PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ANALYSIS OF AQUEOUS LEAF EXTRACT OF COFFEE SENNA (CASSIA *OCCIDENTALIS LINN*)

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ABSTRACT
Phytochemical screening and antibacterial analysis of aqueous leaf extract of Coffee sennawere carried out using standard biochemical procedures. Phytochemical screening of the extracts revealed the presence of alkaloids, tannis, steroids, volatile oils as well as saponin glycosides as the most abundant. Other phytochemicals identified in the extract were flavonoids, saponnins, and cardiac glycoside as moderately present. Balsam and terpenes were minutely present, while anthraquines was absent. Antibacterial screening of leaf extract of coffee senna(Cassia occidentalis) was carried out using agar well diffusion method for bacterial isolates(Bacillus subtilis, Escherichia coli, Salmonella paratyphi, Pseudomonas aeruginosa and Staphylococcus aureus). The result showed that the mean zones of inhibition were higher at 60 and 90 mg/ml against S. paratyphiand S.aureuswith 17.0 and 17.0 mm zones of inhibition respectively. The results showed that the extract of Cassia occidentaliscould be potent in inhibition of the studied microorganisms. Thus, the studied plant materials could be further investigated so as to validate their potent role in the treatment of infectious diseases caused by the tested microorganisms.

Keywords: Phytochemical, antibacterial, screening, leaf, Cassia occidentalis

INTRODUCTION

Plants are the pavement bricks of all the livingorganisms on the earth. They produce a wide range of secondary metabolites such as alkaloids, unsaturated fatty acids, flavonoids, phenols, tannins and terpenes that can be used to treat various chronic and infectious diseases (Reichling*et al.*, 2010).Plants have been used astraditionalmedicine andpharmacopoeialdrugs from ancient times. Most ofthe world's population is dependent on the plant due to its medicinal value and scarcity(Vijay *et al.*, 2013). Medicinal plants have been used for the treatment of illness since ancient periodand numerous plant-derived therapeutic agents for modern medicine have been provided by medicinal plants (GajaLakshmi*et al.*, 2012).Most of the plants exhibit a variety of phytopharmaceuticals, which has important applications in the fields of agriculture, human and veterinary medicine. This plays a major role in developing novel drugs for the treatment and prevention of diseases (Muhammed *et al.*, 2012).Therefore it is very important to have sufficient knowledge regarding herbs not only because of their widespread uses but also because they have the potentials to cause toxic reactions or interact with other drugs (Vijay *et al.*, 2013). Although in traditional medicine Cassia species have been wellknown for their

laxative and purgative properties and for the treatment of skin diseases (GajaLakshmi*et al.*,2012).

Cassia occidentalis is one of the many legumes shrubs the second most important source of human food and animal forage. It is through nitrogen fixation that legumes provide plant tissue that is high in protection. In agricultural practice, the species is used to amend the soil and reduce crop nutrient deficiency. As a green crop, the species provides many benefits to the soil. Nitrogen fixation provides a usable source of nitrogen to the legumes in return for carbohydrate needed by the bacteria. In addition to those plants cultivated for human consumption, many of such crops yield important fodders, as well as green manures (Sadik*et al.,* 2012). Herbs and shrubs have always been helpful to cure diseases. The practice of herbal medicine dates back to very earliest period of known human history. There is evidence of herbs and shrubs having been used in the treatment of diseases and for revitalizing body system in almost all ancient civilizationKar*et al.* (2004).

There is renewed interest in traditional medicine and increasing demand for many drugs from plant sources. Initial screening of plant for possible antibacterial activities typically beings by using crude aqueous or alcohol/ethanol extraction method. Natural products are known to play an important role in both drug discovery and chemical biology (Daniyan*et al.*,2011). There is continuous and urgent need to find or to develop new antimicrobial compounds with a novel mechanism of action for new and emerging infectious diseases (Al-snafi, 2016). Plants have a wide spectrum of biological activities and have to be a major source of traditional medicine because of their valuable physiological and pharmacological properties (Sadik*et al.*,2012).

PATHOGENS AND MICROBES

Because of the presence of microbes in all walks of human life, there is constant interaction between microbes and humans, the vast majority of bacteria in the body are renderedharmless by the protective effects of the immune system. Certain microbes can help us in the fight against other microbes. Microbes are a source of antibiotics and vaccines (Kumburawa*et al.,* 2007). Antibiotics are those substances produced by microorganisms useful in killing or inhibition of other microbes. Vaccines are substances derived from microorganisms and are used to immunize against diseases. Those microbes that normally live in association with human on the various surfaces of the body are known to protect their hosts from infections and otherwise promote nutrition and health. Infectious diseases caused by pathogenic microbes are responsible for more death than any other single cause. There are many ways that bacteria and other microbes can negatively affect human life. Microbes are the agent of food spoilage and decomposition of clothing and sheltering materials (Muhammed *et al.,* 2012).

Cassia occidentalis is a tropical shrub with small multiple branches up to two meters high. It is an annual or biannual herb with ribbed stem and conspicuous green color with an unpleasant smell. The leaves are compound pinnate with 3 – 6 pairs of leaflets. The leaflets are ovate or ovate-lanceolate with terminal pair always larger. There are six to eight pairs of lateral nerves joining in loops to their neighbors. There are two small and three large petals that are yellow in color. There

are small ovate brown seeds is clearly visible chambers at a right angle to the middle axis (Balkeema, 2009). Leaves have petioles that are from one to two inches in length; the stem erect without hairs, cotyledons are broad and round without hair (glabrous) and have distinctive veins. Roots are taproots and fibrous root system and flowers occur in the axial of the upper leaves (Vashishtha*et al.*, 2009).

Leaves of *Cassia occidentalis* exhibit variable lengths and width from the measurement of some plants used. The leaves are broad to the size of about 3 – 5cm to 4.0cm and length of about 5.0cm to 6.0cm. The flower of the plant has been found to be 8.0cm to 10cm inlength, with a breadth of about 0.6cm to 0.7cm. The pods of the plant contain 16 to 22 seeds.

Different parts of *Cassia occidentalis*are employed by local inhabitants of the tropical region for the treatment of typhoid fever. The active principle of many drugs found in plants is phytochemicals.Themedicinal value of these phytochemical is because of the presence of chemical substances that provide definite physiological action on the human body. The leaves are used as a broad spectrum, internal and external anti-bacterial to treat bacterial infections, also used for liver disorder e.g. Lapatitis, detoxification (Ali *et al.*, 2015).

Classification of the Plant (*Cassia occidentalis*)

Based on the classification system, *Cassia occidentalis*is classified as shown below:

i. **Kingdom**: Plantae
ii. **Sub-division:** Spermatophyta
iii. **Family:** Leguminoseae
iv. **Sub-family:** Ceasalpiniaceae
v. **Class:** Angiosperm
vi. **Genus:***Cassia*
vii. **Species:***occidentalis*
viii. **Binomial name:***Cassiaoccidentalis* **(**Linn**)**

The plant "Sanga-Sanga" popularly known by the Hausa speaking people of Sokoto belong to the family Leguminosae, genus *Cassia*andspecies*occidentalis*. *Cassia occidentalis* is a shrub that grows 5 – 8cm. in Nigeria, it is in the same genus as Senna. Furthermore, the *Cassia*group comprises of some species of tree, shrubs, and herbs with numerous species growing in the forest and tropical areas. Many species have been explored medicinally, and these tropical plants have a history in natural medicine as purgative and laxative (Ali *et al.*, 2015). The species has been a subject of recent clinical researches for its beneficial effects on the liver and immune system (Vijay *et al.*, 2013).

Phytochemicals Screening

The biological activity of medicinal plants is closely related to the plant chemicals in it. These chemicals can be classified into major groups such as alkaloids, steroid, tannins, saponin, glycoside

(Kenneth, 2006). The phytochemical effects of ethanol extract of *Cassia occidentalis* were evaluated. Among the key phytochemicals as reported by Hussaini (2001) were:

- **Alkaloids:** Are a group of naturally occurring chemical compounds that contain mostly basic nitrogen atoms with some synthetic compounds of similar structure. They are produced by a large variety of organisms including bacteria, fungi, plant, and animals.
- **Saponins** Are class of chemical compounds, one of many secondary metabolites found in natural sources, found in various plant species but are also isolated from marine organisms.
- **Steroids:** Are the class of chemicals that contains a characteristic arrangement of four cycloalkane rings.

For instance, plant-rich in saponins generally have anti-inflammatoryproperties (Kenner and Requena, 1996). Tannins have been reported to have antibacterial potentials; the basic character of tannins allows them the ability to react with proteins to form stable, water-soluble compounds. Since all animals cell (that of microorganisms inclusive) are made up of protein, there is a possibility that some tannins compounds may be able to selectively react with some vital portentous compounds (such as enzymes) in a pathogenic bacteria thereby inhibiting its life activities (Dangoggo*et al.*, 2006). Also, tannins kill bacteria directly by damaging the cell membrane (Elmarie, 2001).

Antibacterial Activity

Antibiotic is among the most important weapons in fighting bacterial infections and have greatly benefited the health quality of human life since their introduction. However, under the past few decades, these health benefits are under threat as many commonly used antibiotics have less effective against certain illness, not only because many of them produce a toxic reaction. Several workers have investigated a wide range of plant activity against malaria parasite, parasite protozoan, among others (Rai *et al.*, 2003). Drugs derived from natural sources play a significant role in the prevention and treatment of human diseases. Higher plants may give a new source of antibacterial agent with possibly novel mechanisms of action (Vijay *et al.*, 2013).

Aim and objectives of the research

The aim of the research was to determine the antibacterial potential and phytochemical compositions of leaf extract of *Cassia occidentalis*. The aim was achieved through thefollowing objectives

a. To determinethe phytochemical composition of aqueous leaf extract of Coffee senna.
b. To investigate the antibacterial potential of leaf extract of Coffee senna (*Cassia occidentalis)* against some selected pathogenic bacteria.

MATERIALS AND METHODS

The apparatus, solvents, and reagents used in this research are as follows:

Apparatus and Reagents used

Funnel, beakers, test tube, burettes Conical flask, weighing balance, measuring cylinder, wire loop, autoclave, indicator. Among the reagents used were: Sodium hydroxide (NaOH), ferric chloride, concentrated sulphuric acid (H_2SO_4), concentrated hydrochloride (HCL), ethanol, nutrient agar solution, wanger's reagent, Mayer's reagent, Fehlin solution, Benzene, ammonia solution, distilled water.

Test Organisms

Five (5) laboratory isolates of bacteria were collected from the Department of Microbiology of UsmanuDanfodiyo University, Sokoto. The bacteria isolates were:

i. *Staphylococcus aureus*
ii. *Salmonella paratyphii*
iii. *Bacillus subtilis*
iv. *Escherichia coli and*
v. *Pseudomonas aeruginosa*

Collection of Plant Materials

Fresh leaves of *Cassia occidentalis*were collected from Botanical Garden UsmanuDanfodiyoUniversity, Sokoto. The collected species are weed and rain-fed one. The plant took five months to reach its seed stage. After collection, the leaves were cut off and were air-dried and later grounded into powdered form.

Extraction Procedure

Sixty-five grams (65g) of the sample was weighed on weighing balance and placed into a sterilized baker. Two hundred and fifty mills (250ml) distilled water poured into the beaker. The sample was filtered using Whatman filter paper and the mixture was heated ten minutes (10) and kept for 24 hours according to the procedure as described by Charles (2009).

Phytochemical Screening Test

1. **Test for Flavonoids:** Three mills (3ml) aliquot of the filtered extract and one mill (1ml) of 10% sodium hydroxide (NaOH). The presence of yellow color indicates the presence of flavonoids as describes by (El-Olemy*et al.*, 1994).
2. **Test for Tannins:** Five percent (5%) of ferric chloride solution was added drop by drop, into 2 – 3ml of the extract and the color produced was noted, condensed tannins usually give a dark green color, while hydrosable tannins give blue-black color (Harbone, 1988).

3. **Test for Saponins:** Five mills (5ml) of the extract was poured in a test tube plus five mills (5ml) of water and shake strongly. The whole tube was filled for several minutes (Wall *et al.*, 1954).
4. **Test for Glycosides:** Two and a half mills (2.3ml) of 50% H_2SO_4 was added to five ($5cm^3$) of the extracts in a test tube. The mixture was heated in boiling water for fifteen (15 minutes) cooled and neutralized with 10% NaOH, five mills (5ml) of Fehlin solution was added and the mixture was boiled. A brick-red precipitate was observed which indicates the presence of glycosides (Harbone, 1973).
5. **Test for Alkaloid:** About two mills (2ml) of the extract were stirred with two mills (2ml) hydrochloric acid. One mill (1ml) was treated with few drops of Wangers' reagent and the second one mills (1ml) was treated similarly, with Mayer's reagent. Precipitates with either of these reagents were taken as preliminary evidence for alkaloids (Harbone, 1973).
6. **Test for Cardiac Glycosides:** Two mills (2ml) of 3.5% ferric chloride solution was added to the extract and allowed to stand for one minute, one mill (1ml) of concentrated H_2SO_4 was carefully poured down the wall of the tube so as to form a lower layer. Reddish-brown ring indicated the presence of cardiac glycoside (Harbone, 1973).
7. **Test for Steroids:** A half mill (0.5ml) of the extracts was mixed with two mills (2ml) of chloroform. Two mills (2ml) of sulphuric acid was added to form brown color which indicated the presence of steroid ring.
8. **Test for Saponin Glycosides:** Two and a half mills (2.5ml) of the extract was added, two and a half mills (2.5ml) of Fehling solution A and B. A blue-green precipitate showed the presence of saponin glycosides (El-Olemy*et al.*, 1994).
9. **Test for Anthraquines:** Half mill (0.5ml) of the extract was shaken with ten mills (10ml) benzene, and five mills (5ml) of 10% ammonia solution was added. The mixture was shaken and the presence of a pink-red or violet in the ammonical phase indicated the presence of anthraquine (El-Olemy*et al.*, 1994).
10. **Test for Volatile Oil:** One mill (1ml) of the fraction was mixed with dilute hydrochloric acid (HCL). A white precipitate was formed that indicate the presence of volatile oil (Evans, 1980).
11. **Test for Terpenes:** First portion of the chloroform solution from the extract was mixed with one mill (1ml) acetic anhydride. One cm^3 ($1cm^3$) of concentrated sulphuric acid added down the wall of the test tube to form a layer underneath. A reddish-brown color was formed which indicated the presence of terpenes.

Media Preparation

The media used were nutrient age for the growth of bacteria. The media was prepared according to the manufacturer's instructions.

Antibacterial Analysis

Seven gram (7g) of the finely powdered nutrient ager was weighed actively according to the manufacturer's instruction. The weighed ager was dissolved in 100cm^3 of distilled water in a conical flask and was heated on a plate until it was dissolved completely. The flask was covered with cotton wool to prevent contaminants from entering the flaks, and was sterilized at 121^{o}C for about 15 minutes and removed. The six (6) sets of Petri dished were also placed onto hot air oven and sterilized for one hour. The nutrient agar was poured into the Petri dishes, and the dishes with agar were allowed to dry in the oven until the agers solidify. Four wells of 12.0 mm in diameter were made in the plates with a sterile cork borer. The organisms were inoculated media and rubbing them on the prepared ager. The paper discs prepared with organisms were placed in an incubator maintained at 37^{o}C and allowed to incubate for 24 hours. The activity of the extract was seen as a zone of clearance (Charles, 2009).

RESULT

Phytochemical Screening Result

The leaf extract of *C. occidentalis* was tested for the presence of flavonoids, tannins, saponnins, alkaloids, volatile oils, cardiac glycosides, steroids, saponnin glycosides, terpenes, balsam, and anthraquines.

Table 1: Phytochemicals identified in the leaf extract of *C. occidentalis*

Test	Extract Aqueous
Flavonoids	+ +
Tannins	+ + +
Saponins	+ +
Volatile oils	+ + +
Alkaloids	+ + +
Cardiac glycosides	+ +
Steroids	+ + +
Saponin glycosides	+ + +
Terpenes	+
Balsam	+
Anthraquines_ _	

Key:

+ + + = Present in high concentration

+ + = Moderately present

+ = Trace

- = Absent

Result of Antibacterial Sensitivity Test

The result of the antibacterial activity of the leaf extract of *Cassia occidentals*at various concentrations (5, 30, 60 and 90 mg/ml) on the tested pathogenic bacteria (*S. aureus, E. coli, B. subtilis, Salmonella paratyphii, and Pseudomonas aeruginosa*) have been presented in table 2 below:

Table 2: Does dependent antibacterial activity of aqueous leaf extract of *Cassia occidentalis* on bacteria isolates.

Diameter of zone of inhibition (mm)/bacteria species at Different concentration (mg/ml) Organisms	**5.0**	**30.0**	**60.0**	**90.0**
Bacillus subtilis	5.0	10.0	15.0	16.0
Escherichia coli	5.0	__	__14.0	
Staphylococcus auretus	5.0	12.0	17.0	__
Salmonella paratyphii		__15.0	14.0	17.0
Pseudomonas aeruginosa			__	__ 14.0 16.5

Diameter of cork borer used = 12mm; Method: Ager well diffusion technique

DISCUSSION

The result of phytochemical screening of the extract was in Table 1. The result indicated the presence of Alkaloids, flavonoids, tannins, saponnins, steroids, glycosides, cardiac glycosides, terpenes, balsam, etc. In the aqueous extract, alkaloids, are present but with the exception ofanthraquines in aqueous extracts. The presence of these components found in the plant is a strong indication that the plant may have some varied medical potentials. This is because each of the components found in the plant is a strong indication that the pant may some varied medical potentials. This is because each of the components present has record of one therapeutic usage or another.

The study showed dose-dependent antimicrobial activity of aqueous leaf extract of *C. occidentalis* against the isolated pathogenic bacteria *(Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, and Samonellaparatyphii).* The result from this study showed that the *C. occidentalis* could inhibit the growth of the above-mentioned organisms but at higher concentrations (30, 60 and 90 mg/ml). The result showed that the mean of zones of inhibition was higher at 60 and 90 mg/ml against *S. aureus and S. paratyphii* respectively. This is in contrast to what was reported by Kumar *et al.,* (2009) whose result shows that *B. subtilis* was more sensitive to the extract than *S. aureus*. In another report by Paridar (2011), *E. coli* was the most sensitive organism and *B.subtilis* showed least sensitivity while *S. paratyphi* was not sensitive to any of the oils of (fruit, stem, and leaf) against leaf extract of *C. fistula* (L). The phytochemical screening result showed the presence of all the tested phytochemicals exceptanthraquines which is

in contrast to what reported by Thatoi*et al.*, (2008) with the presence of anthraquines in high concentration.

The result of the study showed the fact that *C. occidentalis* could be used regarded as anti-microbial against *B. subtilis, S. aureus, and E. coli.* This corroborates the report of Ali *et al.*, (2015).

CONCLUSION

The phytochemical screening showed that the leaves extract of the *Cassia occidentalis*are rich source of alkaloids, saponins, glycosides, cardiac glycosides, balsams, terpenes, and the antibacterial screening showed that they exhibit inhibitory activities against the test organisms. The result obtained showed that *C. occidentalis*could be exploited in the treatment of infectious diseases caused by the studied microorganisms due to the inhibition properties shown by the leaves extract on the tested organisms

RECOMMENDATION

1. More effort should be geared towards planting this plant in order to exploit its full potential for the betterment of humanity.
2. Since *C. occidentalis*had been found to have medicinal uses, there is a need to conserve it in order to ensure its continuity.
3. More research effort should be directed onto this species, in order to further validate many claims on the species.

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