capsaicin	Antimicrobial Antidiarrhoeal
Alkaloids	Heterocyclic nitrogen compounds	Berberine, Piperine, Palmatine, Tetrahydropalmatine	Antimicrobial, Anthelmintic, Antidiarrhoeal
Lectins and Polypeptides	Proteins	Mannose-specific agglutinin, Fabatin	Antimicrobial
Glycosides	Sugar + non carbohydrate moiety	Amygdalin	Antidiarrhoeal
Saponins	Amphipathic glycosides	Vina-ginsenosides-R5 and -R6	Antidiarrhoeal

Mechanism of Action of Phytochemicals

Different mechanisms of action of phytochemicals have been suggested. They may inhibit microorganisms, interfere with some metabolic processes or may modulate gene expression and signal

transduction pathways [23-25]. Phytochemicals may either be used as chemotherapeutic or chemo preventive agents with chemoprevention referring to the use of agents to inhibit, reverse, or retard tumorigenesis.

Table 3: Mechanism of Action of Some Phytochemicals [22]

Phytochemicals	Mechanism of action
Quinines	Binds to adhesins, complex with cell wall, inactivates enzymes
Flavonoids	Complex with cell wall, binds to adhesions, Inhibits release of autocoids and prostaglandins, Inhibits contractions caused by spasmogens, Stimulates normalization of the deranged water transport across the mucosal cells, Inhibits GI release of acetylcholine.
Polyphenols and tannins	Binds to adhesins, enzyme inhibition, substrate deprivation, complex with cell wall, membrane disruption, metal ion complexation, Makes intestinal mucosa more resistant and reduces secretion, stimulates normalization of deranged water transport across the mucosal cells and reduction of the intestinal transit, blocks the binding of B subunit of heat-labile enterotoxin to GM1, resulting in the suppression of heat-labile enterotoxin-induced diarrhea, astringent action, Increases supply of digestible proteins by animals by forming protein complexes in rumen, interferes with energy generation by uncoupling oxidative phosphorylation, causes a decrease in G.I. metabolism
Coumarins	Interaction with eucaryotic DNA
Terpenoids and essential oils	Membrane disruption, Inhibits release of autocoids and prostaglandins
Alkaloids	Intercalates into cell wall and DNA of parasites, Inhibits release of autocoids and prostaglandins, Possess anti-oxidating effects, thus reduces nitrate generation which is useful for protein synthesis, suppresses transfer of sucrose from stomach to small intestine, diminishing the support of glucose to the helminthes, acts on CNS causing paralysis
Lectins and polypeptides	Blocks viral fusion or adsorption, forms disulfide bridges.
Glycosides	Inhibits release of autocoids and prostaglandins
Saponins	Inhibits histamine release in vitro, Possesses membrane permeabilizing properties, Leads to vacuolization and disintegration of teguments
Steroids	Enhance intestinal absorption of Na ⁺ and water

In this sense chemo preventive phytochemicals are applicable to cancer therapy, since molecular mechanisms may be common to both chemoprevention and cancer therapy [26, 27]. Plant extracts and essential oils may exhibit different modes of action against bacterial strains, such as interference with the phospholipids bilayer of the cell membrane which has as a consequence a permeability increase and loss of cellular constituents, damage of the enzymes involved in the production of cellular energy and synthesis of structural components, and destruction or inactivation of genetic material. In general, the mechanism of action is considered to be the disturbance of the cytoplasmic membrane, disrupting the proton motive force, electron flow, active transport, and coagulation of cell contents [28].

MATERIALS AND METHODS

Chemicals

Fehling's Solution A&B and H₂SO₄ (BDH Laboratory Supplies Poole BH15, ITD, England). Molisch's Reagent & Benedict's Reagent (Made in Italy Packed

by: Hendoz Nig. Ltd), Millions Reagent (EGA-CHEMIE G-Britain). Benzene (Griffen & George Limited London). Ethanol from Sigma-Aldrich Chemical Co. (St. Louis, USA), Vitamin C, Methanol, Chloroform, Ethyl Acetate, Hydrochloric Acid, Sodium Hydroxide, Hexane and all others solvent (Analytical grade) from Merck Co. (Darmstadt; Germany), and Distilled Water.

Materials

Fresh *Cassia ferruginea* root (Schrad D.C) plant was collected during the flowering period (April 2015 to May 2015) from Okehi Local Government Old Quarters Obangede Kogi State, Nigeria. The plant was identified and confirmed at the Biological Department, Federal College of Education Okene Kogi State by Mrs. Aniana S.O.A. a botanist. The roots of *Cassia ferruginea* (Schrad D.C) were collected and washed with pure water, and then; the bark was removed from the core separately.



Fig-2: Cassia ferruginea Root Core Extraction Method

One thousand grams (1kg) of the root bark powdered of *Cassia ferruginea* (Schrad D.C) plant materials (20 mesh~1g) were extracted using percolation process in a mixture of 95%ml ethanol and 5 ml of distilled water at ambient temperature overnight. While the root core was chopped into pieces with a sharp cutlass and air dried for 2-3 weeks, the well dried root core materials was pounded into slightly powdered form (coercive) with mortar and pestle. The extractives was filtered and re-extracted three times for three days.



Fig-3: Root bark extractives



Fig-4: Root core extractives

The combined extract were filtered through a Whatman No. 1 paper and then concentrated in *vacuo* at 40°C using a rotary evaporator, model W2-100

SENCO® @ rpm of 100; Shanghai SENCO technology Co, Ltd Japan. The various extractive concentrates were evaporated to dryness using water bath for some days and residues were obtained in gram for both the root bark and the core of *Cassia ferruginea* plant (Schrad D.C) [29].

Phytochemical Screenings of the Ethanolic Extractives of *Cassia ferruginea* (Schrad D.C) plant

Preliminary Phytochemical screening was done using standard procedures to identify constituents, as described by [30, 31]. It involves testing of different classes of compounds. The methods used for detection of various phytochemical were followed by qualitative chemical test to give idea regarding the nature of constituents present in *Cassia ferruginea* (Schrad D.C) root and core ethanolic extractives. The procedures are as follow;

Tests for simple sugar

Fehling's test

1 ml Fehling's A solution and 1 ml of Fehling's B solution were mixed and boiled for one minute. Now the equal volume of test solution was added to the above mixture. The solution was heated in boiling water bath for 5-10minutes. First a yellow, then brick red precipitate was observed.

Benedict's test

Equal volumes of Benedict's reagent and test solution were mixed in a test tube. The mixture was heated in boiling water bath for 5 minutes. Solutions appeared green showing the presence of reducing sugar.

Molisch's test

Equal volumes of Molisch's reagent and test solution were mixed in a test tube. The mixture was heated in boiling water bath for 5 minutes. Appearance of violet or purple colour ring showing the presence of reducing sugar.

Test for Proteins

Biuret Test

To the small quantity of extract 1-2 drops of Biuret reagent was added. Formation of violet colour precipitate showed presence of proteins.

Million's Test

To the small quantity of extract 1-2 drops of Million's reagent was added. Formation of white colour precipitate showed presence of proteins.



Fig-5: Phytochemical Screenings results (Proteins)

Test for Anthraquinone glycosides

Borntrager's Test

To the 3ml of extract, dil. H_2SO_4 was added. The solution was then boiled and filtered. The filtrate was cooled and to it equal volume of benzene was added. The solution was shaken well and the organic layer was separated. Equal volume of dilute ammonia solution was added to the organic layer. The ammonia layer turned pink showing the presence of Anthraquinone glycosides.

Test for Cardiac glycosides (Keller-Killiani Test)

To the 5ml of extract, 1ml of conc. H_2SO_4 , 2ml of Glacial acetic acid and 1 drop of $FeCl_3$ solutions was added. Appearance of Brown ring shows the presence of cardiac glycosides.

Test for Coumarins

To the 2ml of extract 10% NaOH was added and shake well for 5mm shows the yellow colour.

Tests for Quinone

To the 2ml of extract conc. H_2SO_4 added and shake well for 5 mm shows the Red colour.

Test for steroids

Salkowski Test

To 2ml of extract, 2ml of chloroform and 2ml of conc. H_2SO_4 was added. The solution was shaken well. As a result chloroform layer turned red and acid layer showed greenish yellow fluorescence.

Test for alkaloids

Hager's Test

To the 2-3ml of filtrate, 1ml of dil. HCl and Hager's reagent was added and shake well. Yellow precipitate was formed showing the presence of alkaloids.

Mayer's Test

To the 2-3ml of filtrate, 1ml of dil. HCl and Mayer's reagent was added and shake well. Formation of yellow precipitate, showed the presence of alkaloids.

Dragendroff's Test

To the 2-3ml of filtrate, 1ml of dil. HCl and Dragendroff's reagent was added and shake well.

Formation of orange-brown precipitate showed the presence of alkaloids.

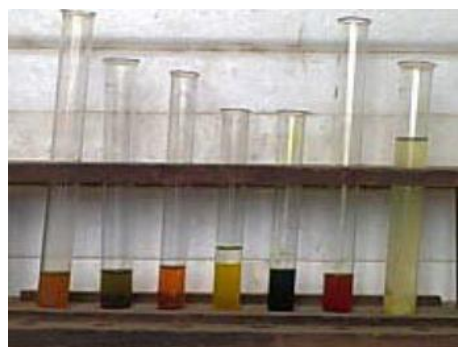


Fig-6: Phytochemical Screenings results (alkaloids)

Wagner's reagent test

To the 2-3ml of filtrate, 1ml of dil. HCl and Wagner's reagent was added and shake well. Formation of reddish-brown precipitate showed the presence of alkaloids.

Test for Flavonoids

With Lead Acetate

To the small quantity of extract lead acetate solution was added. Formation of yellow precipitate showed the presence of flavonoids.

Test for Tannins and Phenolic compounds

$FeCl_3$ Solution Test

On addition of 5% $FeCl_3$ solution to the extract, deep blue black colour appeared.

Lead Acetate Test

On addition of lead acetate solution to the extract white precipitate appeared.

Test for Saponins

Foam Test

To 1ml extract 20ml distilled water was added and shakes well in measuring cylinder for 15min. Then 1cm layer of loam was formed.



Fig-7: Phytochemical Screenings results (Foam Test)

RESULTS AND DISCUSSIONS

The Phytochemical analysis for ethanolic extractives of *Cassia ferruginea* (Schrad D.C) was determined. It revealed the presence of all tested phytochemical compounds such as Alkaloids, Steroids, Saponins, Phenolic, Tannins, Fixed Oil& Fat, Proteins, Anthraquinone Glycosides, Cardiac Glycosides, Flavonoids, Quinone and Coumarins except Carbohydrate as shown in Table 4. As it is expected for ethanolic solvent used being an active component extraction [29]. Therefore, the presence of these secondary compounds validates the use of *Cassia ferruginea* root (Schrad D.C) bark and its core as herbal

drugs anywhere they are found. Plant synthesizes a great variety of chemical substances. To date, some 12,000 different substances have been identified, and there surely are many more to be discovered and analyses. Of all these chemical substances, the active principles are those which produce a specific effect on the human body depending on their chemical nature.

The absence of simple sugar in both the root barks and root core may be the fact that photosynthesis of such components only takes place in the leaf of a plant [32]. These involved two phases:

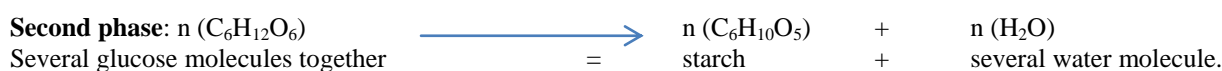
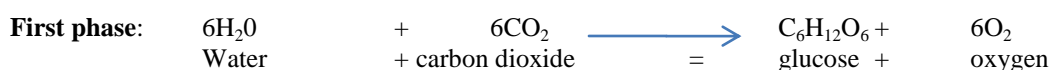


Table 4: Preliminary Screening of *Cassia ferruginea* (Schrad D.C) plant root

S/N O	Constituent	Chemical	Root bark	Root core
1	Alkaloids	Hager's Reagent	+	+
		Dragendroff's Reagent	+	+
		Mayer's Reagent	+	+
		Wagner's Reagent	+	+
2	Carbohydrate & reducing sugar	Fehling's Regent	-	-
		Benedict's Regent	-	-
		Molisch's Regent	-	-
3	Steroids	Salkowski Regent	-	+
4	Saponins	Foam	+	+
5	Phenolics& Tannin	FeCl ₃ Sol.	+	+
		Lead Acetate	+	+
6	Fixed oil & fats	Spot test	+	+
7	Proteins	Biuret Reagent	+	-
		Million's Regent	+	-
8	Anthraquinone glycosides	Borntrager's Reagent	+	-
9	Cardiac glycosides	Keller-Killiani Reagent	+	+
10	Flavonoids	Lead Acetate	+	+
		Extract + NH ₃	+	+
11	Quinone	Extract + Conc. H ₂ SO ₄	+	-
12	Coumarins	Extract + 10% NaOH	+	-

Key: + = present - = absent

The root is the organ in charge of absorbing water and minerals from the soil, and pumping them up to the leaves in order to feed the whole plant. most plants usually produce starch, inuline and other

sugars(also known as carbohydrates) which are stored in the roots, while some root of other plant synthesize alkaloids, glycosides, vitamins and other phyto-constituents. In some cases only the root bark is usually

useful, because the active components are more concentrated in it. However distribution of such component can be between the root bark and the root core. Attentions are mainly to the study of the root bark

only while the other important components found in the root core are neglected. However, they have shown different physical properties after extraction and evaporation as in Table 5;

Table 5: Showing the Physical Properties of the Crude Extractives from *Cassia ferruginea* (Schrad D.C) plant root

Extract	Colour	Texture	Solubility	Weight (g)
Root bark	Brown	Creamy	Polar solvent	1.8
Root core	Golden yellow	Solid	Polar solvent	1.0

Phytochemicals are known to possess antimicrobial properties as reported [29]. This showed that the whole *Cassia ferruginea* (Schrad D.C) root is rich in chemical constituents as investigated in this study. These principles have been known for many years to exhibit biological activity, such as effects on the central nervous system, and antibacterial, antitumour, and anthelmintic activity. Thus, the presence of these phyto-constituents in the *Cassia ferruginea* (Schrad D.C) root bark and root core such as alkaloids which act as antiparasitic and treating gout [33]; tannins act against diarrhea [34]; steroid compounds as hormone stimulator and regulator [35]; and in the treatment of prostate cancer [36]; Saponins as anti-inflammatory, hypocholesterolemic and immune stimulating [37]; flavonoid prevent ulcer [38]; cardiac glycoside as potential anticancer [39]; and phenolic compound possess anti-cancer ability [40] has indeed support the acclaimed wonders and their various uses traditionally. Thus, natural agent such as, water, the sun, air and Phytochemical constituents (medicinal herbs) which are gotten from healthy food, as well as the adoption of healthy habits (physical exercise, adequate rest, good mental health and trust in God) may

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do more for health than all powerful, chemically synthesized medicines or aggressive treatment.

CONCLUSION

The result from this study on Nigeria *Cassia ferruginea* (Schrad D.C) root bark and root core lends strong support to their uses. This is the first time quantitative phytochemical analysis of constituents is carried out on *Cassia ferruginea* (Schrad D.C) root bark and root core as compared. More so, the root core of the plant possess additional medicinal important component i.e. steroid apart from the ones present in root bark. The root core will be centered areas for further scientific research findings in isolating their active component. Thus, pharmacological screening of this medicinal plant (*Cassia ferruginea* (Schrad D.C) root core will thereby provide a scientific basis for the continued potential sources of new, effective and safe drugs.

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