EVALUATION OF *IN-VITRO* ANTIMICROBIAL ACTIVITIES AND PHYTOCHEMICAL CONSTITUENTS OF *Cassia occidentalis*

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ABSTRACT

The research was carried out to evaluate the in-vitro antimicrobial activity and phytochemical constituents of Cassia occidentialis. Cassia leaves were collected from Kacha town in Niger State and extracted using methanol, hexane, chloroform and water extraction methods. Serial concentrations: 50, 60, 70, 80, 90 and 100 % methanol, hexane, chloroform and aqueous extracts were prepared and sterilized. The bacterial isolates used; E. coli, P. multocida, S. typhi, S. typhimurium, S. pyogenes, S. pneumoniae and K. pneumoniae were authenticated using biochemical and serological methods. The suspension (0.5) of each bacterial isolate was prepared in isotonic sodium chloride. The disc agar diffusion method was performed on 70 Mueller-Hinton agar plates, 10 per microorganism , using serial diffusion concentration: 500, 600, 700, 800, 900 and 1000 mg of hexane, methanol, chloroform and water. The results showed that all the extracts of Cassia occidentalis have antimicrobial activity on E. coli at concentrations between 900 – 1000 mg. E. coli was most susceptible to hexane extract at concentration ranges between 500 – 1000 mg, there was no antimicrobial activity exhibited against the other tested microorganisms. Phytochemical analyses showed the presence of alkaloid, tannin, saponin, glycoside and flavonoid, steroid was absent.

Keywords: Evaluation, *In-vitro,* Antimicrobial activity, Phytochemical properties, *Cassia occidentalis*

INTRODUCTION

Cassia occidentalis (Caesalpiniaceae) is a small tree growing 5 – 8 metres in height (Blumgarten, 1937). The leaves are compound, composite, paripinnate with 5 – 8 pairs of leaflets usually oval. Inflorescence occurs as auxiliary or terminal, yellow, short cluster of flower. Fruit is a pod, narrow, flat, slightly curved about 15 cm long with 10 – 12 seeds, brownish at maturity (Mann, 2003).

Traditionally, its roots, leaves, flowers and seeds are used as laxative and purgative (Todd, 1967). It is a vermifuge, anticonvulsant and used against chicken pox (Mann, 2003). Other uses include febrifuge, extrusion of guinea worm (Iwu, 1993) and black quarter (Ndi *et al.,* 2000). Previous studies have shown that its leaves exhibited *in-vitro* antibacterial, antimalarial and antihepatotoxic properties (Gasquet, 1993; Percez, 1994; Saraf, 1994). Seeds are brewed into a coffee like beverage for asthma and the flower infusion is used for bronchitis in the Peruvian Amazon (Akinloye *et al.,* 2003).

Phytochemically, the aqueous extract of *Cassia occidentalis* contained tannins, anthraquinones, sterol, cardiac glycosides, saponin and alkaloids (Muyibi *et al.,* 2000). The changing pattern of bacterial aetiology of infection and their altered sensitivities to antimicrobial agents employed in their treatment call for intensive regular exploration of indigenous plants. This will help us identify plants with antimicrobial value that will not only serve as resource for our indigenous pharmaceutical industries but will also serve as an alternative complementary medicine (Saganuwan and Gulumbe, 2006). Orji *et al*. (2003) reported that a particular characteristic of a plant is that different chemical substances are obtained in members of even the same species in different areas.

The objectives of this study was to evaluate the in-vitro antimicrobial activity and photochemical constituents of *Cassia occidentalis* of Nupe land as soil nature and environmental factors like climate, weather and humidity may affect phytochemical properties of plant grown different soil textures and environments.

MATERIALS AND METHODS

Plant: The fresh leaves of *Cassia occidentalis* were collected from Katcha, the headquarter of Katcha Local Government Area of Niger State, but identified and authenticated in Herbarium of Biological Science Department of Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

Extraction: The leaves of *Cassia occidentalis* were collected fresh, pounded with mortar and pestle then dried in the sun for 30 minutes to 1 hour and grounded into powder using mortar and pestle.

The extraction was carried out as described by Saganuwan and Gulumbe (2006). Serial concentrations; 50, 60, 70, 80, 90 and 100% of the hexane, methanol, chloroform and water extracts were prepared and sterilized through 0.45 µm membrane filter paper (Cheesebrough, 1985).

Collection of Bacterial Isolates: Bacterial isolates of *S. pyogenes* and *S. pneumonia*e were obtained from Microbiology Laboratory of Sokoto specialist hospital. *K. pneumoniae* and *E. coli* were supplied by Microbiology Laboratory, College of Health Sciences, Usmanu Danfodiyo University all in Sokoto. But *P. multocida* was isolated in Veterinary Public Health Laboratory, Usmanu Danfodiyo University Sokoto, whereas *S. typhi* and *S. typhimurium* were donated by Department of Veterinary Public Health and Preventive Medicine, University of Nigeria Nsukka, Nigeria. The isolates were authenticated by chemical and serological tests as described by Cheesebrough (1985), preserved on blood agar slant and stored at 4 ^{0}C until ready to use.

Disc Diffusion Method: The isolates of *K. multocida*, *S. typhi*, *S. typhimurium*, *K. pneumoniae*, *E. coli*, *S. pyogenes* and *S. pneumoniae* were subcultured overnight at 37 ^{o}C on nutrient agar plates, ten plates per microorganism. The suspensions of each bacterial isolate were prepared as described by John *et al.* (1999) in isotonic sodium chloride solution. Dried Petri dish, ten per each microorganism of Mueller-Hinton agar were flooded with the appropriate suspension of the bacterial isolates.

Sterile 6 mm diameter absorbent filter papers (punched out from No. 1 whatman paper) were impregnated with the appropriate concentrations; 500, 600, 700, 800, 900 and 1000 mg of the hexane, chloroform, methanol and water extracts and placed on the corresponding inoculated 70 plates. Ten each of *S. typhi*, *S. pyogenes*, *S. pneumoniae*, *K. pneumoniae*, *E. coli*, *P. multocida* and *S. typhimurium*. After the incubation at 37 ^{0}C for 24 hours, all the plates were observed for zones of growth inhibition and the diameters of the zones measured in millimeter (mm) using calibrated ruler.

RESULTS

E. coli showed diametric zones of inhibition at concentrations between 900 – 1000 mg of 10.0 and 17.0 mm with average mean of 4.5 mm as there was no any zone of inhibition shown on other microorganisms by the hexane extract of *Cassia occidentalis*. Diametric zones of inhibition were shown on *E.* coli by the chloroform extract at concentration ranges between 900 – 1000 mg of 10.0 and 16.0 mm with average mean of 4.3 mm. No zone of inhibition shown on other microorganisms by the chloroform extracts (Table 1).

E. coli showed diametric zones of inhibition at concentration ranges between 900 – 1000 mg of 10.0 and 15.0 mm with average mean of 4.16 mm as there was no any zone of inhibition shown on other microorganisms by the methanol extract. Diametric zones of inhibition of 8 mm and 10 mm were shown on *E. coli* at concentration ranges between 900 – 1000 mg with average mean of 3.67 mm as there was

Table 1: Serial concentrations of the hexane, chloroform, methanol and water extracts, and their corresponding diametric zones of inhibition

Conc. of the extracts (mg)	Diametric zone of inhibition (mm)													
	Hexane							Chloroform						
	S. ty	S. typhim	E. col	S. pneu	K. pneu	P. mul	S. pyo	S. ty	S. typhim	E. col	S. pneu	K. pneu	P. mul	S. pyo
500	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
600	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
700	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
800	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
900	0.0	0.0	10.0	0.0	0.0	0.0	0.0	0.0	0.0	10.0	0.0	0.0	0.0	0.0
1000	0.0	0.0	17.0	0.0	0.0	0.0	0.0	0.0	0.0	16.0	0.0	0.0	0.0	0.0
Mean: 750	0.0	0.0	4.5	0.0	0.0	0.0	0.0	0.0	0.0	4.3	0.0	0.0	0.0	0.0
	Methanol							**Water**						
500	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
600	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
700	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
800	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
900	0.0	0.0	10.0	0.0	0.0	0.0	0.0	0.0	0.0	8.0	0.0	0.0	0.0	0.0
1000	0.0	0.0	15.0	0.0	0.0	0.0	0.0	0.0	0.0	14.0	0.0	0.0	0.0	0.0
Mean: 750	0.0	0.0	4.16	0.0	0.0	0.0	0.0	0.0	0.0	3.67	0.0	0.0	0.0	0.0

Keys: S. ty = Salmonella typhi, S. typhim = Salmonella typhimurium, E. col = E. coli, S. pneu = Streptococcus pneumoniae, K. pneu = Klebsiella pneumoniae, P. mul = Pasteurella multocida, S. Pyo = Streptococcus pyogenes

Table 2: Phytochemical constituents of *Cassia occidentalis* methanol, hexane, chloroform and aqueous leaf extracts

Extract	Alkaloid	Tannin	Glycoside	Flavonoid	Steroid	Saponin
Methanol	+	+	+	+	-	+
Hexane	+	+	+	+	-	+
Chloroform	+	+	+	+	-	+
Water	+	+	+	+	-	+

no zone of inhibition shown on other microorganisms by the aqueous extract of *Cassia occidentalis* (Table 1).

Phytochemical analyses revealed the presence of alkaloid, tannin, glycoside, flavonoid and saponin in *Cassia occidentalis* methanol, hexane, chloroform and aqueous leaf extracts as there was no steroid present in all the four extracts (Table 2).

DISCUSSION

The antimicrobial activity exhibited by hexane, chloroform, methanol and aqueous leaf extracts of *Cassia occidentalis* on *E. coli* at concentrations between 900 – 1000 mg agrees with the findings of (Gasquet, 1993; Percez, 1994; Saraf, 1994) that the leaves exhibited in-vitro antibacterial, antimalarial and antihepatotoxic properties. The plant may be used for the treatment of colibacillosis caused by *E. coli* which occurs in all species of newborn farm animals as major cause of death and economic loss in this age group (Radostits *et al.,* 2000). Leeflang (1993) had earlier observed that indigenous knowledge and practices will be useful in the promotion of animal health and meat production in the near future in Nigeria. Furthermore, Tamboura *et al.* (2000) had also reported that ethno veterinary medical health care will be the only alternative to western veterinary therapy. These ethnoveterinary remedies which rely on local plants or easily available materials are practical, effective and cheap (Tamboura *et al.,* 2000). However, lack of antimicrobial activities exhibited by all the extracts of *Cassia occidentalis* and generally at concentrations between 500 – 1000 mg on *P. multocida*, *S. typhi*, *S. typhimurium*, *S. pyogenes*, *S. pneumoniae* and *K. pneumoniae* is suggestive of limited antimicrobial activity of the plant. This was not pointed out by (Gasquet, 1993; Percez, 1994; Saraf, 1994). More so, uniformity of antimicrobial activity exhibited by hexane, chloroform, methanol and aqueous extracts of *Cassia occidentalis* leaf on only *E. coli* may confirm the limited antibacterial activity of the plant even *in-vivo.* Nonetheless, there is need to separate the chemical constituents of the plant leaf and then test each component on the microorganisms.

The results of qualitative analyses of all the four extracts confirmed the presence of alkaloid, tannin, glycoside and saponin as reported by Muyibi *et al.* (2000) that the aqueous extract of *Cassia occidentalis* contained tannins, anthraquinone, sterol, cardiac glycoside, saponin and alkaloid. But there was no steroid as it also contained flavonoid.

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