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Research Article

ACUTE TOXICITY, PHYTOCHEMISTRY AND ANTIBACTERIAL ACTIVITY OF AQUEOUS AND ETHANOLIC LEAF EXTRACTS OF *CASSIA ALATA* LINN

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ABSTRACT
Cassia alata Linn is an important medicinal plant as well as an ornamental flowering plant that has diverse reported medicinal values. This study focused on the evaluation of the phytochemical components, antibacterial as well as acute toxicity studies. The leaves of the plant were collected, dried, ground and extracted using 95% ethanol and water. The extracts were used for acute toxicity study, phytochemical screening and antibacterial assay using cup-plate method. The result from this study revealed the presence of saponins, anthraquinones, cardiac glycosides, flavonoids, reducing sugars and terpenes. The concentrations of these bioactive components were slightly higher in ethanol than in aqueous leaf extract. The antibacterial effects produced by the extracts was dose dependent at the tested doses (20 mg to 160 mg) on *Staphylococcus aureus, Escherichia coli* and *Salmonella typhi*. The aqueous leaf extract showed higher activity on *Escherichia coli* than ethanol extract at 160 mg ($p<0.05$), whereas ethanol leaf extract had higher activity than aqueous leaf extract on *Salmonella typhi* at the same dose ($p<0.05$). The MIC for aqueous leaf extract ranged between 3.50 mg and 25.15 mg, while that of ethanol leaf extract was from 1.41 mg to 3.55 mg on the organisms tested. This study showed that ethanol and aqueous leaf extracts contain some secondary bioactive metabolites that may be responsible for the observed antibacterial activity and thus supports the traditional use of the plant in the management of typhoid fever and other infections caused by susceptible organisms.
Keywords: Phytochemical, antibacterial, Aqueous, Ethanol, *Cassia alata*

INTRODUCTION

Cassia alata Linn a native to South America has now naturalized in many tropical countries in Africa including Nigeria [1]. It is commonly known as "Rai dore" in Hausa, "Asuwon oyinbo" in Yoruba, "Omirima" in Igbo [2] and "Whu shil-shili" in Kilba. It is an erect tropical perennial herb which belongs to the family fabaceae. Several parts of *Cassia alata* have been used in traditional medicine because it contains some chemical components which have diverse pharmacological activities [3, 4, 5]. *Cassia alata* obtained from Nigeria has shown to contain some phytochemical groups in their leaves and roots that has been reported to be useful in treating constipation, food poisoning, burns, wounds, and ringworm as well as eczema [4, 5].

The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including plants [6]. The extensive use of decoction of *Cassia alata* in Nigeria traditionally for the treatment of typhoid fever and several other infections caused by bacteria, fungi and parasites [6, 7] could be due to therapeutic failure of most frequently used antibiotics [6] , in addition to the significant number of people in North Eastern Nigeria living below poverty level. To the best of our knowledge no study was carried out in our environment that evaluated the scientific rational for the traditional use of this plant in the management of reported diseases. Therefore, this study was carried out in order to determine the phytochemical constituents and antimicrobial activity of ethanol and aqueous leaf extract of this plant.

METHODOLOGY

Source of Plant Material, Collection and Authentication

The leaf of *Cassia alata* Linn were collected in the month of September, 2011 from Hong, Hong local government area of Adamawa state, Nigeria and was identified by Mr. Mbaya of the Department of forestry and Wild life, University of Maiduguri at which the voucher specimen number (23697) was assigned and deposited in the Department.

Preparation of the Leaf Extracts

The fresh leaves of *Cassia alata* were air dried at room temperature and were then ground into powder using pestle and mortar and sieved to obtain a fine powder. Two hundred grams each of the powder was weighed into containers labelled A and B. The two samples were subjected to maceration using 800 ml of ethanol and 1500 ml of water so as to obtain the ethanol and aqueous extracts respectively. The mixtures were both stirred and kept for 24 hours at which it was filtered to obtain residues. The residues were soaked in 300 ml and 500 ml of ethanol and water respectively and kept for another 24 hours. This procedure was repeated three times, and the combined filtrate was transferred to a rotor-vapour machine to obtain the crude ethanol and aqueous extracts. The crude extracts were then evaporated to dryness. The weights of the ethanol and aqueous extracts obtained were 16.4 g (8.2%) and 12.5 g (6.25%) respectively.

Source of the Microorganisms

Clinical isolates of the test organisms; *Staphylococcus aureus* and *Escherichia coli* were obtained from the Department of Microbiology, University of Maiduguri Teaching Hospital (UMTH), while *Salmonella typhi* was obtained from the Department of Veterinary Microbiology, Faculty of Veterinary Medicine University of Maiduguri.

Acute Toxicity Studies

Thirty adult albino rats and 30 adult albino mice of both sexes were first quarantined and acclimatized for 3 days in Pharmacology Laboratory, Faculty of Pharmacy, University of Maiduguri. They were then divided into 6 groups each (i.e. six groups for mice and six groups for rats). Acute toxicity studies were conducted in two phases. In the first phase, the animals were grouped into six groups with each group having two animals. Groups 1 to 3 were administered ethanol extract of *Cassia alata* in the doses of 1000 mg, 100 mg and 10 mg respectively, while groups 4 to 6 were administered aqueous

extract at the same dose levels to both the rats and mice. The extracts were administered intraperitoneally to the animals and were observed for 24 hours. In the second phase, the animals were grouped into six groups with each group containing 3 animals each. Groups 1 to 3 were administered 800 mg, 400 mg and 200 mg respectively of the ethanolic and aqueous extracts. Observation for 24 hours was also made in each case. Distilled water was used as a control in each case. The LD_{50} was determined at the end of the second phase using modified Lorke's [8] method.

Phytochemical Analysis of the Leaf Extracts

The preliminary phytochemical analysis of each of the leaf extracts were conducted based on the standard method of Trease and Evans [9] and Sofowora [10].

Antibacterial Assay of the Plant Extracts

The media used for this research work was a preparative nutrient agar (double layer). Each of the layers contains 30 ml of nutrient agar, making a total of 60 ml of nutrient agar for each plate. The media was then sterilized by autoclaving at 121^{o}C for 15 minutes and was allowed to cool at which it was used for the antimicrobial assay. A sterilized cork borer of 6 mm in diameter was used in boring holes on the agar plates, with each media containing 5 holes in which 160 mg, 80 mg, 40 mg and 20 mg of the reconstituted ethanol and aqueous extracts of 500 mg/ml stock solution was poured into the four holes respectively. Distilled water was added to the fifth hole as a negative control, while disc of 30 µg Rifampicin was used as positive control.

A broad culture was diluted with peptone water to match turbidity of McFarlan standard number 3 which was used as inoculums for the microorganisms. One millilitre of the bacterial inoculums was transferred onto the pure agar plates using a sterile syringe in each case. The agar plates were then slanted to ensure uniform spread on the surface of the plates. Zero point four millilitre of the appropriate diluted extract was administered into 4 holes on each plate with varying strength of 160, 80, 40 and 20 mg for both ethanol and aqueous extracts. Similarly, 0.4 ml of distilled water was then administered in to the fifth hole on the media plate, while 30 µg Rifampicin disc was used as control. Five different media plates were prepared for each organism. One hour was allowed for diffusion before incubation of plates at 37^{o}C for 24 hours. The clear zones of inhibition for the respective strength of both extracts were measured in millimetres using a metre rule.

Determination of the Minimum Inhibitory Concentration

The values for the minimum inhibitory concentration of each extract were obtained by extrapolation from the plot of the log strength (per hole) of the extract against the clear zones of inhibition in millimeters [11].

Statistical Analysis

Student t-test was used in the analysis to determine the level of significance of the various bacterial zones of inhibition observed. P-value less than 0.05 were considered significant.

RESULT ANALYSIS

Acute Toxicity Study of Ethanol and Aqueous Leaf Extracts of *Cassia alata*

The LD_{50} obtained from acute toxicity study was 566 mg in both rats and mice for aqueous extract, while the LD_{50} for ethanol extract were 566 mg and 895 mg in rats and mice respectively (Table 1).

Qualitative Phytochemistry of Ethanol and Aqueous Leaf Extracts of *Cassia alata*

The result obtained from the phytochemistry showed that both the ethanol and aqueous extracts of *C. alata* contained glycosides, reducing sugars, flavonoids, terpenes and saponins, whereas tannins and alkaloids were not detected in both extracts. Anthraquinone was detected in the ethanol but was absent in the aqueous extract (Table 2).

Quantitative Phytochemistry of Ethanol and Water Leaf Extracts of *Cassia alata*

Phytochemistry showed that terpenes were present in high concentration, whereas glycosides, flavonoids, and anthraquinones were seen in moderate concentration in the ethanol leaf extract of *Cassia alata*. However, reducing sugars and saponins were found in low concentration in the extract. Terpenes and glycosides were in moderate concentration, whereas flavonoids, saponins and reducing sugars were seen in low concentration in the aqueous leaf extract (Table 3).

Antibacterial Screening of Aqueous Leaf Extract of *Cassia alata*

The result from the antibacterial assay of the aqueous extract showed that at 20 mg there was no growth inhibition against *Staphylococuss aureus* and *Salmonella typhi,* whereas there was activity on *Escherichia coli.* The extract at 40 mg and above and the positive control (Rifampicin) showed activity against all the organisms studied. However, the extract showed a higher activity against *E. coli* at 160 mg than Rifampicin with statistically significant difference ($p<0.05$) (Table 4).

Antibacterial Screening of the Ethanol Leaf Extract of *Cassia alata*

The effect of ethanol leaf extract against *Salmonella typhi* and *Escherichia coli* was dose dependent, whereas a decrease in activity was observed on *Staphylococuss aureus* at increasing dose of the ethanol extract. The positive control (Rifampicin) showed activity on all the organisms studied. However, 160 mg of ethanol leaf extract showed significantly higher activity than Rifampicin against *Escherichia coli* ($p<0.05$) (Table 5).

Minimum Inhibitory Concentration of Ethanol and Water Leaf Extract of *Cassia alata*

The minimum inhibitory concentration (MIC) of the ethanol leaf extract of *Cassia alata* for *Escherichia coli* and *Salmonella typhi* were obtained from the graph by extrapolation of a plot of log dose versus zones of inhibition (mm) as 3.55 mg and 1.41mg respectively. The MIC for *Staphylococuss aureus* could not be determined by graphical method because the effect of the extract was not dose dependent on the organism. The MIC of aqueous leaf extract of *Cassia alata* for *Staphylococuss aureus, Escherichia coli* and *Salmonella typhi* were 25.12 mg, 3.55 mg and 22.39 mg respectively (Table 6).

DISCUSSION

The acute toxicity study of the ethanol and aqueous leaf extracts revealed some behavioral changes in most mice and rats at 3 hours after intraperitoneal administration of the extracts at 1000 mg/kg. The toxic signs disappeared in most of those that received a dose of 100 mg after 24 hours. No adverse changes were observed in rats and mice treated with less than 100 mg/kg of the extracts. The LD_{50} of 566 mg/kg in rats and 895 mg/kg in mice for ethanol extracts obtained in the present study could likely be attributable to the environmental changes the laboratory animals were subjected to during the study period. This however, contradicts with the result of acute toxicity obtained by Pieme *et al* [12] that showed higher LD_{50} of 18.5 g/kg even though the route of administration was oral, in addition to the mixed solvent

system (hydro-ethanolic) used by Pieme [12] and his colleagues.

The result obtained from this study showed that the ethanol and aqueous leaf extracts of *Cassia alata* was similar to the report presented by El-Mahmoud and Doughari [6]. However, the absence of tannins and alkaloids contradicts the finding of El-Mahmoud and Doughari [6] that earlier reported the presence of these bioactive metabolites. The use of methanol and chloroform solvent system by El-Mahmoud and Doughari [6] for extraction could be the likely explanation for the disagreement. Anthraquinone was detected in the ethanol extract while the aqueous extract did not, may be attributed to the higher water polarity compared to ethanol. The presence of these phytochemical component detected in both the extracts may be responsible for the observed antimicrobial activity of the plant leaf extracts. This finding conforms to the report of Anyanwu and Dewet [13] in which similar constituents were found to exhibit antiprotozoal and antibacterial activities. Flavonoid has also been reported by Jouad *et al* [14] to possess greater potential benefit to human health. Quantitatively, ethanol leaf extract was found to extract most of the bioactive components than the water extract which could be explained by the polarity difference of the two solvents used. The inhibiting activities exhibited by the extracts on the microorganism tend to agree with the reports of Levin *et al* [15] and El-Mahmoud and Amey [16], that linked antimicrobial properties of plants to the presence of bioactive secondary metabolites like saponins, flavonoids, glycosides, diterpenes etc. Mbuh and his colleagues [17] investigated the antibacterial activity of leaf extracts of *Psidium guajava* and found out that the bioactive compounds in the plant inhibited the growth of *Escherichia coli, Staphylococuss aureus, Shigella species, Proteus mirabilis* and *Klebsiella pneumoniae.* The ethanol and aqueous crude leaf extracts of *Cassia alata* inhibited the growth of *Escherichia coli, Salmonella typhi* and *Staphylococuss aureus* to varying degrees dose dependently. The activity of the leaf extracts against both gram negative and gram positive bacteria is an indication that a broad spectrum antibacterial compound could be present in the extracts studied. In the present study, distilled water used as control did not show any activity. However, the standard antibiotic (Rifampicin 30 µg) consistently displayed much activity when compared with the extracts on *Escherichia coli* and *Staphylococuss aureus*. This may be attributed to the fact that Rifampicin is a pure compound compared with the doses of crude leaf extracts of *Cassia alata* used. This could further be supported by the report of El-Mahmood and Amey [16] in which crude extracts having a mixture of plant constituent interfered with antimicrobial activity via degradation and decomposition especially on long term storage. Interestingly, the activity of both aqueous and ethanol leaf extracts of *Cassia alata* on *Escherichia coli* showed a higher statistically significant difference ($p<0.05$) than the positive control (Rifampicin 30 µg) at the highest dose tested (160 mg). The ethanol leaf extract of *Cassia alata* at 160 mg was found to inhibit the growth of *Salmonella typhi* significantly higher than the aqueous leaf extract at the same dose of 160 mg ($p<0.05$). Conversely, 160 mg aqueous leaf extract inhibited the growth of *Escherichia coli* significantly higher ($p<0.05$) than the ethanol extract of *Cassia alata* at the same dose tested. The use of the decoction of this plant traditionally has been justified especially the use of aqueous leaf extract at 160 mg on infectious diseases caused by *Escherichia coli*. The higher activity demonstrated by ethanol extract (organic solvent) in this study showed that most of the bioactive principles in *Cassia alata* are extracted in ethanol.

The minimum inhibitory concentration (MIC) of the aqueous and ethanol leaf extracts against organisms tested in this study agrees with the reports of Makinde et al [18] and Emeruwa [19] in which the effects of most crude leaf extracts vary widely in their degree of susceptibility to antimicrobial agents. High MIC value is an indication of low activity while low MIC value is an indication of high activity. In this study, *Salmonella typhi* had the lowest MIC value for the ethanol extract, thus suggesting highest susceptibility to the efficacy of the crude extract and hence justifying ethnomedical use of the leaf extract for typhoid fever and other gastrointestinal infections. The aqueous extract showed the highest MIC value on *Staphylococuss aureus* thus suggesting the lowest susceptibility of the organism to the extract.

CONCLUSION

Cassia alata has been known to contain saponins, reducing sugars, flavonoids, terpenes, anthraquinones and glycosides. The presence of some of these bioactive metabolites could be responsible for the observed antibacterial activities on the susceptible organisms studied. Ethanol leaf extract had better antibacterial activity against *Salmonella typhi*, while aqueous leaf extract was better on *Escherichia coli*.

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REFERENCES

1) Irwin HS, Barneby RC. The American *Cassinae*: Synoptical versions of leguminosae, tribe cassieae, sub tribe cassinae in the New York. Botanical garden 1982, 35(2): 455-918.

2) Arbonnier M. Trees, shrubs and lianas of West African dry zones. CIRAD, Margrat publishers, Gmbh, MNHN, Paris, France. 2004, Pp 573.

3) Sule WF, Okonko IO, Ojezele MO, Nwanze JC, Alli JA, Adawale OG et al. Invitro antifungal activity of *Cassia alata* leaf extract. Advances in Applied Science Research 2010, 1(2): 14-26.

4) Zhongguo Z. Studies on chemical constituents from leaves of *C. alata*. Chinese Article 2009, 34(7): 861-3.

5) Adedayo O, Anderson WA, Mooyoung M, Snieckus V, Patil PA, Kolawale DO. Phytochemical and antibacterial activity of *Senna alata* flower. Pharmaceutical Biology 2001, 39(6): 408-412.

6) El-Mahmoud AM, Doughari JH. Phytochemistry and activity of *Cassia alata*. African Journal of Pharmacy and Pharmacology 2008, 2(7): 124-129.

7) Owoyale JA, Olatunji GA, Oguntoye SO. Antifungal and antibacterial activities of an alcoholic extract of *Cassia alata* leaves. Journal of Applied Science and Environmental Management 2005, 9(3): 105-107.

8) Lorke D. A new approach to practical acute toxicity testing. Archives of Toxicology 1983, 54(4): 275-287.

9) Trease GE, Evans WC. Pharmacognosy. BaillaiereTindals, London. 1989, Pp687-689.

10) Sofowora A. The state of medicinal plants research in Nigeria.University Press, Ibadan, Nigeria, 1978, pp. 86.

11) Timothy SY, Galadima IH, Wazis CH, Maspalma DI, Bwala AY, Reuben U et al. Antibacterial and Phytochemical screening of N-butanol and Ethyl acetate leaf extract of *Byrsocarpus coccineus* Schum and Thonn. Sahel Journ of Vet Science 2011, 10(2): 21-26.

12) Pieme CA, Penlap VN, Nkegoum B, Taziebou CL, Tekwu EM, Etoa FX et al. Evaluation of acute and sub acute toxicities of aqueous ethanol extract of leaves of *Cassia alata*. African Journal of Biotechnology 2006, 5(3): 283-289.

13) Anyanwu GI, Dewet A. Pharmacological and phytochemical screening of *Hyptis suaveolens poit (Lamiaceae)* for bioactivity in rodent. Nigerian Journal of Botany 2005, 18:190-196.

14) Jouad H, Lacalle-Duboi MA, Lyoussi B, Eddouks M. Effect of the flavonoids extracted from *Spergularia purpurea pers* on arterial blood pressure and renal function in normal and hypertensive rats. Journal of Ethnopharmacology 2001, 76(2): 159-163.

15) Levin MD, Vandon-Berghe DA, Marten T, Vilientmick A, Lomwease EC. Screening of higher plants for biological activity. Planta Medica 1979, 36: 311-312.

16) El-Mahmood AM, Amey JM. In vitro antibacterial activity of *Parkia biglobosa* root bark extract against some microorganisms associated with urinary infections. African Journal of Biotechnology 1997, 6(11): 1272-1275.
17) Mbuh FA, Asika IS, Doughari JH. Studies on antibacterial activities of leaf extracts of *Psidium guajava L*. Best Journal 2008, 5(1): 44-47.
18) Makinde AA, Igoli JO, Ta`ama L, Shaibu SJ, Garba A. Antimicrobial activity of *Cassia alata*. African Journal of Biotechnology 2007, 6(13): 1509-1510.
19) Emeruwa KC. Antimicrobial substances from *Carica papaya* fruit extracts. Journal of Natural Products 1982, 45(2): 123-127.

Table 1: Acute toxicity study of aqueous and ethanol leaf extracts of *C. alata*

Extract	*LD_{50} (mg/kg)*	
	Rat	*Mice*
Aqueous	566	566
Ethanol	566	895

LD_{50} = Lethal dose that kills 50% of laboratory animals

Table 2: Qualitative phytochemistry of ethanol and aqueous leaf extracts of *Cassia alata*

S/N	*Phytochemical components*	*Extracts*	
		Ethanol	*Aqueous*
1	Glycosides	+	+
2	Reducing sugars	+	+
3	Flavonoids	+	+
4	Terpenes	+	+
5	Anthraquinones	+	_
6	Tannins	_	_
7	Alkaloids	_	_
8	Saponins	+	+

+ = present - = absent

Table 3: Quantitative phytochemistry of ethanol and aqueous leaf extracts of *Cassia alata*

S/No.	Phytochemical component	Results	
		Ethanol	Water
1	Terpenes	+++	++
2	Glycosides	++	++
3	Flavonoids	++	+
4	Anthraquinones	++	-
5	Reducing sugars	+	+
6	Saponins	+	+

+ = present in low concentration
++ = present in moderate concentration
+++ = present in high concentration

Table 4: Antibacterial assay of aqueous leaf extract of *Cassia alata* showing the zones of inhibition (mm) (n=5)

Organism	**Water extract**				
	Control	**20mg**	**40mg**	**80mg**	**160mg**
Staph. aureus	27.20±0.84	N.I	11.60±1.14*	14.00±1.58*	18.20±0.84*
S. typhi	23.60±3.65	N.I	12.60±1.14*	16.20±0.84*	20.00±1.58
E. coli	26.80±1.92	18.20±0.84*	20.00±1.58*	27.40±1.14	37.00±1.58*

*indicates a significant P value (T-test) with the control, Control = 30µg Rifampicin,
Staph. aureus = Staphylococcus aureus, E. coli = Escherichia coli, *S. typhi = Salmonella typhi*

Table 5: Antibacterial assay of ethanol leaf extract of *Cassia alata* showing the zone of inhibition (mm) (n=5)

Organism	**Ethanol extract**				
	Control	**20mg**	**40mg**	**80mg**	**160mg**
Staph. aureus	27.20±0.84	23.60±1.14*	25.00±1.58	18.80±1.92*	17.20±1.79*
S. typhi	23.60±3.65	15.80±0.84*	18.20±0.84*	21.60±1.14	26.00±1.58
E. coli	26.80±1.92	21.4±2.30*	18.40±4.72*	24.60±2.40*	29.40±2.07*

*indicates a significant P value (T-test) with the control, Control = 30µg Rifampicin,
Staph. aureus = Staphylococcus aureus, E. coli = Escherichia coli, *S. typhi = Salmonella typhi*

Table 6: MIC of ethanol and aqueous leaf extracts of *Cassia alata*

S/No.	Test organism	MIC (mg)	
		Ethanol	Water
1	*Staph. aureus*	-	25.12
2	*E. coli*	3.55	3.55
3	*S. typhi*	1.41	22.39

MIC = Minimum inhibitory concentration
Staph. aureus = Staphylococcus aureus,
E. coli = Escherichia coli,
S. typhi = Salmonella typhi

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