



## Antiulcerogenic Activity of Eight Chromatographic Fractions of Ethyl Acetate Leaf Extracts of *Securidaca longepedunculata* fres. (Polygalaceae) and *Luffa cylindrica* (L.) Roem. (Cucurbitaceae)

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### ABSTRACT

*Securidaca longepedunculata* (SL) and *Luffa cylindrica* (LC) leaf extracts have been shown to exhibit significant antiulcer effect on ethanol-induced gastric lesions. This study examined the antiulcerogenic effect of chromatographic fractions of ethyl acetate extracts of the selected plants. The ethyl acetate extracts of (SL) and (LC) were fractionated using Vacuum Liquid Chromatography (VLC). Antiulcer screening was carried out on the eight chromatographic fractions obtained. The fractionation afforded four fractions (A, B, C and D) each for *Securidaca longepedunculata* and *Luffa cylindrica*. Twenty groups of 5 rats each were treated with 50 and 100 mg/kg *b.w* of LCA1-LCD2 and SLA1-SLD2, respectively. Fraction B of LC (LCB2, 100 mg/kg *b.w*) gave the highest percentage of ulcer inhibition (94.9%) while fraction D of SL (SLD2, 100 mg/kg *b.w*) showed the highest percentage protection (92.3%), against ethanol-induced gastric ulcer. Chromatographic fractions of the ethyl acetate extracts may also possess antiulcerogenic property.

**Keywords:** Antiulcerogenic, Chromatographic fractions, *Securidaca longepedunculata*, *Luffa cylindrica*.

### INTRODUCTION

Peptic ulcers are deep gastrointestinal erosion disorder that involves the entire mucosal thickness, penetrating the muscular mucosa<sup>1,2</sup>. Peptic ulcers represent one of the most important diseases of the digestive system and a medico-social problem of global economic importance, the latter due to its broad geographical distribution, as well as high incidence, morbidity and drug consumption.

It is estimated that at some time in their life nearly 20% of all people may suffer from peptic ulcers, caused by factors such as stress, diet, smoking, alcohol and certain types of drugs<sup>3,4</sup>. The drugs currently used in the treatment of gastric ulcers are antacids, anticholinergics, proton pump inhibitors and H<sub>2</sub>-receptor antagonists. However, there are innumerable adverse effects caused by these allopathic medicines<sup>4,5</sup>, indicating the need for more effective and safer anti-gastric ulcer agents with less



side effects. In this context, metabolites derived from plants used in traditional medicine have provided an important basis for the discovery and development of modern therapeutic drugs<sup>4,6</sup>.

*Securidaca longepedunculata* Fres. (Polygalaceae) (Fig. 1), called violet tree (English), is a small plant, with sweetly smelling and bright coloured flowers. The plant is widespread throughout tropical Africa. In many parts of Africa, the plant is employed in traditional medicine principally for its psychotropic properties<sup>7,8</sup>. Other uses include treatment of rheumatic conditions, many inflammatory conditions, treatment of several sexually transmitted diseases, fever, headache, constipation, stomach pain, malaria, skin infections, peptic ulcer disease and also as sexual enhancer<sup>8,9,10,11</sup>. The pulverized root is also a functional agent in the control of pest during grain storage<sup>8,12,13</sup>.

*Luffa cylindrica* (L.) Roem. (Cucurbitaceae) (Fig. 2), known as sponge gourd or bath sponge, has been researched on for its anti-inflammatory, fungus-destroying, analgesic and sedation, anti-myocardial ischemia, antiulcer, anti-asthma and expectorant effects among others<sup>14</sup>. *Luffa cylindrica* has been found to be a unique vegetable which also belongs to a family of cucumber and marrow and also known as a vegetable sponge or sponge gourd<sup>15,16</sup>. In addition to being used as edible vegetable, *Luffa* also finds a wide application in packing medium, shoes mats, sound proof linings, bath sponges, utensil cleaning sponges, adsorbent for removal of heavy metal [such as Nickel, Lead, Chromium, Copper, etc] in waste water, and immobilization matrix for plant, algae, bacteria and yeast<sup>16,17</sup>. However, in spite of the wide medicinal application of these two plants, there is scarcity of information in literature on their application as antiulcer agents. Therefore, the aim of this study was to investigate the antiulcer activity of the chromatographic fractions of the ethyl acetate extracts of the selected medicinal plants.



**Fig. 1.** *Securidaca longepedunculata*  
Source: Adesiyan Village, Ido Local Government Area (LGA), Oyo State, Nigeria (Collected January, 2014)



**Fig. 2.** Leaves of *Luffa cylindrica*  
Source: Igboloye, Ota, Ado-Odo Ota (LGA) Ogun State, Nigeria (Collected January, 2014)

## MATERIALS AND METHODS

### Vacuum Liquid Chromatography

#### Vacuum Liquid Chromatography of ethyl acetate leaf extract of *Securidaca longepedunculata*

The ethyl acetate leaf extract (91.37 g) of *Securidaca longepedunculata* was prepared into slurry with ethyl acetate and adsorbed onto silica gel. This was allowed to dry. A vacuum column chromatography cup and a vacuum pump were used for the chromatographic set up. The column chromatography was performed on silica gel (TLC grade, without the binder) (Merck; Germany) and successively eluted with increasing polarities of solvents starting from 100% n-hexane, n-hexane/dichloromethane (DCM), DCM/ethyl acetate (EtoAC) to 100% methanol (MeOH). Fractions were collected and pooled based on their TLC profiles and the pooled fractions were concentrated. Antiulcer screening was thereafter carried out on each of the pooled fraction.

#### Vacuum Liquid Chromatography of ethyl acetate leaf extract of *Luffa cylindrica*

The ethyl acetate leaf extract (79.87 g) *Luffa cylindrica* was prepared into slurry with ethyl acetate and adsorbed onto silica gel. This was allowed to dry. A vacuum column chromatography cup and a vacuum pump were used for the chromatographic set up. The column chromatography was performed on silica gel (TLC grade, without the binder) (Merck; Germany) and successively eluted with increasing polarities of solvents starting from 100% n-hexane, n-hexane/DCM, DCM/EtoAC to 100% MeOH. Fractions were collected and pooled based on their TLC profiles and the pooled fractions were concentrated. Antiulcer screening was thereafter carried out on each of the pooled fraction.

### Thin Layer Chromatography

Analytical thin layer chromatography (TLC) was conducted with Si gel 60 F254 plates (10 x 5 and 5 x 1 Merck, Germany).

### Experimental Animals for Bioassays

One hundred albino Wistar rats of both sexes with average weight 130 g were used for the experiments. These animals were purchased from the animal house of the Department of Physiology, University of Ibadan, Oyo State, Nigeria. The rats were kept in wooden cages with wooden shaven beddings to prevent coprophagy. The male rats were separated from the female rats to prevent copulation. The rats were supplied water *ad libitum* and fed standard feed. They were kept under standard conditions of temperature and humidity. The animals were acclimatized for a period of one week in the animal house of the Department of Physiology, University of Ibadan, Oyo State, Nigeria. The ethical standard for animal handling and treatment was followed.

### Animal Treatment

One hundred rats was divided into 20 groups of 5 rats each with average weight 130 gram. The treatment groups comprised:

Normal Control: Normal saline treated animals

Positive Control (Ulcer Group): Animals challenged with Absolute Ethanol

Negative Control (Cimetidine Group): 50 & 100 mg/kg *b.w* cimetidine treated animals and ulcerated SL Chromatographic Fractions Pretreated animals prior to Ulcer Induction

SLA1 & SLA2: 50 & 100 mg/kg *b.w* extract

SLB1 & SLB2: 50 & 100 mg/kg *b.w* extract

SLC1 & SLC2: 50 & 100 mg/kg *b.w* extract

SLD1 & SLD2: 50 & 100 mg/kg *b.w* extract

LC Chromatographic Fractions Pretreated animals prior to Ulcer Induction

LCA1 & LCA2: 50 & 100 mg/kg *b.w* extract

LCB1 & LCB2: 50 & 100 mg/kg *b.w* extract

LCC1 & LCC2: 50 & 100 mg/kg *b.w* extract

LCD1 & LCD2: 50 & 100 mg/kg *b.w* extract

### Experimental Protocol for Ulceration

Wistar rats were fasted overnight but given water *ad libitum*. The chromatographic fractions and cimetidine were given to the animals orally.

Fifty minutes later, 1 mL of absolute ethanol was administered orally. The animals were sacrificed after 1 h and their stomachs excised and opened along the greater curvature, rinsed with 1.15% KCl and ulcer scoring/severity grading was done.

### Quantification of Ulceration

Ulceration index which is the extent of ulceration in the rat and percentage inhibition was done and calculated according to the methods described by Kayode *et al.*,<sup>8</sup> and Lee *et al.*,<sup>18</sup>

## RESULTS

The fractions obtained from the Vacuum Liquid Chromatography (VLC) of SL and LC are illustrated in Table 1 and 2. The VLC of LC afforded four fractions with the yield (g) in the following order LCD > LCC > LCB > LCA. Similarly, the VLC of SL gave four fractions with the yield in this pattern SLD > SLC > SLB > SLA (Table 2). Groups treated with LCB1 and LCB2 (from Fraction LCB) gave the highest percentage ulcer inhibition of 92.3% and 94.9%, respectively. The LCC1 has the lowest percentage ulcer inhibition (Table 3). Animals pretreated with SLD1 and SLD2 showed the highest percentage gastric ulcer inhibition (87.2% and 92.3%) among the groups pretreated with the chromatographic fractions of *Securidaca longepedunculata* (Table 4). The lowest of the SL treated groups was the SLA1 with 64.8% (Table 4). The standard antiulcer drug, cimetidine, used as negative control revealed 64.1% and 71.8% for 50 and 100 mg/kg *b.w*, respectively (Table 3 and 4).

**Table 1: Vacuum Liquid Chromatography of Ethyl acetate Extract *Luffa cylindrica* (79.87 g)**

VLC Pooled			
Fractions (Weight g)		Solvent Mixture	Ratio Per 500 mL
LCA 1-5	(7.92)	Hexane/DCM	70:30
LCB 6-12	(10.88)	DCM/Hexane	90:10
LCC 13-18	(15.60)	DCM/Ethyl acetate	60:40
LCD 19-34	(40.87)	Ethyl acetate/Methanol	70:30

**Table 2: Vacuum Liquid Chromatography of Ethyl acetate Extract *Securidaca longepedunculata* (91.37 g)**

VLC Pooled			
Fractions (Weight g)		Solvent Mixture	Ratio Per 500 mL
SLA 1-4	(7.78)	Hexane	80:20
SLB 5-12	(8.73)	DCM/Hexane	90:10
SLC 13-18	(21.29)	Ethyl acetate/DCM	60:40
SLD 19-32	(29.53)	Ethyl acetate/Methanol	70:30

**Table 3: Effect of VLC fractions *Luffa cylindrica* on absolute ethanol-induced gastric ulcer**

Experimental Group	(Weight mg/kg <i>b.w</i> )	Inhibition (%)
LCA1	(50)	61.5
LCA2	(100)	92.3
LCB1	(50)	92.3
LCB2	(100)	94.9
LCC1	(50)	38.5
LCC2	(100)	46.2
LCD1	(50)	46.2
LCD2	(100)	61.5
Ethanol	(1 mL)	0.0
Cimetidine	(50)	64.1
Cimetidine	(100)	71.8
Normal control	(1 mL Normal Saline)	-

**Table 4: Effect of VLC fractions *Securidaca longepedunculata* on absolute ethanol-induced gastric ulcer**

Experimental Group	(weight mg/kg <i>b. w</i> )	Inhibition (%)
SLA1	(50)	64.8
SLA2	(100)	71.8
SLB1	(50)	82.1
SLB2	(100)	92.3
SLC1	(50)	71.8
SLC2	(100)	76.9
SLD1	(50)	87.2
SLD2	(100)	92.3
Ethanol	(1 mL)	0.0
Cimetidine	(50)	64.1
Cimetidine	(100)	71.8
Normal control	(1 mL Normal Saline)	-

## DISCUSSION

Induction of gastric ulcers using ethanol are commonly used for the assessment of gastroprotection<sup>19</sup>. Oral administration of absolute ethanol solution to the control group clearly produced the necessary mucosal injury. Absolute ethanol solution (1 mL/ 130 g *b.w*) induced gastric ulcer in all treated animals and this agrees with reports in other previous studies<sup>8,18,20</sup>. Pretreatment with 50 mg/kg and 100 mg/kg of LCA1 – LCD2 and SLA1 - SLD2 caused significant ulcer inhibition in varying degrees. The significant percentage inhibition of

ulceration shown by the groups pretreated with the chromatographic fractions of *Luffa cylindrica* and *Securidaca longepedunculata* suggests that these treatment agents have gastro protective properties<sup>8,19,20,21,22</sup>. The present study demonstrates that the VLC fractions of the ethyl acetate leaf extracts of *Securidaca longepedunculata* and *Luffa cylindrica* are possible potent gastro protective and antiulcer agents against ethanol-induced gastric ulcer. The obtained results show that these fractions induced significant antiulcer effect in ethanol-induced gastric lesions. We speculate that the gastro protective and antiulcer effects of these fractions from the selected plants could be partly attributed to free radical scavenging property of the plant, inhibition of gastric acidity and strengthening of the gastric mucosal barrier through antioxidant enzyme induction. In summary, our data suggest that the antiulcer effect of these plants<sup>8</sup> appears to be retained in the chromatographic fractions of the ethyl acetate extracts. The data obtained for the effect of treatment of the chromatographic fractions on ethanol-induced ulcer in rats provide a scientific platform for the validation of the folkloric and public use of these plants in ulcer disease treatment and management. The data given may correlate with the results obtained for other indigenous medicinal plants used traditionally for gastric ulcer treatment<sup>23,24,25,26,27,28,29</sup>. The results obtained from this study could influence the development of a new drug for gastric ulcer treatment.

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## Conflict of interest

The authors declare that there are no conflicts of interest.

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