Evaluation of Phytochemical Components of Aqueous Root extracts of *Cassia alata* and Its Pharmacological Effects on Isolated Rat Jejunum.

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ABSTRACT

Cassia alata Linn is one of the most important species of the genus *Cassia*, which is now widely distributed in the tropics. The root of *Cassia alata* had been preferentially used early in this century in the South-South part of Nigeria, especially as laxative and abortifacient by women, with apparent success.This study evaluated the phytochemical components of *Cassia alata* root using standard methods of screening; also the stimulatory effects of the aqueous extract of *Cassia alata* root on isolated tissue from rat jejunum were studied by suspending each tissue piece in a 25 ml organ bath using *in vitro* experimental procedures. It also proffers into the possible mechanism of its action by comparison to known standard agonists and antagonists. Phytochemical screening of the roots of *Cassia alata* confirmed the presence of alkaloids, flavonoids, reducing compounds, glycosides and polyphenols. The stimulatory effect of the aqueous extract of *Cassia alata* root (8×10^{-4} -8×10^{-7} g/ml) on the rat jejunum were not attenuated by Atropine, propranolol or phentolamine. This suggests to a greater extent specific interaction with specific receptor, and to a lesser extent, the involvement of non-specific interaction with specific receptor at higher concentration of the extract. The aqueous extract exhibited marked dose-dependent spasmodic effect on drug-induced contractions of the jejunum. Cassia alata root could be therefore be a possible plant source of laxative drugs.

KEY WORDS: *Cassia alata (RCAE)*, Phytochemical components, Laxative, Stimulatory effect.

INTRODUCTION

There are over 33 *Cassia species Linn* (Leguminosae caesalpinodeae family) growing in Nigeria, especially in the Southern part (Abo et al., 1999). The plant *Cassia alata* is a herb commonly found in cool areas. It often grows up to 15 feet tall; and it has green alternate leaves, with even-pinnately leaflets. The flowers are on a long pedicle with yellow colour and bloom from the bottom to the end with 4-5 petals. It is widely distributed from tropical America to India and Bangladesh (Kirtikar and Basu), Indonesia and Malaysia (Corner), (Smith and Au, 1979). It is a pan tropical shrub, native to tropical Americas (Adnan et al., 2011).

The plant is differently named in different countries. The names of some of the Nigeria *Cassia species* include *C. alata, C. fistula, C. hirsute, C. occidentalis, C. podocarpa, C. siamea, C. sieberiena, C. autifolia*, just to mention a few. *Cassia alata* is commonly called 'Udokaya' in South-South Nigeria, precisely in Cross River and Akwa Ibom States (Abo et al., 1999). It is locally known as dadmardan in Bangladesh and India. Chinese: Chi jia jue ming, Spanish: bajagua, mocote. It is also known as Candelbra Bush, Empress, Candle plant, Ringworm tree or Candle tree in English (Adnan et al., 2011).

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The crude extract of *Cassia alata* root was often applied as rectal enema to induce abortion and for laxative effects. The leaves and sometimes the roots were ground to powder, and then mixed with local alcoholic and then applied on the skin to decorate the skin by women during cultural ceremonies.

Some studies and investigations have been carried out on the seeds, leaves and barks of *Cassia alata* but only a few studies have been reported on the roots. *C. alata* is one of the most important species of the genus *Cassia* which is rich in anthraquinones and polyphenols (Palanichamy and Nagarajan, 1990; Yagi *et al*., 1998). The leaves of *C. alata* have been qualitatively analyzed for the presence of primarily five pharmacologically active anthraquinones: Rhein, aloe-emodin, chrysophanol, emodin, and physcion (Smith and Ali, 1979), as well as the flavonoid kaempferol (Rao *et al*., 1975). Rhein and chrysophanol are known to be present in the roots (Co, 1989), in addition to two other quinine pigments (Tiwaari and Yadava, 1981). These anthraquinones derivatives are well known to exhibit a variety of biological activities (Thomson, 1987). Previously, *C.alata* extracts were analyzed for the presence of kaemferol-3-o-gentiobioside in various parts of the plant (leaves, flowers, rachis, stem, and seed) using high performance liquid chromatography (HPLC) (Moriyama et al., 2003).

The antimicrobial properties of most of the *Cassia species* growing in Nigeria have been studied. However, comprehensive report of biological evaluations of the phytochemical screening of the root of *Cassia alata* growing in Nigeria is scanty. There are only few available report of detailed investigations carried out on effect of *C.alata* on Jejunum smooth muscle contractile activities. Thus, this study investigated the phytochemical components of *Cassia alata* root and the pharmacological effects of the root extract on isolated rat jejunum.

MATERIALS AND METHODS

Plant collection and identification

Fresh roots of *Cassia alata* were collected from Itak Ikot-Akap, in Ikono local government area of Akwa Ibom state. The plants were identified and authenticated by Dr.(Mrs) Margaret Bassey, the taxonomist at the Department of Botany and ecological studies, University of Uyo as *C. senna alata* by its morphological characters, including numerical value of its stomatal index, and the plant sample was deposited at the Herbarium unit of the Department, at the university of Uyo, Nigeria with Voucher No: 1059.

Plant Extraction

Some quantity of *Cassia alata* was dried and grounded to powder. A quantity of the grounded sample (100 g) was weighed and extracted with 250 ml distilled water at 100^{o}C for 8-10 hours. On evaporation of the extract using a rotary vacuum evaporator a brownish solid mass was obtained. The percentage yield of the extract was 21.3%.

Animals

Six (6) healthy Wistar rats of both sexes (140-200 g) were purchased from the animal house of the department of pharmacology and toxicology, university of Uyo. The animals were fed with standard rodent feed for laboratory animals, and all of them were allowed free access to drinking water. All the animals were kept in standard condition of temperature (22^{o}C), with good ventilation. They were all shielded from direct sunlight, and the environment was kept clean to ensure good sanitary condition. The "principles of laboratory animal care" (National Institute of Health-NIH publication No. 85-23) guidelines and procedures were followed in this study. The Ethical Committee of the postgraduate Committee of the department of pharmacology and toxicology, University of Uyo, approved this study.

The six rats were divided into 2 groups (3 rats per group). Group 1 rats which served as control were given normal saline intraperitoneally (I.P.); while each of the three (3) rats in group 2 were pretreated by receiving one dose of 8×10^{-4}, 8×10^{-5}, 8×10^{-7}g/ml extract via I.P. route respectively, for three consecutive days. The rats were fasted overnight and sacrificed on the fourth day by stunning and were subsequently used for the *in vitro* organ bath study of jejunum smooth muscle activity.

Drugs/ Chemical

Acetylcholine Chloride and Histamine diphosphate were obtained from Sigma chemical co. (USA), Propranolol from Macclesfield (Great Britain). The chemicals used were Sodium chloride and glucose (M and B, England), Calcium chloride (copharm), Magnesium chloride (Hopkin Williams, U.K), Barium chloride (BDA), Sodium bicarbonate (Sigma USA). All chemicals were of analytical grade and were dissolved in deionized distilled water at the required concentrations.

Phytochemical Screening of *Cassia alata* root
The extract was subjected to phytochemical analysis for identification of constituents using the methods of Harborne (1984) and Sofowora (1984).

Effect of *Cassia alata* root extract on Jejunum muscle contraction in rat
This involves the preparation of isolated tissues and experimentation using isolated tissue preparations. Six (6) adult rats of both sexes were used for this experiment.

The animals were stunned and bled and immediately the abdominal region of each animal was opened by midline incisions and the jejunum were quickly removed and placed in petridish containing Tyrode solution. Tubular segments of 2-3 cm long were cut from the small intestine; the 5 cm portion nearest to the jejunum was discarded and suitable length (2-3 cm long) of the remaining portion of the intestine (jejunum) was utilized to study the smooth muscles activity using different agonist, antagonist and extract. The tissue strips in each preparation was suspended vertically under a tension of about 1 g in 25 ml Organ bath containing tyrode solution that was continuously aerated with atmospheric air and maintained at 37^{o} C and connected to the thread attached to a fixed pin in the tissue bath and the other end also connected to a lever writing on a kymograph drum.

The composition of normal Tyrode solution in (mM/L) as described by P.C. Unekwe was NaCl: 13.8, KCl: 5.7, $CaCl_{2:}$1.8, $NaHCO_3$: 2.5, Na_2PO4: 0., $MgSO_{4:}$1.1, D-glucose: 5, PH 7.8.

In this an equilibration period of 60 minutes usually preceded drug application; during this period, the tyrode solution was replaced at 10 minutes intervals in order to prevent accumulation of metabolites (Unekwe et al., 2007).

In this set of experiments , contractile responses to acetylcholine (2.2×10^{-9} to 2.2×10^{-6}), Barium chloride, and Calcium chloride were conducted separately before the addition of aqueous *Cassia alata* root extract into the organ bath. The initial observations with various agonists served as the control values, which was used to compare the effects of extract on the agonist-induced contraction for graded dose-response relationships. Other antagonists were added to the fluid bathing the tissues and in each case, the effects were observed for about 0.5 - 2 minutes and this was followed by 3-5 washings.

Statistical Analysis
Results are presented as Mean ± Standard error of the mean (Mean±SEM). Differences between mean values were assessed using the analysis of variance (ANOVA), Students't'-test. Values of ($p<0.05$) were regarded as significant.

RESULTS
Results of Phytochemical screening of *Cassia alata* root are shown in Table 1.

Table 1: Results of Phytochemical Screening of *Cassia alata* Root.

S/NO	Chemical constituent	
1.	Alkaloid	+
2.	Cardiac glycosides	+
3.	Saponins	-
4.	Tannins	-
5.	Flavonoids	+
6.	Reducing Compound	+
7.	Polyphenol	+++
8.	Phlobatanins	-
9.	Anthraquinones	-
10.	Hydroxymethyl Anthraquinones	-

Keys

+ Slight Presence; + +Strong Presence; +++ Very Strong Presence; - Not Present

Table 2: Effect of Water Extract of *Cassia alata* Root on Rat Jejunum Responses to Graded Concentration of Barium Chloride.

	Control Responses	**Test Responses (g/ml)**					
FBC $BaCl_2$ (g/ml)	**-log Conc.**	**Max height (mm)**	**% max height (mm)**	**FBC Root**	**-log**	*** max height (mm)**	**% max height**
$8x10^{-6}$	5.1	4.8± 0.1	38± 0.9	$8x10^{-7}$	6.1	5 ± 0.1	21 ±0.5
$8x10^{-5}$	4.1	5.7± 0.3	45± 2.6	$8x10^{-6}$	5.1	8 ± 0.5	33 ±2.1
$8 x10^{-4}$	3.1	7.4±0.6	59± 4.5	$8x10^{-5}$	4.1	16± 0.9	67 ±3.4
$8 x10^{-3}$	2.1	11.3± 0.4	90± 2.9	$8x10^{-4}$	3.1`	12.8±0.1	53±0.8

Control Response**:** Graded concentration of Barium Chloride (g/ml)
Test Response**:** Responses in presence of 0.8mg/ml of root of *Cassia alata* extract
* $\overline{X}$ ± SEM of 4 values; Maximum height = 11.3 ± 0.4
* FBC-Final Bath Concentration

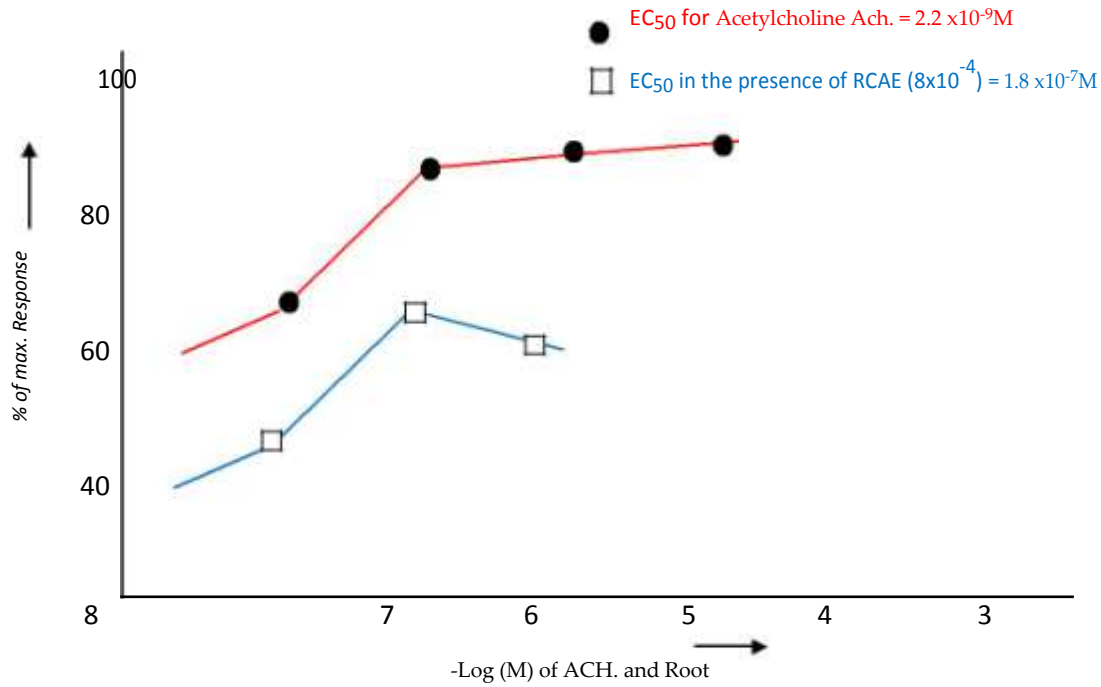

Figure 1**:** Graded Log Concentration – response curves for acetycholine on rat jejunum (controls) in the presence of *C. alata* root. Each point represents the means ± S.E.M. of 4 values.

DISCUSSION

The phytochemical screening of the roots of *Cassia alata* confirmed the presence of alkaloids, flavonoids, reducing compound, glycosides and polyphenols as some of the chemical constituents in the extracts (Table 1). *C.alata* is one of the most important species of the genus *Cassia* which is rich in

anthraquinones and polyphenols (Palanichamy, S.and Nagarajan, 1990; Yagi et al., 1998). Rhein and chrysophanol are known to be present in the roots (Co, 1989), in addition to two other quinone pigments.These anthraquinones derivatives are well known to exhibit a variety of biological activities (Thomson, 1987). Also, previously published data had reported that a number of alkaloids elicit pronounced pharmacological effects on the gastrointestinal tract smooth muscle. The mechanism of action and the active ingredients in *Cassia alata* responsible for some of its effects may be related to the alkaloid content. The alkaloids stimulate specific receptors present in the GIT smooth muscles (Gilman et al., 1980). In addition, anthraquinones stimulate colonic peristalsis and are used as laxatives (Meyers et al., 1984).

The aqueous extract of *Cassia alata* also showed some pharmacological actions on isolated jejunum smooth muscles activities. Administration of low concentration produced significant increase in the height of contraction on already contracting smooth muscles; this was shown nearly in all the test responses of the extract in the jejunum isolated tissue of non pregnant rat. It is possible that the effects encountered in this work may be due to the presence of alkaloids, glycosides, and possibly polyphenol compounds in the aqueous extracts of *Cassia alata root.* The extract also exhibited significant spasmodic effect on contraction of smooth muscle in response to increased Ca^{2+} concentration in a high Ca^{2+}- free media; this also indicated that *Cassia alata* may be acting by interfering with calcium ion mobilization during smooth muscle depolarization and contraction. Figure 1 shows that the root extract was acting as a competitive antagonist with respect to acetylcholine effect, hence, pre-administration of the extract significantly reduced the height of acetylcholine induced contraction.

The influence of the extracts on the jejunum smooth muscle preparations was tested using acetylcholine (Ach.) and barium chloride as spasmogens. The extract exhibited marked dose-dependent spasmodic effect on drug-induced contractions of the jejunum smooth muscle preparations tested. The log dose-response curves of acetylcholine (Ach.) were shifted to the right in the presence of the extract ($8x10^{-4}$g/ml) with increasing Kd_{50} (EC_{50}) values which is a reversible inhibition, but with decreasing Δ_{max} ($P<0.01$). Administration of low concentration of aqueous extracts of *Cassia alata* root consistently produced significant increases in the amplitude of contraction. The stimulatory effect of the extract ($8x10^{-4}$g/ml) on the rat jejunum smooth muscle was not attenuated by Atropine ($4.8x10^{-6}$mg/ml), propranolol ($1.5x10^{-3}$mg/ml) or phentolamine ($1x10^{-4}$g/ml). But pre-administration of Aminophylline ($5x10^{-3}$mg/ml) significantly attenuated the contraction of extract ($8x10^{-4}$g/ml) - induced spasms on the rat isolated tissues ($P<0.05$-0.01). The extract did not seem to activate cholinergic muscarine receptor. The evidence above suggests to a greater extent specific interaction with specific receptor, and to a lesser extent, the involvement of non-specific interaction with specific receptor at higher concentration of the extract (Gilman *et al.,* 1980). The results of the experiments in this study closely support the recent report by Tologbonse et al that *C.alata* is one of the species that is now increasingly being used by herbalists as laxative, arbotifacient and in the treatment of various skin diseases (Tologbonse, *et al*., 2015).

CONCLUSION

Our findings therefore suggest that the aqueous extract of Cassia *alata* root is phytochemically rich; the phytochemical screening of the root confirmed the presence of Alkaloids, Flavonoids, reducing compound, glycosides and polyphenols as some of the chemical constituents. The aqueous extract also exhibited marked dose-dependent spasmodic effect on drug-induced contractions of the jejunum; hence, it could be a possible plant source of laxative drugs.

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