Volume 2 No.4, April 2012 ISSN 2224-3577
International Journal of Science and Technology

http://www.ejournalofsciences.org

Phytochemical and Some Antmicrobial Activity of Cassia Occidentalis L. (Caesalpiniaceae)

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ABSTRACT

Cassia occidentalis L. (Caesalpiniaceae) was exhaustively extracted with n-hexane and subsequently with methanol. The methanol portion was subsequently partition with chloroform, ethyl acetate and n-butannol. The phytochemical studies of the partition portion were done using standard protocols. The zone of inhibition (ZI), Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined. The antimicrobial screening revealed that the extract exhibited varying activity against different microbes. These activities observed could be attributed to the presence Active metabolites contained in the extract.

Keywords: *Cassia Occidentalis, Phytochemical, Antimicrobial activity, MIC, ZI, MBC.*

1. INTRODUCTION

The development of resistance to current antibiotics by disease causing microbes has reinforced research for discovery of new ones. Current trends in drug development process are focused on natural sources, especially source of plant origin due to some proven correlation between the folkloric medicinal uses of some of these plants to biological activity. However, the use of plant materials to prevent and treat infectious diseases successfully over the years has continued to attract the attention of scientists worldwide [1][2][3].

Infectious diseases usually present clear symptoms, a likelihood that enables traditional healers to recognize and develop effective therapies against them. Traditional medical practitioners constitute a large proportion of the population of the people of Northern Nigeria who rely heavily on the use of traditional plants for physical and psychological health needs [4]. Despite of the tremendous progress in human medicines, infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health. There impact is particularly large in developing countries due to relative unavailability of medicines and the emergence of widespread drug resistance [5]. During the last two decades, the development of drug resistance as well as the appearance of undesirable side effects of certain antibiotics [6] has lead to the search of new antimicrobial agents mainly among plant extracts with the goal to discover new chemical structures, which overcome the above disadvantages [7]. Current research on natural molecule and products primarily focuses on plants since they can be sourced more easily and be selected based on their ethno-medicinal uses [8]. *Cassia occidentalis* L. called as Kasmard in Sanskrit, Kasondi in Hindi, Coffee Senna in English, Rai Daure, belongs to family Caesalpiniaceae [9]. It is an ayurvedic plant with huge medicinal importance. Leaves of *C. occidentalis* plant have ethno medicinal importance like paste of leaves is externally applied on healing wounds, sores, itch, cutaneous diseases, bone fracture, fever, ringworm, skin diseases and throat infection [10],[11]. Previous pharmacological investigations showed that *C. occidentalis* leaf extracts have antibacterial [12],[13] Saga, antimalarial [14], antimutagenic [15,16], antiplasmodial [15] anticarcinogenic [17] and hepatoprotective [18] activity. Moreover, studies on this plant showed that the nature and amount of the Phytochemical varies according to the season and geographical location [19].

In this study we examined the antimicrobial properties of the six extracts portion of *Cassia occidentalis* leave against some selected microorganisms using bioassay method.

2. MATERIALS AND METHOD

Plant Material

The plant materials were collected from a farm land in Zaria, Nigeria. The shrub was taxonomically authenticated at the herbarium Biological science Department A.B.U Zaria by comparing the herbarium sample and voucher deposited (No 1047).

Preparation of the Extracts

The air-dried powdered leaf of the plant (530g) was successively extracted with n-hexane and subsequently with 95% ethanol using cold maceration. The ethanol portion of the extract (40g) was suspended in water (500ml) and sequentially partition with Chloroform, Ethyl acetate and n- Butanol. The various partition portions were concentrated using a rotary evaporator [20].The phytochemical screening of the various partition portion of the extract was conducted using standard protocol [21,22].

Tested Microorganisms

Antimicrobial activity of *Cassia occidentalis* leaves extract was investigated against five bacterial isolates and one fungal isolate, which were obtained from Ahmadu Bello university Teaching Hospital Microbiology laboratory. The

bacteria used for the research work include *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella spp, Escherichia coli, Bacilllus subtilis* and the fungi used for the research work was *Candida albicans.* The tested microorganisms were cultured on Nutrient agar (for bacteria at 35+2^{0}c for 24 h) for the bacteria isolates and on Potatoes Dextrose Agar were used for the fungal isolates for fungus at 28+20^{o}C for 48-72 h [23].

Inoculumn Preparation

A loopful of isolated colonies was inoculated into 4 ml peptone water and incubated at 37^{0}C for 4 h. The turbidity of actively growing bacterial suspension was adjusted to match the turbidity standard of 0.5 McFarland units prepared by Mixing 0.5 ml of 1.75% (w/v) barium chloride dehydrates with 99.5 ml 1% (v/v) sulphuric acid. This turbidity was equivalent to approximately 1.2×10^8 colony-forming units per milliliter (cfu/ml). The grown suspension was used for further testing [24]

Antimicrobial Bioassay

The antimicrobial activities of the extracts were determined by the Kirby-Bauer agar diffusion method according to NCCLS standards [25, 26].Nutrient Agar and Potato Dextrose agar was used for the antimicrobial activity test. Under aseptic conditions in the Biosafety chamber, 15ml of Nutrient agar medium was dispensed into pre-sterilized Petri dishes to yield a uniform depth of 4 mm and inoculated by the bacterial and fungal culture, respectively. The sterile discs (Diameter 6mm) were impregnated with different concentration (400mg/ml, 200mg/ml, 100mg/ml, 50mg/ml) of the *Cassia occidentalis* leave extract and dried for 10-15 minutes. The dried discs were placed on Nutrient agar surface with flamed forceps and gently pressed down to ensure contact with the agar surface. Streptomycin of (40mg/ml) was used as positive control. The discs were spaced far enough to avoid reflections wave from the edges of the Petri dishes and overlapping rings of inhibition. Finally, the Petri dishes were incubated for 18 to 24 hours at 37^{0}C for bacteria and 28^{0}C for 48 to 72 hours for fungus. The diameter of zone of inhibition as indicated by clear area which was devoid of growth of microbes was measured and recorded.

Determination of Minimum Inhibitory Concentration (Mic) and Minimum Bacteriacidal/Fungicidal Concentration (Mbc/Mfc)

Minimum inhibitory concentration (MIC) was determined for each partition portion of the extract showing antimicrobial activity against test pathogens using broth micro dilution method [18]. The MIC values were taken as the lowest concentration of the extracts in the well of the test tube that showed no turbidity after incubation. The turbidity of the wells in the test tube was interpreted as visible growth of microorganisms. The minimum bactericidal and/fungicidal concentration (MBC,/MFC) was determined by sub culturing from each well showing no apparent growth. Least concentration of extract showing no visible growth on sub culturing was taken as MBC/MFC.

3. RESULTS

Table 1: Preliminary Phytochemical Screening of the Leaves Extract of Cassia Occidentalis

CONSTITUENTS	TEST	Ps	Es	CL	EtOAC	n-But	AQ
carbohydrate	Molisch	-	+	-	-	-	++
	Fehling's	-	++	-	-	-	+++
	Barfoed	-	+	-	-	-	++
	Benedict	-	+	-	-	-	++
Alkaloids	Mayer's	-	-	-	-	-	-
	Wagner	-	-	-	-	-	-
	Dragendorff	-	-	-	-	-	-
	Hager's	-	-	-	-	-	-
Flavonoids	Lead Acetate	-	++	+	+	+ +	-
	Shinoda	-	++	+	+	++	-
	Tetraoxosulphuric acid	-	+	+	-	+ +	-
Glycosides	Borntrager's	-	++	-	+	+	++
	Legal	-	+	+	+	+	++
Saponin	Froth test	-	++	-	+	+ +	+++
Cardiac	Keller	-	+	-	++	+	+ +
Glycosides	Killiani	-	++	-	+	+	++
Tannins	Gelatin test	-	+	+	-	+	++
	Alkaline reagent test	-	+	-	-	+	++

Phenolic compound	Ferric Chloride test	-	++	+	+	++	+

Antimicrobial Zone of Inhibition Results of *Cassia Occidentalis* Leave Extract using Disc Diffusion Method

Table 2: Pet Ether Extract

ISOLATES	400mg/ml	200mg/ml	100mg/ml	50mg/ml	40mg/ml(control)
Pseudomonas auregenosa	22	15	13	8	32
Escherichia coli	10	8	R	R	22
Klebsiella spp	R	R	R	R	R
Candida albican	10	R	R	R	26
Bacillus subtilis	28	10	R	R	34
Staphylococcus areus	18	R	R	R	24

R=Resistant. Control=Streptomycin

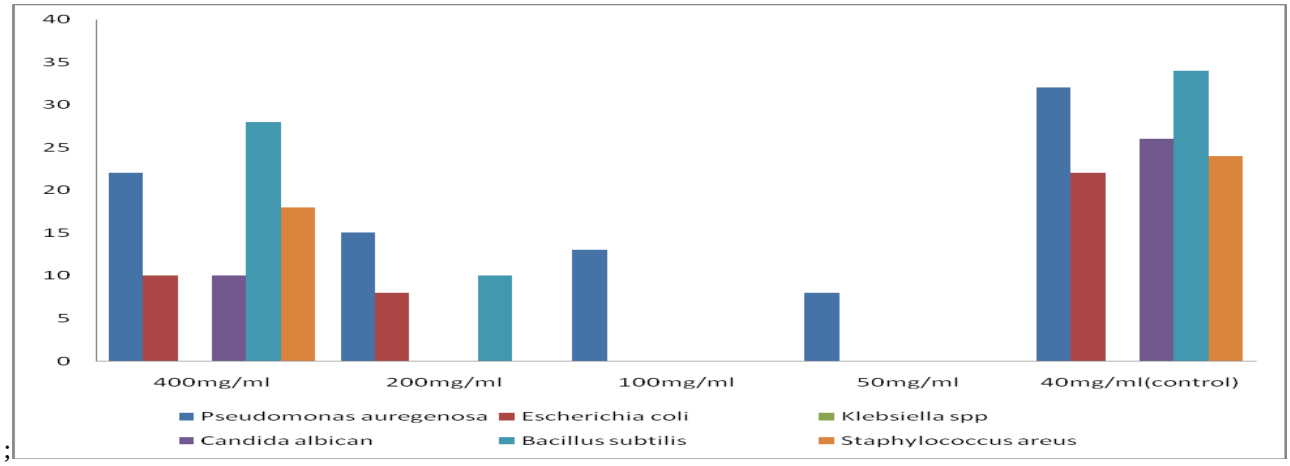

Fig 1: Pet Ether Extract

Table 3: Ethanolic Extract

ISOLATES	400mg/ml	200mg/ml	100mg/ml	50mg/ml	40mg/ml(control)
Pseudomonas auregenosa	22	14	R	R	34
Escherichia coli	16	10	R	R	20
Klebsiella spp	R	R	R	R	R
Candida albican	20	12	8	R	24
Bacillus subtilis	24	14	10	R	30
Staphylococcus areus	8	R	R	R	28

R=Resistant. Control=Streptomycin

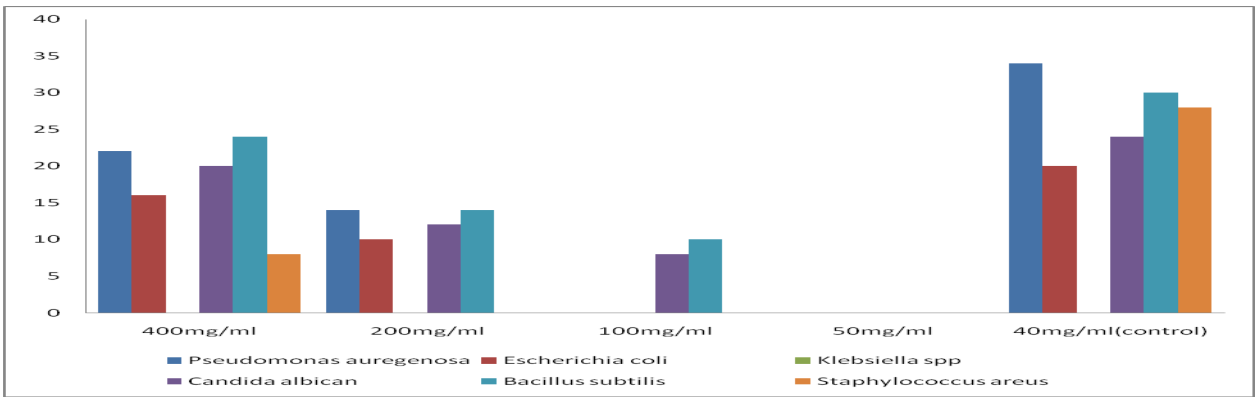

Fig 2: Ethanolic Extract

Table 4: Chloroform Extract

ISOLATES	400mg/ml	200mg/ml	100mg/ml	50mg/ml	40mg/ml(control)
Pseudomonas auregenosa	28	20	16	8	32
Escherichia coli	16	12	8	R	20
Klebsiella spp	R	R	R	R	R
Candida albican	22	18	14	8	28
Bacillus subtilis	24	18	16	10	32
Staphylococcus areus	12	8	R	R	28

R=Resistant. Control=Streptomycin

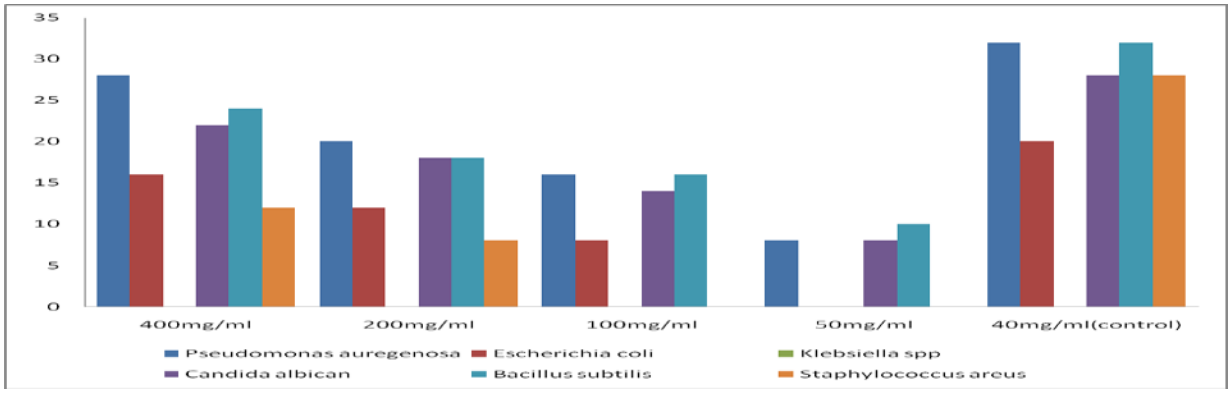

Fig 3: Chloroform Extract

Table 5: Ethyl Acetate Extract

ISOLATES	400mg/ml	200mg/ml	100mg/ml	50mg/ml	40mg/ml(control)
Pseudomonas auregenosa	22	14	R	R	36
Escherichia coli	14	10	6	R	20
Klebsiella spp	R	R	R	R	R
Candida albican	18	14	8	R	22
Bacillus subtilis	28	24	10	R	32
Staphylococcus areus	10	8	R	R	29

R=Resistant. Control=Streptomycin

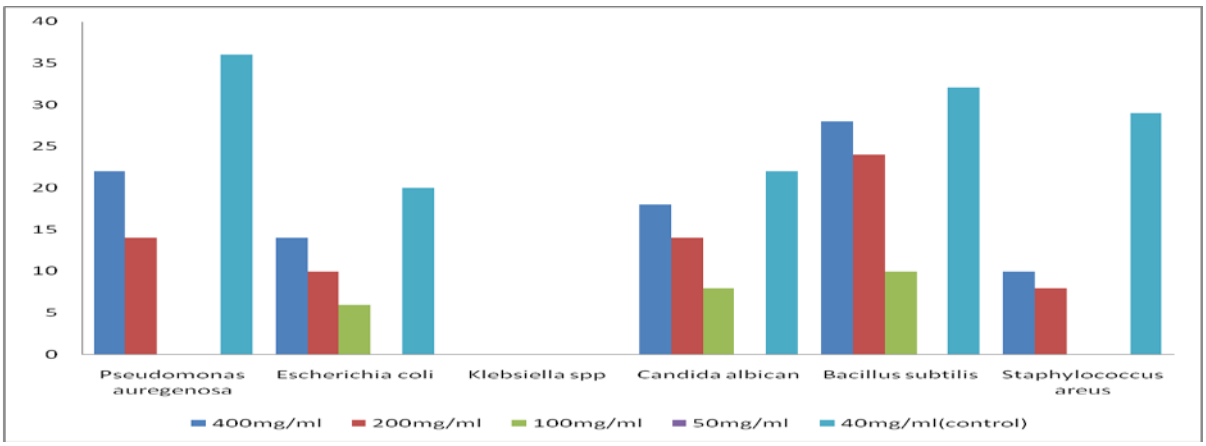

Fig 4: Ethyl Acetate Extract

Table 6: n-Butanol Extract

ISOLATES	400mg/ml	200mg/ml	100mg/ml	50mg/ml	40mg/ml(control)
Pseudomonas auregenosa	26	20	16	10	34
Escherichia coli	16	12	8	R	22
Klebsiella spp	R	R	R	R	R
Candida albican	22	24	18	14	24
Bacillus subtilis	18	15	10	6	30
Staphylococcus areus	22	16	R	R	30

R=Resistant. Control=Streptomycin

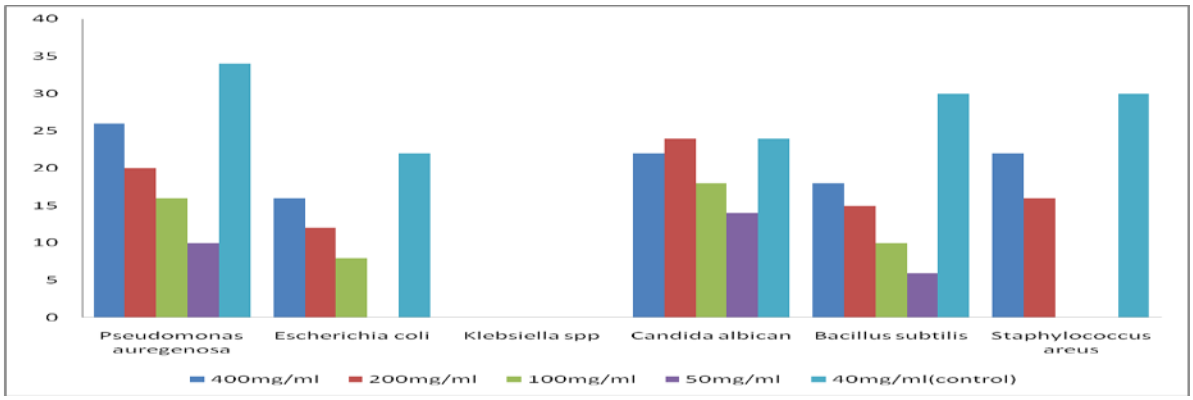

Fig 5: n-Butanol Exract

Table 7: Aqueous Extract

ISOLATES	400mg/ml	200mg/ml	100mg/ml	50mg/ml	40mg/ml(control)
Pseudomonas auregenosa	10	8	R	R	36
Escherichia coli	12	R	R	R	20
Klebsiella spp	R	R	R	R	R
Candida albican	14	12	8	R	26
Bacillus subtilis	22	18	12	R	28
Staphylococcus areus	8	R	R	R	28

R=Resistant. Control=Streptomycin

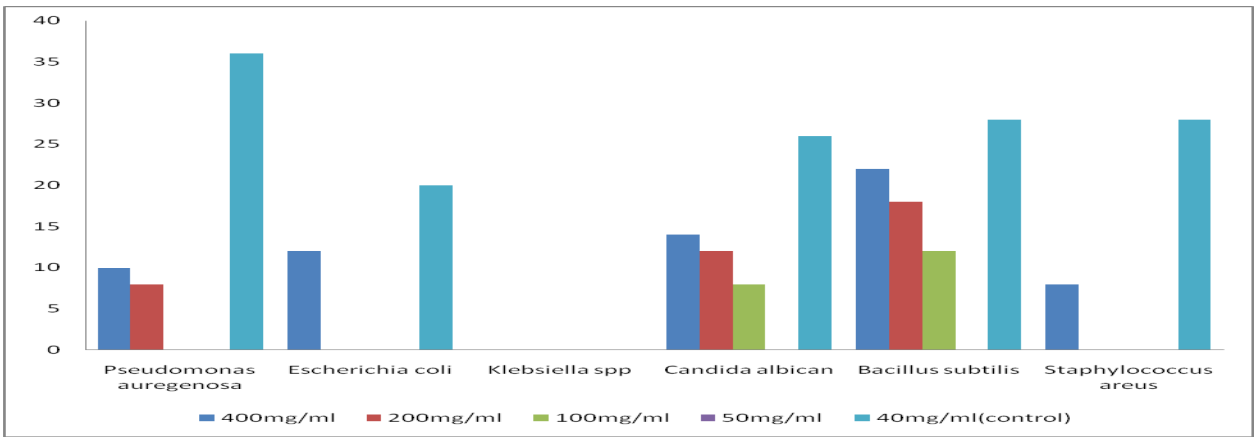

Fig 6: Aqueous Extract

Antimicrobial Minimum Inhibition Concentration (MIC) Results of *Cassia occidentalis* Leave Extract

Table 8: Pet Ether Extract Portion

	ISOLATES	300mg/ml	200mg/ml	100mg/ml	50mg/ml	25mg/ml
1	*Pseudomonas auregenosa*	-	-	-	**0***	**+++**
2	*Escherichia coli*	-	**0***	++	+++	++++
3	*Candida albican*	**0***	++	+++	**+++**	++++
4	*Bacillus subtilis*	-	**0***	++	**++**	+++
5	*Staphylococcus aureus*	**0***	+	++	**+++**	+++

Key: - = No Growth (No Turbidity), 0* = MIC, + = Low Growth, ++=Moderate Growth, +++= High Growth

Table 9: Ethanolc Extract Portion

	ISOLATES	300mg/ml	200mg/ml	100mg/ml	50mg/ml	25mg/ml
1	*Pseudomonas auregenosa*	**0***	++	+++	+++	+++
2	*Escherichia coli*	-	**0***	+++	+++	+++
3	*Candida albican*	-	-	**0***	++	+++
4	*Bacillus subtilis*	-	-	**0***	+	+++
5	*Staphylococcus aureus*	**0***	++	+++	+++	+++

Key: - = No Growth (No Turbidity), 0* = MIC, + = Low Growth, ++=Moderate Growth, +++= High Growth

Table 10: Chloroform of the Extract Portion

	ISOLATES	300mg/ml	200mg/ml	100mg/ml	50mg/ml	25mg/ml
1	*Pseudomonas auregenosa*	-	-	-	0*	++
2	*Escherichia coli*	-	-	-	-	0*
3	*Candida albican*	-	-	-	0*	+
4	*Bacillus subtilis*	-	-	-	-	0*
5	*Staphylococcus aureus*	-	0*	-	0*	+++

Key: - = No Growth (No Turbidity), 0* = MIC, + = Low Growth, ++=Moderate Growth, +++= High Growth

Table 11: Ethyl Acetate Extract Portion

	ISOLATES	300mg/ml	200mg/ml	100mg/ml	50mg/ml	25mg/ml
1	*Pseudomonas auregenosa*	-	0*	++	+++	+++
2	*Escherichia coli*	-	-	0*	++	++
3	*Candida albican*	-	-	0*	++	++
4	*Bacillus subtilis*	-	-	-	0*	++
5	*Staphylococcus aureus*	-	0*	++	++	+++

Key: - = No Growth (No Turbidity), 0* = MIC, + = Low Growth, ++=Moderate Growth, +++= High Growth

Table 12: n-Butanol Extract Portion

	ISOLATES	300mg/ml	200mg/ml	100mg/ml	50mg/ml	25mg/ml
1	*Pseudomonas auregenosa*	-	-	-	-	**0***
2	*Escherichia coli*	-	-	-	**0***	+
3	*Candida albican*	-	-	-	**0***	**+**
4	*Bacillus subtilis*	-	-	-	-	**0***
5	*Staphylococcus aureus*	-	**0***	++	**+++**	**+++**

Key: - = No Growth (No Turbidity), 0* = MIC, + = Low Growth, ++=Moderate Growth, +++= High Growth

Table 13: Aqueous Extract Portion

	ISOLATES	300mg/ml	200mg/ml	100mg/ml	50mg/ml	25mg/ml
1	*Pseudomonas auregenosa*	**-**	**0***	++	++	**0***
2	*Escherichia coli*	**-**	**0***	++	**++**	+++
3	*Candida albican*	**0***	-	**+**	**++**	+++
4	*Bacillus subtilis*	-	**-**	**0***	++	**+++**
5	*Staphylococcus aureus*	**0***	+	++	**+++**	+++

Key: - = No Growth (No Turbidity), 0* = MIC, + = Low Growth, ++=Moderate Growth, +++= High Growth

Antimicrobial Minimum Bactricidal Concentration (MBC) Results of *Cassia occidentalis* Extract

Table 14: Pet Ether Extract Portion

	ISOLATES	300mg/ml	200mg/ml	100mg/ml	50mg/ml	25mg/ml
1	*Pseudomonas auregenosa*	-	-	0*	+++	+++
2	*Escherichia coli*	-	0*	++	+++	++
3	*Candida albican*	_	0*	++	++	+++
4	*Bacillus subtilis*	_	0*	+++	+++	+++
5	*Staphylococcus aureus*	0*	++	+++	+++	+++

Key: - = No Growth (No Turbidity), 0* = MBC, + = Low Growth, ++ = Moderate Growth, +++= High Growth

Table 15: Ethanolic Extract Portion

	ISOLATES	300mg/ml	200mg/ml	100mg/ml	50mg/ml	25mg/ml
1	*Pseudomonas auregenosa*	0*	++	++	+++	+++
2	*Escherichia coli*	0*	++	+++	+++	+++
3	*Candida albican*	-	0*	++	++	+++
4	*Bacillus subtilis*	-	0*	++	+++	+++
5	*Staphylococcus aureus*	0*	++	+++	+++	+++

Key: - = No Growth (No Turbidity), 0* = MBC, + = Low Growth, ++ = Moderate Growth, +++= High Growt

Table 16: Chloroform Extract Portion

	ISOLATES	300mg/ml	200mg/ml	100mg/ml	50mg/ml	25mg/ml
1	*Pseudomonas auregenosa*	-	0*	+	+++	+++
2	*Escherichia coli*	-	-	-	0*	++
3	*Candida albican*	-	-	0*	++	+++
4	*Bacillus subtilis*	-	-	-	0*	++
5	*Staphylococcus aureus*	-	-	0*	+++	+++

Key: - = No Growth (No Turbidity), 0* = MBC, + = Low Growth, ++ = Moderate Growth, +++= High Growth

Table 17: Ethyl Acetate Extract Portion

	ISOLATES	300mg/ml	200mg/ml	100mg/ml	50mg/ml	25mg/ml
1	*Pseudomonas auregenosa*	0*	+	++	+++	+++
2	*Escherichia coli*	-	0*	0*	++	++
3	*Candida albican*	-	0*	0*	+++	+++
4	*Bacillus subtilis*	-	-	0*	++	+++
5	*Staphylococcus aureus*	0*	++	+++	+++	+++

Key: - = No Growth (No Turbidity), 0* = MBC, + = Low Growth, ++=Moderate Growth, +++= High Growth

Table 18: n-Butanol Extract Portion

	ISOLATES	300mg/ml	200mg/ml	100mg/ml	50mg/ml	25mg/ml
1	*Pseudomonas auregenosa*	-	-	-	0*	++
2	*Escherichia coli*	-	-	0*	++	+++
3	*Candida albican*	-	-	0*	+	++
4	*Bacillus subtilis*	-	-	0*	++	+++
5	*Staphylococcus aureus*	0*	+	++	+++	+++

Key: - = No Growth (No Turbidity), 0* = MBC, + = Low Growth, + = Moderate Growth, +++= High Growth

Table 19: Aqueous Extract Portion

	ISOLATES	300mg/ml	200mg/ml	100mg/ml	50mg/ml	25mg/ml
1	*Pseudomonas auregenosa*	0*	++	+++	+++	++++
2	*Escherichia coli*	0*		0*	++	++
3	*Candida albican*	-	-	0*	+	++
4	*Bacillus subtilis*	0*	++	++	++	++++
5	*Staphylococcus aureus*	0*	++	+++	+++	++++

Key: - = No Growth (No Turbidity), 0* = MBC, + = Low Growth, ++ = Moderate Growth,+++= High Growth

4. DISCUSSION

Antimicrobial activity of the extracts of *C. occidentalis* was first time investigated against *P. aeruginosa*, *K. pneumoniae*, and *C. albicans*. However, investigations have already been done on *E. coli* [10,12,18] and *S. aureus* [10].The results of this study showed remarkable variations in the effectiveness of the leaves extract against *E. coli*. In previous studies, for leaves extract, *E. coli* was found to be sensitive [10,12] and in some experiments resistant [17]. Saganuwan and Gulumbe [12] observed that the *E. coli* was sensitive to methanol, hexane, chloroform and aqueous extract of leaves of *C. occidentalis* (collected from Niger state) at a concentration range 900-1000 mg/ml Similarly, Jain and his coworkers [10] observed that the metabolite rich fraction of (anthraquinones) leaves, pods, flowers and callus were effective against *E. coli* (Inhibition zone 22 mm). In contrast, our study revealed that the petroleum ether and ethanolic extract of leaves of *C. occidentalis* (collected from Zaria, Kaduna state of Nigeria) was effective against *E. coli* at concentration of 400 mg/ml. The inhibition activities were not observed in the chloroform and aqueous extracts of leaves of *C. occidentalis* against *E. coli*

in the present study while Saganuwan and Gulumbe [12] observed the activities in these extracts. These differences in the plant extracts activities may be due to spatial and temporal variations of the plants. Infections caused by *P. aeruginosa*, especially those with multidrug resistance, are among the most difficult to treat conventional antibiotics. In our study, the growth of *P.aeruginosa* was remarkably inhibited by the aqueous extract of the leaves of *C. occidentalis*

5. CONCLUSION AND RECOMMENDATION

The results obtained from this study is interesting looking at the fact that the crude extracts of Cassia *occidentalis* was effective on some microorganisms such as *Staphylococcus aureus, Escherichia coli, Bacillus subtilis and Candida albicans* which are commonly involved in urinary tract infection [18] and diarrhea diseases which is one of the principal cause of death in infants[18]. The extracts was found to be ineffective on *Klebsiella spp* that was used in this study, therefore more research work should be done to exploit other plants with the view to discover ethno-medicinal plant that could be effective against *Klebsiella spp*. Finally, attempts should be made to conduct *in vivo* studies with the extracts so as to confirm the present *in vitro* findings as the diameter of the zone of inhibition is not only affected by sensitivity of the microorganisms alone but also the concentration of the extract on the discs is very important and this is why *in vivo* findings such as toxicity of the plant to humans at different concentration is very essential in the use of the plant for treatment.

REFERENCES

[1] Kunle O. F and Egharevba, H O (2009).preliminary studies on vernonia ambigua: Prhytochemistry and Antimicrobial screening of the whole plant. Ethnobotanical Leaflets, 13: 1216-21.

[2] Kunle O. F and Egharevba, H O (2009).preliminary studies on vernonia ambigua: Prhytochemistry and Antimicrobial screening of the whole plant. Ethnobotanical Leaflets, 13: 1216-21.

[3] Roopashre T S, Raman D, Shobha RRH, Narendra C. (2008). Antibacterail activity of antipsorriatic herbs: Cassia tora, Momordica charantia and Calendula officialis. International Journal of Research in National Products 3: 20-28.

[4] Begum S, Hassan SI, Siddiqui BS, Shaheen F, Ghayur AH (2002).Triterpeneoids from the leaves of Psidium guajava. Phytochemistry.61:399-403.

[5] Dabai, Y. U. ,Muhammad, s. Antibacterial activity of some nigerian medicinal plants. SCIENCE WORLD Journal Vol 3 (NO2) 2008. SWJ:43-44

[6] Zampini IC, Cuello S, Alberto MR, Ordonez RM, Almeida RD, Solorzano E, Isla MI, 2009. Antimicrobial activity of selected plant species from the Argentine puna against sensitive and multiresistant bacteria. Journal ofEthnopharmacology, 124: 499-505.

[7] kemo PO, Bais HP, Vivanco JM, 2003. *In vitro* activities of *Maesa lanceolata* extracts against fungal plant pathogens. Fitoterapia, 74: 312-316.

[8] Bouamama H, Noel T, Villard J, Benharref A, Jana M, 2006. Antimicrobial activities of the leaf extract of two Moroccan *Cistus* L species. Journal of Ethnopharmacology, 104: 104-107.

[9] Arora DS, Kaur GJ, 2007. Antibacterial activity of some Indian medicinal plants. Journal of Natural Medicine, 61:313-317.

[10] Jain SC, Sharma RA, Jain R, Mittal C, 1998. Antimicrobial screening of *Cassia occidentalis* L *in vivo* and *in vitro*.Phytotherapy Research, 12: 200-204.

[11] Burkill, H. M. (1995), The useful plants of West Tropical Africa. Royal botanic garden kewl (u,k)

[12] Saganuwan AS, Gulumbe ML, 2006. Evaluation of *in vitro* antimicrobial activities and phytochemical constituents of *Cassia occidentalis*. Animal Research International, 3: 566-569.

[13] Tona L, Ngimbi NP, Tsakala M, Mesia K, Cimanga K, Apers S, De Bruyne T, Pieters L, Totté J, Vlietinck AJ, 1999.Antimalarial activity of 20 crude extracts from nine African medicinal plants used in Kinshasa Congo. Journal of Ethnopharmacology, 68: 193-203.

[14] Jafri MA, Subhani MJ, Javed K, Singh S, 1999. Hepatoprotective activity of leaves of *Cassia occidentalis* against paracetamol and ethyl alcohol intoxification in rats. Journal of Ethnopharmacology, 66: 355-61.

[15] Sharma N, Trikha P, Athar M, Raisuddin S, 2000. In vitro inhibition of carcinogen induced mutagenicity by *Cassia occidentalis* and *Emblica officinalis*. Drug and Chemical Toxicology, 23: 477-84.

[16] Tona L, Cimanga RK, Mesia K, Musuamba CT, De Bruyne T, Apers S, Hernans N, Miert SV, Pieters L, Totté J, Vlietinck AJ, 2004. *In vitro* antiplasmodial activity of extracts and fractions from seven medicinal plants used in the Democratic Republic of Congo. Journal of Ethnopharmacology, 93: 27-32.

[17] Yadav JP, Arya V, Yadav S, Panghal M, Kumar S, Dhankhar S, 2009. *Cassia occidentalis*: A review on its ethnobotany,phytochemical and pharmacological process. Fitoterapia, doi:10.1016/j.fitote.2009.09.008.

[18] Basri DF, Fan SH. 2005. The potential of aqueous and acetone extracts of galls of Quercus infectoria as antibacterial agents. Indian J Pharmacol 37(1):26-29.

[19] Bauer AW, Kirby WM, Sherris JC, Turck M, 1966. Antibiotic susceptibility testing by a standardized single disc method. American Journal of Clinical Pathology, 45: 493-496.

[20] Shu – Xiang, Z., Tadato, T., Seiichi, Y., Chao – Mei, M., Min – Chuan, W., Shao-Qing, C. and Yu-Ying, Z. (2003). Glycosyl Flavonoids from the roots and Rhizomes of Asarum Longerhi Zomatosum. J.

[21] Sofowora, A. (2008). Medicinal plants and Traditional Medicine in Africa,Spectrum Books Limited Nigeria. P 10_37

[22] Richard, J. P. C. (1998). Natural Product Isolation. Glaxo Welcome Research and Development Steven age Hertz U. K. Humana press Totowa New Jersey P. 209 – 230, 243 – 361.

[23] Osadebe, P.O. and Ukwueze, S.E. (2004). A comparative study of the phytochemical and Antimicrobial properties of the Eastern Nigerian species of Africa Mistletoe (Loranthus Micranthus) Sourced from different host trees – J. Beol. Res. Biotechnol 2(1): 18 – 23.

[24] NCCLS, 2000. Performance standards for antimicrobial disk susceptibility tests: Approval standard M2-A7 7th edition. Pennsylvania: Clinical and Laboratory Standards Institute.

[25] Singh B, Sahu PM, Sharma MK, 2002. Anti-inflamatory and antimicrobial activities of triterpenoids from *Strobilanthes callosus* Nees. Phytomedicine, 9: 355- 359.

[26] Ndumbe P, Koulla S, Adiogo D , Ongolo SN,Abong T.Cours de microbiologie.Universite de Yaounde I 92/93,1993:258.

[27] Syder JD,Merson MH.The magnitude of the global problem of acute diarrhoeal disease a review of active surveillance data.Bulletin of the World Health Organizatio .1982;60:605-613