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Evaluation of Phytochemical Constituents and Antibacterial Activity of Methanolic and Aqueous Leaf Extracts of Coffee Senna (*Cassia occidentalis* L.)

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Cassia occidentalis commonly known as Coffee senna is a herbaceous plant that belongs to the family fabaceae. The plant is called "Sanga-Sanga" in Hausa, "Ewe Oriesi" in Yoruba, and "Akidi Agbara" in Igbo. Coffee senna is a pan-tropical plant. This research was aimed at evaluating the phytochemicals and antibacterial activity of extracts of leaves of Coffee Senna. Plant leaf samples were collected behind Department of Biological Sciences Federal University Gusau. The samples were air dried and grounded into powder using mortar and pestle. The phytochemical analysis of both methalonic and aqueous leaf extracts revealed the presence of Cardiac glycosides, Steroids, Flavonoids, Alkaloids, Saponin glycosides, Saponins glycosides, Tannins and Anthraquines. Volatile oils, Balsams and glycosides were absent in the extracts. Methanolic and aqueous extracts of leaves of *Cassia occidentalis* were screened for their antimicrobial activity against two bacterial strains by disk diffusion assay. The pattern of inhibition varied with the solvent used for extraction and the microorganism tested. Among these extracts, methanol and aqueous extracts showed significant antimicrobial activity against the tested microbes. The most susceptible microorganism was *Salmonella typhi* 22 mm zone of inhibition in both the extract (Methanol and aqueous extract), then, followed by *Streptococcus epidermidis* with 20 mm zone of inhibition in both the extract (Methanol and Aqueous). The study concludes that *Senna occidentalis* leaf extracts studied showed high inhibition of bacterial growth, therefore the plant can be used in ethno-medicine and the pharmaceutical industries.

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1. Introduction

Coffee senna (*Cassia occidentalis*) is a medicinal plant belongs to the family of caesalpiniaceae, subfamily caesalpinioideae. Coffee senna is a slender upright short-lived herb with 0.5-2.5 m tall and distinguished fetid odour. It is once a compound leave consisting of 3-7 pairs of leaflets (2-10 cm long and 2-3 cm wide) with pointed tip; amounted gland at the base of leaf stalks no glands between leaflets. There is a conspicuous dark coloured gland near the base of the stalk of each leaf. *Cassia occidentalis* is known as "ewe ori esi" in Yoruba and Sanga sanga or Rai dore in Hausa. It is an ayurvedic plant with huge medicinal importance (Bagega *et al*., 2018). Traditionally, the use of plants as source of herbal preparation for the treatment of various ailments is based on experience passed from generation to generation. The knowledge of medicinal plants by traditional healers is jealously guarded with utmost secrecy for economic reasons. Many traditional herbal practitioners hide the identity of plants used for the treatment of different ailments largely for the fear of patronage, should the patient learn to cure himself (Sase *et al*., 2020). Medicinal plants have been used over the years to treat various type of acute and chronic diseases. Medicinal plants contain a lot of bioactive constituents or phytochemical compounds which are secondary metabolites so called because they are not required for growth, respiration, transpiration or any primary function in plants (Musa *et al*., 2018).

The ethno-medicinal property of these indigenous medicinal plants lies within the rich array of chemical compounds that trigger a

cascade of events within the human body that boosts normal body functions including immuno-stimulation. Some of these compounds include alkaloids, flavonoids, tannins and phenolic compounds (Gali *et al*., 2016). Standardization of medicinal plants is an essential issue to be considered as a therapeutic drug for safety in health care (Tohani *et al*., 2020). Phytochemical are nonnutritive plant chemicals that have protective or disease preventive properties. Plant produces these chemicals to protect itself, but recent research demonstrates that many phytochemicals can protect humans against diseases. There are many phytochemicals in fruits and herbs and each works differently (Geetha, 2017). Infectious diseases are disorders caused by pathogenic microorganisms like bacteria, viruses, fungi, protozoa and multi cellular parasites. These diseases are also called as transmissible diseases since they can be transmitted from one person to another via a vector or replicating agent. Infectious diseases account for about half of the deaths in tropical countries especially Nigeria (Satha *et al*., 2012). This study was aimed to investigate the phytochemical constituents and antibacterial activity of the leaf extracts of *Cassia occidentalis*. Many researches has been carried out on phytochemical screening and antibacterial activity of *Cassia occidentalis* leaf extract, but little has been done on this plant in the study area. This research would provide information on the effectiveness of *Cassia occidentalis* leaves on the treatment of bacterial diseases in the study area.

2. Materials and Methods

2.1 Study Area

This research was conducted at Biochemistry and the Microbiology laboratories of Federal University Gusau, Zamfara State Nigeria. Gusau local government area is the capital of Zamfara state, located in the North-Western part of Nigeria.

2.2 Sample Collection and Preparation

Fresh leaves of *Cassia occidentalis* Leaves were collected during rainy season in the month of August 2021 behind Department of Biological Sciences of Federal University Gusau Zamfara State. The plant specimens were identified and authenticated by Sharhabilu Aliyu Gusau, a taxonomist and also in charge of the Herbarium of the Department of Biological Sciences Federal University Gusau. The voucher number obtained is FUG/BIO/HEB/21/0072. The leaves were shade dried for one week and grounded into powder using mortar and pestle, the powder was kept in clean polythene bag for the analysis.

Plate 1: *Cassia occidentalis* growing behind Department of Biological Sciences Federal University Gusau Zamfara State Nigeria.

2.3 Preparation of Extracts

Dried leaf materials were extracted successively with Soxhlet extractor at temperature of 100ºC. Each of the solvent; aqueous and the methanol were allowed to remain in contact with the plant material for 12 hours; the extracts were evaporated to dryness using rotary evaporator.

2.4 Phytochemical Screening of *Cassia occidentalis* Leaf Extracts

The extracts were analyzed for the presence of Alkaloids, Tannins, Saponins, Flavonoids, Glycosides, Anthraquinones, Cardiac Glycosides, Steroids, Saponins Glycosides, Balsam using the method as described by Sofowara (1993) Rahilla (1994).

2.5 Antibacterial Screening of *Cassia occidentalis* Leaf Extract

(a) ***Test organisms***

The two bacterial strains used in this study were *Salmonella typhi and Staphylococcus epidermedis* obtained from Microbiology laboratory Department of Microbiology Federal University Gusau Zamfara State Nigeria. *Salmonella typhi* is a gram-positive bacterium and *Staphylococcus epidermedis* is gram-negative bacterium.

(b) ***Preparation of Suspension***

The test organisms were suspended with sterile wire loop into sterile test-tube containing of sterile normal saline and mix thoroughly. The suspensions were Mcferland turbidity standard.

(c) ***Inoculum Preparation***

Pure culture was subculture and maintained on nutrient agar plates regularly. The culture was inoculated on sterile nutrient agar plate and place in an incubator at 37^{o}C for 24 hours. Inoculum was prepared by inoculating the pure bacteria cultured in nutrient both and incubates overnight at 37^{o}C.

(d) ***Antibacterial Bioassay***

The bacteria were streaked using sterile swab stick on the surface of Muellerhinton agar plate. Sterile circular filter paper was immersed in different concentration prepared of both test samples and standard. Then the pieces were placed on the surface of Muellerhinton agar all over with suitable gaps in between and incubated for dish and allowed to incubate for 24 hours at 37^{o}C. The plates were observed for diameter of the zone of inhibition of test and standard samples were measured in millimeters and recorded.

3. Results and Discussion

Phytochemical constituents of the leaf extracts of Coffee senna (*Cassia occidentalis*) methanolic and aqueous leaf extracts were analyzed. Results of methanolic *Cassia occidentalis* leaf extracts showed the presence of flavonoids (+), tannins (+), cardiac glycosides (+), alkaloids (+), steroids (+), saponin (+), saponin glycosides (+) and anthraquinones (+). Glycosides (-), balsams (-) and volatile oils (-) are absent. Results of aqueous leaf extract of Cassia occidentalis showed the presence of flavonoid (+), tannins (+), cardiac glycoside (+), alkaloids (+) and steroids (+). Saponin (-), glycosides (-), balsams (-), anthraquinones (-) and volatile oils (-) are absent (Table 1). In this study, phytochemical constituents of the plant leaves were found to contain flavonoids, tannins, cardiac glycosides, alkaloids and steroids while saponins, glycoside, saponins glycosides, balsams, anthraqunines and volatile oil were absent in the tested extracts. This present study was similar to finding of Yadav *et al*. (2009) and found that, a large number of alkaloids were found in stem, leaves and fruits of *Cassia occidendali*s from Ethiopia. The chemical constituents of the plants vary depending on the species, variety and part of the plant, condition of the plant growth (Soil, water and temperature), and with the age of the plant. The phytochemistry of the plant also varied to the geographical regions, season and time of collection and different climatic conditions (Chaudhury, 1999).

Table 1: Qualitative phytochemical constituents of the methanolic and Aqueous leaf extracts of Coffee senna (*Cassia occidentalis*)

S/N	Parameters	Methanolic extract Result	Aqueous extracts Result
1.	Flavonoid	+	+
2.	Tannins	+	+
3.	Saponins	+	-
4.	Glycoside	-	-
5.	Cardiac glycoside	+	+
6.	Saponin glycoside	+	-
7.	Alkaloid	+	+
8.	Steroids	+	+
9.	Balsams	-	-
10.	Anthraquinones	+	-
11.	Volatile oil	-	-

Key: + = present, - = absent

Results of antimicrobial activity of *Cassia occidentalis* leaf extracts were evaluated. Methanol extract was found very effective against *Salmonella typhi* with diameter zone of inhibition of (13 mm, at 50 mg), (18 mm,at 100 mg) and (22 mm,at 150mg) followed by the *staphylococcus epidermidis* (13 mm at 50 mg) (17 mm at 100 mm) and (20 mm at 150 mg) respectively (Table 2). The aqueous leaf extract *Cassia occidentalis* was found active against *Salmonella typhi* with diameter zone of inhibition of (9 mm at 50 mg). (13 mm at 100 mg). then, followed by *Staphylococcus epidermidis* with (8 mm at 50 mg) (13 mm at 500 mg) and (19 mm at 150mg) Diameter zone of inhibition respectively (Table 3). Furthermore, the result of this research showed remarkable variations in the effectiveness of the leaves extract against *S. typhi* and *S*. *epidemidis.* This study revealed that, *S. typhi* and *S. epidemidis* found to be sensitive at concentration of 150mg/mL against *S. typhi* (Inhibition zone 22 mm in both the extracts (methanolic and aqueous extract) respectively. This present finding agrees with the findings of Saganuwan and Gulumbe. (2006) who worked on evaluation of *in intro* antimicrobial activities and phytochemical constituents of *C. occidentalis,* and observed that, *S. Typhae* and *S. epidermidis* were sensitive to methanol and aqueous extracts of *C. occidentalis* leaves. Similarly, Jain *etal*. (1998) worked on antimicrobial screening of *C. occidentalis* L. *in vivo* and *in vitro and* observed that, the metabolite refraction of (anthraquines) leaves, pods, flower and callus were effectives against *E. Coli* (inhibition Zone 22mm). In contrast, the previous study revealed that, the petroleum either and methanolic extract of the leaves *C.*

occidentalis, was effective against *E. coli* at concentration of 400mg/ml with 5 and 11mm inhibition zone respectively (Isah and Mujib, 2013).

Table 2: Anti-bacterial activity of methanolic leaf extracts of *Cassia occidentalis*

Test Organisms	Extract at Different Concentration (mg)		
	50g/mL DIZ (mm)	100g/mL DIZ (mm)	150g/mL DIZ (mm)
Salmonella typhi	13	18	22
Streptococcusepidemidis	13	17	20

Key: DIZ Diameter Zone of Inhibition in mm.

Table 3: Anti-bacterial activity of aqueous leaf extract of *Cassia occidentalis*

Test Organisms	Extract at Different Concentration (mg)		
	50mg/mL DIZ (mm)	100mg/mL DIZ (mm)	150mg/mL DIZ (mm)
Salmonella typhi	9	13	22
Streptococcus epidermidis	8	13	19

Key: DIZ Diameter Zone of Inhibition in mm.

4. Conclusion

It could be concluded that, C*assia occidentalis* studied showed the presence of phytochemicals of both aqueous and methalonic leaf extracts. Anti-bactrial activity of the aqueous and methanolic leaf extracts showed higher zones of inhibition at concentration of 100 mg/mL and 150 mg/ml against *Salmonella typhi* and *Staphylococcus epidermedis.* Therefore, the plant can be used as a source of crude drug in pharmaceutical industries in the production of antibacterial drugs.

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Conflict of interest

The authors declare that there is no conflict of interest.

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