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# Anti-ulcer Activity of Ethanol Root Extracts of *Cassia sieberiana D.C.* in Albino Rats

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Author HB designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors ZM and UAK supervised the work, managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aims:** The study was aimed at investigating the anti-ulcer activity of *Cassia sieberiana* fractionated root extracts using ethanol induction model in laboratory rats.
**Study Design:** This is an experimental laboratory report on phytochemical screening, acute toxicity and anti-ulcer investigations that will provide scientific information on ulcerogenic potential of *Cassia sieberiana* root.
**Place and Duration of Study:** The work was carried out at the Department of Pharmacognosy/ Drug Development and Department of Pharmacology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria from April, 2012 to February, 2013.
**Methodology:** *Cassia sieberiana* root were extracted with absolute ethanol for 48 h using cold maceration method .Reconstituted in aqueous solution, fractionated with ethylacetate and butanol. The extracts were subjected to phytochemical, toxicity experiment and anti- ulcer evaluation using ethanol – induced gastric ulcer in laboratory rats. A standard anti-ulcer agent, cimetidine was used as reference. The data were analyzed by one-way Analysis of Variance at ($p<0.05$).
**Results:** The total solid recovered from crude extract of *C. sieberiana* root yielded 80 g. The

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extract was dark brown in color with pleasant smell and pasty inconsistency. The weight of various fractions determined were ethylacetate (14.01 g), butanol (9.98 g), Aqueous (7.80 g). Preliminary phytochemical studies revealed the presence of anthraquinone saponins, glycosides, tannins, steroid, flavonoids in the ethylacetate fraction. $LD_{50}$ was above 5000 mg/kg and did not cause mortality in all the tested rats. Ethanol triggered severe gastric ulcers with mean ulcer index (53.8±2.4 mm) and pretreatment with *C. sieberiana* extracts of ethylacetate fraction (EAF) and n-butanol fraction (NBF) at (200, 400 and 800 mg/kg) and cimetidine (100 mg/kg) produced a significant ($p < 0.05$) dose dependent anti-ulcer activity with increase in percentage preventive index (72%, 91% and 100%) for EAF and (46%, 81%and 97%) for NBF fractions. cimetidine has 88% preventive index.

**Conclusion:** This study demonstrated that *C. sieberiana* has anti-ulcer potentials and it justified the traditional uses of this plant root in ulcer treatment.

*Keywords: C. sieberiana; plant extract; rats; anti-ulcer activity.*

## 1. INTRODUCTION

Peptic ulcer is one of the most common, chronic gastrointestinal disorders in modern era. Now it has become a common global health problem affecting a large number of people world- wide and also still a major cause of morbidity and mortality [1,2]. It is now considered to be one of the modern age epidemics affecting nearly 10% of world population [3]. About 6,000 die of ulcer related complication. Peptic ulcer causes significant morbidity which is mainly related to pain and hospitalization for complication [4]. Many studies indicate that plant products are potential agents for healing ulcers and largely preferred because of the absence of unwanted side effects and their effectiveness [5]. *Cassia sieberiana* belongs to the family Fabaceae (Leguminosae – caesalpinioideae). *Cassia sieberiana* is widely used for the treatment and prevention of various diseases. The phytochemical analysis of the roots had shown the presence of flavonoids, anthracenic derivates and non- hydrolysable tannins [6]. Previous studies showed that ethanolic root extract of *C. sieberiana* had an anti-parasitic effect, myorelaxant and anti-spasmodic activity [7]. Analgesic and Anti-inflammatory activity of aqueous root extract of *C. sieberiana* were also investigated [8]. The extract from various parts of the plant have been shown to possess some pharmacological activities like; anti-malaria [9], anti-diabetic [10], anti-oxidant [11] and laxative activity [12].These pharmacological activities are related to the presence of bioactive compounds such as alkaloids and flavonoids identified in the pods, roots, leaves, and stem bark of the plant. The root of this plant also contains many other natural products such as anthracenic derivatives and tannins. Flavonoids have been found previously in the roots of *C. sieberiana* through classical isolation and characterization methods [13]. Epiafzelechin has also been isolated from the Root Bark of *C. sieberiana* [14]. Only very few scientific data are available in support of gastro protective effect of *C. sieberiana* based on the traditional uses. The aim of present study was to investigate the anti-ulcer activity of root extracts in ethanol induced rat model.

## 2. MATERIALS AND METHODS

### 2.1 Solutions, Chemicals and Reagent

Freshly prepared solutions and analytical grade chemicals were used in all the experiments.

### 2.2 Collection and Identification of Plant Sample

The fresh plant of *C. sieberiana* was collected along Giwa road, Kaduna State, in April 2010. The plant was taxonomically authenticated by U S Gallah with Voucher specimen number 900202 deposited at the Herbarium, Department of Biological Sciences, Ahmadu Bello University (ABU) Zaria, Nigeria.

### 2.3 Preparation of Plant Materials

The roots, was removed from the plant and washed in clean water to removed sand, sliced into pieces and air dried for two weeks. It was then pulverized into coarse powder with mortar and pestle and stored in cellophane bags at room temperature until required for experiment [15].

Fractionation of plant sample was adapted from Cho et al. [16].

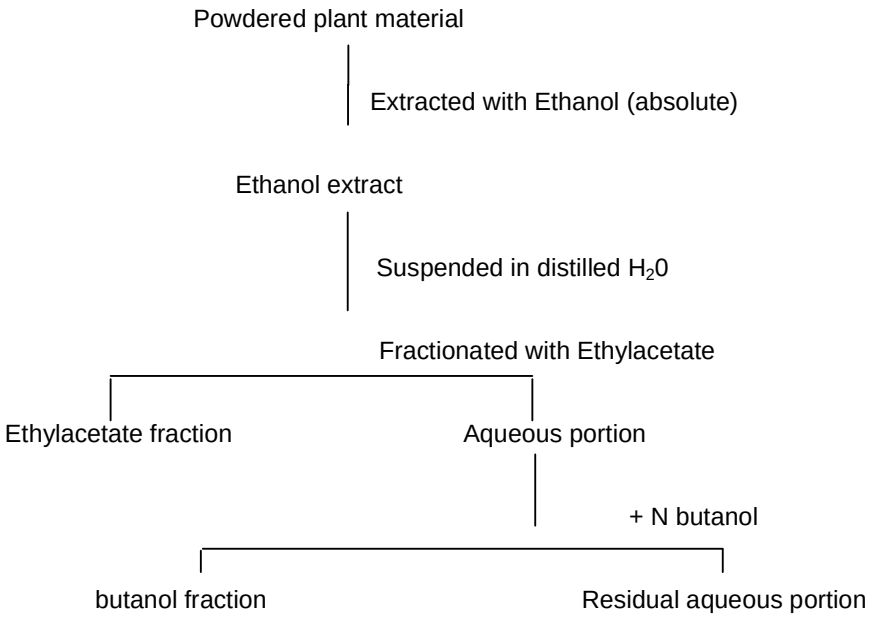


**Fig 1. Fractionation of C sieberiana powdered root**

## 2.4 Preliminary Phytochemical Screening

The phytochemical examination of *C. sieberiana* was performed by the standard methods [17-19].

## 2.5 Evaluation of Anti-ulcer Activity of Extracts of *Cassia sieberiana*

### 2.5.1 Experimental animals

A total of 45 male wister rat weighing 100 – 170 g bred in the laboratory animal unit of the Faculty of Pharmaceutical Science A.B.U Zaria. Were used for the experiment, house under similar condition of temperature and relative humidity, light, dark cycle. They were maintained on ad libitum food and water. The rats were kept in stainless steel wire mesh cages which separated them from their faeces to prevent coprophagy, they were fed on standard diet, grower mesh (ECWA feeds, Jos) and water *ad. Libitum.* Ethical rules guiding the use of animals for experimentation were strictly adhered to [20].

### 2.5.2 Acute toxicity study of extracts of *Cassia sieberiana*

The lethal dose ($LD_{50}$) of the *C. sieberiana* root extract was determined by modified method of Lorke [21] the study was carried out in two phase. The first phase requires nine rats which were divided into three groups of three rats each and were treated with the extracts at doses of 10, 100 and 1000 mg/kg body weight orally. They were observed for 24 hr for signs of toxicity. In the second phase, the procedure was repeated using three groups of three rats each and treated with the extracts at doses of 1600, 2900 and 5000 mg/kg bodyweight *(i. p).* The oral median lethal dose ($LD_{50}$) was calculated as the geometric mean of the minimum toxic dose and maximum tolerated dose using the second phase. This procedure was repeated for ethylacetate, butanol and aqueous extracts.

### 2.5.3 Experimental designed for ulcer studies

Ethanol-induced ulcer, were evaluated in rats as described by [22]. The rats were fasted for 48 hour to produce significant effect of the drug. Forty five adult rats were weighed and marked and randomly assorted into 9 groups (1-9) with each group containing 5 rats each. The separated groups were given distilled water (1 ml), ethanol (1 ml), standard drugs as positive control (cimetidine 100 mg/kg), *C. sieberiana* extract of ethylacetate fraction, (200, 400, 800 mg/kg) and butanol fraction, (200, 400, 800 mg/kg). After 30 mins of administration 1 ml of absolute ethanol was administered orally to all the rats. The animals were sacrificed by cervical dislocation and dissected after one hour. Their stomach were carefully removed, each stomach was cut open through the greater curvature with

a scissor and rinsed, stretched lightly and spread on a filter paper for proper viewing and assessment of ulcers. The stomachs were examined for ulcer macroscopically. The extent of the mucosal damage were measured by using a calibrated meter rule (in millimeters) and the ulcer indices measurement was done from left to right of each tissue. The average mucosal damage was determined and the ulcer index (U.I) was calculated. The effectiveness of the extract and drugs was calculated using the following formula [23].

Preventive index % =

$$\frac{U.I of\ negative\ control - U.I\ of\ treated}{U.I\ of\ negative\ control} \times 100$$

Tissues were then kept in air tight containers and preserved with formalin for reference & further study.

## 2.6 Statistical Analysis

One way Analysis of variance (ANOVA) was carried out to test for significant differences between the means of samples and control. A difference was considered statistically significant when $p < 0.05$.Using SPSS Version 20, followed by multiple comparisons using Duncan Multiple Range Test (DMRT) to separate the means.

# 3. RESULTS

## 3.1 Preparation of the Extract

The total solid of *C. sieberiana* crude extracts recovered from maceration was 80 g. The extract was dark brown in color with pleasant smell and pasty inconsistency. The weight of various fractions determined were ethylacetate (14.01 g), butanol (9.98 g), Aqueous (7.80 g).

## 3.2 Phytochemical Screening

Phytochemical constituents are; Anthraquinones, saponin, sterols, tannins, triterpenes, flavonoid, glycoside. Ethylacetate fraction revealed the presence of anthraquinones, flavonoid, tannins, glycoside and steroid/terpenoids. The n-butanol fractions showed the presences of tannins, and saponin. While the aqueous fractions show the presence of saponin. These secondary plant metabolites are known to posses various pharmacological effects and may be responsible for various action of *Cassia sieberiana.*

## 3.3 Acute Toxicity

Acute toxicity study ($LD_{50}$): The extracts (ethylacetate, nbutanol and aqueous) are characterized by a very low degree of toxicity. Their acute toxicity $LD_{50}$ in albino rats was found to be above 5000 mgkg-1.

## 3.4 Antiulcer Evaluation

*Cassia sieberiana* extract produced significant dose- dependent antiulcer activity in all the dose levels, Ethylacetate portion produced a significant reduction in the mean ulcer index of 15.2±0.68, 4.6±0.21, 0.0±0.0 at 200, 400, 800 mg/kg respectively when compared with 53.8±2.40 mg/kg in Ethanol treated rats. The butanolic extract shows significant reduction in the mean ulcer index of 10.27±0.46, 1.4±0.06 at 400 and 800 mg/kg respectively. The preventive index % of various groups are Ethanol (0) Cimetidine (88), Ethylacetate 200, 400, and 800 mg/kg (72, 91 and100) respectively. butanol 200, 400, and 800 mg/kg (49,81,97) respectively.

**Table 1. Phytochemical screening of the crude extract, ethylacetate, N-butanol and aqueous fraction of *C sieberiana***

| Constituent | Crude extract | EAF | NBF | AQ |
|---|---|---|---|---|
| Anthraquinones | + | + | - | - |
| Tannins | + | + | + | - |
| Flavonoids | + | + | - | - |
| Saponins | + | - | + | + |
| Terpernoids/Steroid | + | + | + | - |
| Glycoside | + | + | - | - |
| Alkaloid | - | - | - | - |

*NB: Ethylacetate, (EAF), Butanol, (NBF), Aqueous, (AQ), present (+), Absent, (-)*

**Table 2. Effect of *Cassia sieberiana* ethylacetate & n-butanol extract on ethanol induced gastric ulcer**

| Treatment | Dose | MUI± SEM | PI |
|---|---|---|---|
| Distilled water(normal) | 1 ml | 0.0±0.0[a] | -- |
| Ethanol (-ve control) | 1 ml | 53.8±2.40[e] | 0 |
| Cimetidine(+ve control) | 100 mg/kg | 6.6±0.30[b] | 88 |
| Ethylacetate | 200 mg/kg | 15.2±0.68[c] | 72 |
| Ethylacetate | 400 mg/kg | 4.6±0.21[b] | 91 |
| Ethy lacetate | 800 mg/kg | 0.0±0.0[a] | 100 |
| Butanol | 200 mg/kg | 27.4±2.40[d] | 46 |
| Butanol | 400 mg/kg | 10.2±0.46[c] | 81 |
| Butanol | 800 mg/kg | 1.4±0.06[a] | 97 |

*N.B: MUI-Mean ulcer index. SEM-Standard error mean. PI- Preventive index, -ve- Negative, +ve- Positive. Mean with same alphabets along the column are not differences at p < 0.05, N = 5 per group*

The Control rat stomach shows severe ulcer lesions, red streaks were seen on the stomach wall when induced with 1 ml ethanol, Fig. 3.1. The Rat stomachs were highly protected with cimetidine (100 mg/kg) in ethanol induced ulceration only point ulcers were determined as seen in Fig. 3.2. Rat stomachs wall were found to be completely protected with ethylacetate 800 mg/kg in ethanol induced ulceration, this show 100% preventive index as seen in Fig. 3.3. Ethylacetate 200 mg/kg shows fairly protection on rat stomachs, red streaks ulceration were also seen, due to low dose of the extract as observed in Fig. 3.4. Butanol 800 mg/kg shows significance reduction in gastric ulcer lesion as seen in Fig. 3.5.

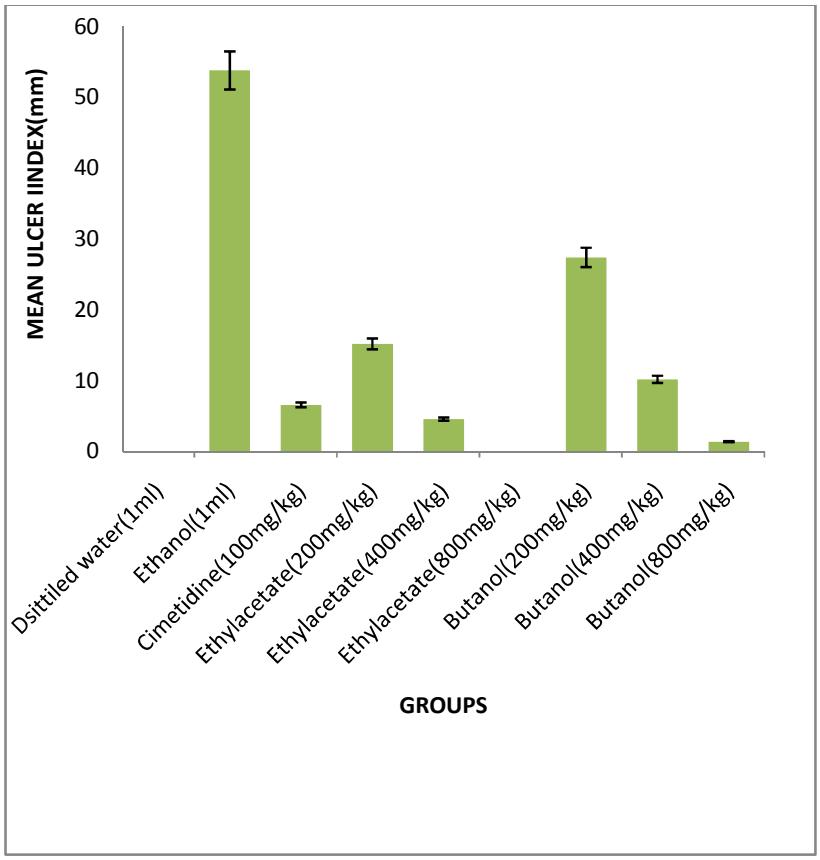


**Fig. 2.1. Ulcer indices of various groups**

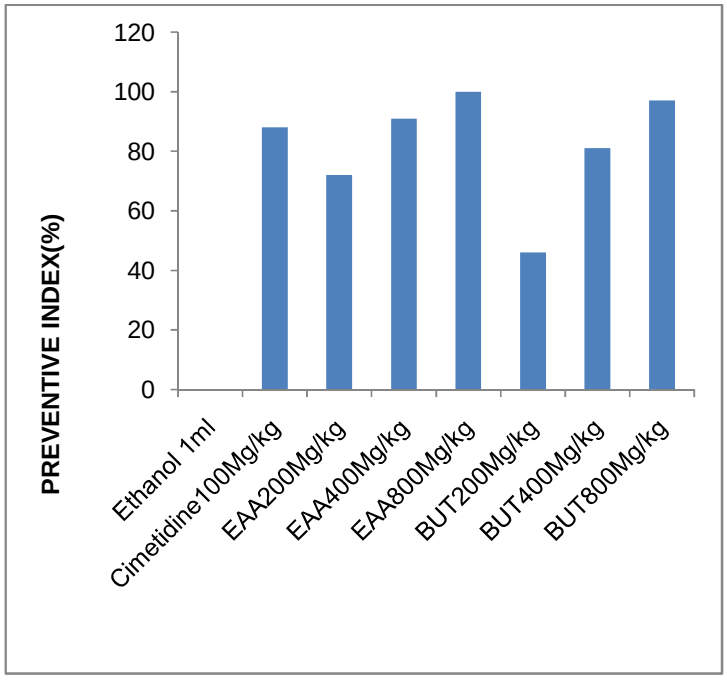


**Fig. 2.2. Preventive index of various groups**

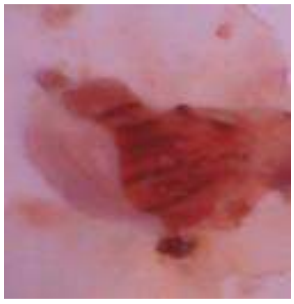

**Fig. 3.1. Control: Ethanol (1 ml)**

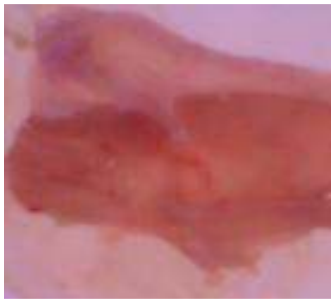

**Fig. 3.2. Cimetidine (100 mg/kg)**

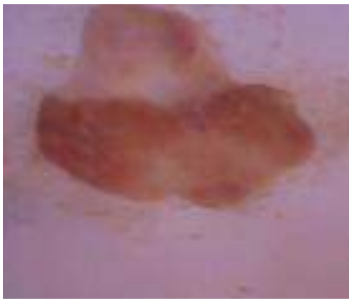

**Fig. 3.3. Ethylacetate (800 mg/kg)**

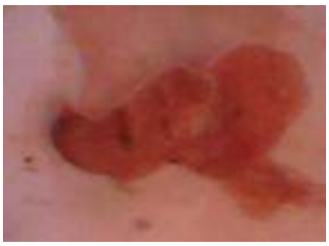

**Fig. 3.4. Ethylacetate (200 mg/kg)**

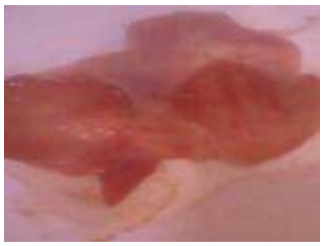

**Fig. 3.5. Butanol (800 mg/kg)**

## 4. DISCUSSION

The root extracts of *C. sieberiana* was subjected for phytochemical investigation and findings supported the previous work of Ajayi et al. [13] with the presence of anthraquinone. $LD_{50}$ studies was performed up to the dose level of 5000 mg/kg, the root extract of *C. sieberiana* was safe and did not show any clinical sign of toxicity. Also no possible side effect is associated with the use of this plant. This is Supported by previous toxicity studies of *C. sieberiana* extracts

[13,24,25,]. *C. sieberiana* extract produced significant anti-ulcer activity in all the dose levels as showed in Table 1. Ethanol triggered severe gastric ulcers with mean ulcer index (53.8±2.4 mm) and pretreatment with *C. sieberiana* extracts of ethylacetate fraction (EAF) and n-butanol fraction (NBF) at (200, 400 and 800 mg/kg) and cimetidine (100 mg/kg) produced a decrease in mean ulcer index with increase in dose of the extracts. Ethylacetate fraction at 400 mg/kg and butanolic fractions at 800 mg/kg also shows better gastro – protective effect over cimetidine (Fig. 2.1). Ethylacetate fraction shows complete gastro protective effect with preventive index of 100% at 800 mg/kg over 88% with cimetidine, preventive index percentage increases with increase in dose of the extracts, it produced a dose dependent effect (Fig. 2.2). These may be due to the facts that the extracts produced its effect by forming a cytoprotective barrier, which may be useful against peptic ulcers. Therefore *C. sieberiana* fractionated root extracts possesses strong gastrocytoprotective properties against ethanol-induced gastric ulcers. These observations confirmed to the findings of Nartey et al. [11] but with lower percentage inhibition of the extract given as 27.50%, 50.00% and 85.38% respectively for animals pretreated with 500 mg/kg, 750 mg/kg and 1000 mg/kg body weight of *C. sieberiana* root bark. Analytically more content of the terpenoids, tannins and flavonoids (Anti-ulcer compound) have been found in ethylacetate portion than butanol fraction which supported to the greater activities of ethylcetate portion of *C. sieberiana* extract. The roots of *C. sieberiana* were found to contain flavonoids and tannins which were extracted into aqueous solution [8]. Some phytochemical compounds such as flavonoid groups may prevent or suppress ulcerogenic process. This is in agreement with previous reports which shown that Cassia singueana leaf has flavonoid compound which exhibit a gastro protective effect against ethanol –induced stomach ulcers [23]. Ethanol is widely used to induced ulcers [26]. This are done by suppressing the protective action of the mucus secreted by mucus membrane the increased synthesis of mucus can be explained as the probable cytoprotective mechanism in this case [27]. *C. sieberiana* extract (CSE) suppressed ulcerogenic tendencies of ethanol in the effect suggestive of ant-ioxidant potential [23] have reported that plant drugs containing saponins, terpenoids or amino acid have anti-ulcer activity. Presence of flavonoid & phenolic compound are known to be in anti-ulcer activity. Tannins may prevent ulcer development due to their protein precipitation and also constricting effect [28]. Their astringing action can help precipitating micro proteins on the ulcer site forming an impervious layer over the lining that hinders induced gastric ulcer in rats as evidenced by the guts secretions and protects the underlying mucosa from reduction in the ulcer score. Cytoprotection by drugs has been considered to be due to the generation of prostaglandins by anti-ulcer drugs when used in their non anti-secretory doses [29]. The protective effect of Root extract of gastric ulcers many be due to the strengthening of gastric mucosa [30] or by other mechanisms like increased gastric and duodenal alkaline secretion [31] by increased luminal prostaglandin levels [32].

Flavonoids have been reported to possess significant anti-ulcer activity in various experimental models of gastric and duodenal ulceration [33]. Mahran et al. [34] have reported that plant drugs containing saponins, terpenoids or amino acid have anti-ulcer activity. Various phyto-chemicals like flavanoids, tannins, saponins, terpinoids showed their anti-ulcer activity due to their cytoprotection, antisecretory and antioxidant property [35].

## 5. CONCLUSIONS

Root extracts of *C. sieberiana* exhibited a significant anti-ulcer activity in experimental rats. This laid credence to traditional use of the plant root in ulcer treatment. Further studies on *C. sieberiana* extract are however recommended to isolate the antiulcer compounds.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

The Principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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