

## Gastroprotective potentials of *Annona senegalensis* ethanol leaf extract in wistar rats

Anthony Uchenna Megwas<sup>1\*</sup>, Godwin Christian Akuodor<sup>2</sup>, Leo Clinton Chukwu<sup>3</sup>, Edmund Maduabuchi Okorie<sup>1</sup>, Desmond Onyebuchi Eke<sup>4</sup>, Anuli Nkechinyere Chukwumobi<sup>5</sup>, Ikoro NC<sup>1</sup>, Prince Chiazor Uneke<sup>2</sup>

<sup>1</sup> Department of Optometry, School of Health Technology, Federal University of Technology, Owerri, Nigeria.

<sup>2</sup> Department of Pharmacology and Therapeutics, Faculty of Medicine, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Nigeria.

<sup>3</sup> College of Medicine, Chukwuemeka Odumegwu Ojukwu University, Amaku, Awka, Nigeria.

<sup>4</sup> Department of Pharmacology and Therapeutics, Faculty of Medicine, Ebonyi State University, Abakaliki, Nigeria.

<sup>5</sup> Department of Internal Medicine, Chukwuemeka Odumegwu Ojukwu University Teaching Hospital, Awka, Nigeria.

Received May 3, 2021; Accepted June 11, 2022; Published online June 30, 2022

---

### Abstract

The leaves of *Annona senegalensis* are traditionally used for the treatment of different diseases in Nigeria, including gastrointestinal disorders. The anti-diarrhoeal and ulcer-protective activities of its ethanol extract were determined in Wistar rats. This extract was investigated for castor oil- induced diarrhoea, intestinal transit time and castor oil induced enteropooling in rats. The leaf extract was also tested for its ulcer protective potentials in acetylsalicylic acid, ethanol and stress- induced ulceration in rats. The phytoconstituents and oral acute toxicity of the leaf extract were also assessed. The extract significantly reduced the frequency of fecal dropping in castor oil- induced diarrhea. It also inhibited the masses and volumes of intestinal accumulation in castor oil induced enteropooling in rats. The distance travelled by charcoal meal was as well reduced. The phytochemical tests revealed the presence of alkaloids, saponins, tannins, flavonoids, terpenoids, and anthraquinone. However, the LD<sub>50</sub> was greater than 5000 mg/kg in rats. The ethanol leaf extract of *A. senegalensis* exhibited strong

---

\*e-mail: [tonymegwas@yahoo.com](mailto:tonymegwas@yahoo.com)

antidiarrhoeal and ulcer protective activities, thus justifying its folkloric use by the local population against gastrointestinal disorders.

**Keywords:** Antidiarrhoeal, Ulcer protective, *Annona senegalensis*, Leaf extract, Rats

---

## 1 Introduction

Herbal medicines are an important part of the culture and traditions of African people. Plants are valuable sources of natural products for maintaining human health for decades (Mohammed et al., 2015). Today, most African populations depend on herbal medicines for their health care needs. The World Health Organization has long recognized and drawn the attention of many countries to the ever increasing interest of the public in the use of medicinal plants and their products in the treatment of various ailments (WHO, 1999; Tarkang et al., 2015). These plants, which are found in our environment enjoy wide acceptability by the population and serve as affordable alternatives to orthodox medicine (Akuodor et al., 2012). Such medicinal plants can be explored since it has been shown that they are important sources of new chemical substances with potential therapeutic effects. Many plants are known to have some pharmacological activities in the treatment of diarrhoea, ulcer, pain, inflammation and fever among others (NRC, 2008; Akuodor et al., 2011; Akuodor et al., 2012)

*Annona senegalensis* PERS (Annonaceae) is a small fire-resistant plant commonly found in the savanna region of northern Nigeria. Its flowers are aromatic which are used to flavor food.

In traditional medicine, this plant has a long history of medicinal usage for the treatment of different diseases. Medicinally, the leaves, roots and stem are used in Nigeria for the treatment of cancer, Antimicrobial, anticonvulsant and Trypanosomiasis (Ezugwu and Odoh, 2003; More et al., 2008; Sowemimo et al., 2007; Ogbodoyi et al., 2007). Other applications include neurological disorder and testicular function indices (Okoli et al., 2010; Nwonuma et al., 2015).

This study therefore, investigated the antidiarrhoeal and ulcer protective effects of the ethanol leaf extract of *A. senegalensis* to confirm its ethnomedicinal claims by the local population.

## 2 Materials and Methods

### 2.1. Plant collection and identification

Fresh leaves of *A. senegalensis* were collected from Suleja, Niger State, Nigeria. The plant was identified by a taxonomist in the department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria.

### 2.2. Extraction procedure

The leaves were washed, shade dried at room temperature in the Department of

Optometry, School of Health Technology, Federal University of Technology, Owerri and subjected to size reduction to a coarse powder by using mortar and pestle. Four hundred grams (400 g) of the powder was macerated in 2.5 L of ethanol for 24 hours with frequent agitation until the soluble matter has dissolved. The mixture was strained, the marc pressed and the filtrate concentrated to dryness by gentle heating over a water bath set at low temperature (40 °C) to obtain a semi-solid brownish extract. The extract was stored at 4 °C in a refrigerator till when needed for the assay.

### **2. 3. Experimental animals**

Adult Wistar rats (200 – 250 g) of either sex were used in this study. The animals were sourced and maintained at the Animal house of Federal University of Technology, Owerri, Imo State. The animals were acclimatized for 2 weeks prior to the tests. Animals were maintained under standard environmental conditions at 40 – 45% relative humidity for 12 h each of dark and light cycle and fed with a standard pellet rat diet obtained from Oladokun feed, Ibadan, Nigeria and clean water was supplied *ad libitum*. The study protocol was carried out as per the rules and regulations of the Institutional Animal Ethical Committee, School of Health Technology, Federal University of Technology, Owerri (FUTO/SOHT/REC/02/0101) as well as the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH, 2011).

### **2. 4. Acute toxicity test**

The LD<sub>50</sub> of *A. senegalensis* leaf extract was tested to determine the safety of the agent according to Organization of Economic Community Development method with slight modification (OECD, 2002). The studies were done in two phases. Nine rats, randomized and divided into three groups with three animals each were used in the first phase. The rats were orally administered with 100, 600, and 1000 mg/kg of the leaf extract respectively. They were observed for the first 4 and 24 hrs for signs of toxicity and mortality. This was followed by the second phase in which 2000, 3000 and 5,000 mg/kg of the extract was administered to the next three groups of one rat per cage. The signs of toxicity and mortality were also monitored for 24 hours, 48 hours and 72 hours respectively.

### **2. 5. Antidiarrhoeal studies**

#### **2. 5. 1. Castor oil-induced diarrhoea test**

Thirty (30) Wistar albino rats of either sex with the weight range of 200-250 g, were divided into five groups of six in each cage (Akuodor et al., 2011). The rats were fasted for 24 hours prior to the test, but allowed free access to clean water. Group 1 was treated with 20 ml/kg of normal saline, which served as control. Groups 2, 3 and 4 received different doses of the extract (100, 200 and 400 mg/kg) respectively. Group 5 received standard drug (Loperamide 4 mg/kg). All doses were administered orally. The animals were kept singly in cages lined with transparent paper. One hour after treatment, each animal was challenged with 1mL of castor oil. These animals were observed for 4 hours for the presence of diarrhoea defined as wet and unformed stool. The frequency of characteristic diarrhoea (droppings) was recorded and compared with the control. The percentage inhibition of diarrhoea was calculated following the

formula:

$$\text{Percentage inhibition of defecation} = 100 - \left( \frac{\text{FNE}}{\text{FNC}} \times 100 \right).$$

Where FNE = Mean Faecal number of each experimental group and FNC = Mean faecal number of the control group.

### 2. 5. 2. Gastrointestinal transit test

The method as described by Essiet et al., (2017) was employed to assess the effect of this extract on gastrointestinal transit in Wistar rat. The animals (rats) fasted as described previously, were divided into five groups of six rats each. The first group (negative control) was orally given 20 mL/kg normal saline. The second, third and fourth groups received 100, 200 and 400 mg/kg of *A. senegalensis* leaf extract. The fifth group (positive control) received atropine sulphate (5mg/kg). At one hour after drug administration, 1ml of charcoal meal (10 % charcoal suspension in 5 % tragacanth) was orally given to all animals in the study. One hour after charcoal meal, all the animals were anaesthetized and sacrificed. The small intestine of each rat was dissected out and the distance traveled by charcoal meal from pylorus to the caecum was measured and expressed as a percentage of the length of small intestine and determined according to the expression of Bakare et al., (2014).

$$\text{P1\%} = \frac{\text{LM}}{\text{LSI}} \times 100$$

$$\% \text{ inhibition} = \frac{\text{P1\% (control)} - \text{P1\% (treatment)}}{\text{P1\% (control)}}$$

Where P1 = Peristaltic index, LM = Length of charcoal meal, LSI = Length of small intestine

Castor oil – induced intestinal fluid accumulation test.

### 2. 5. 3. Castor oil-induced enteropooling study

The method as reported by Bakare et al. (2014) was used in this study. The animals were fasted for 24 hours prior to the experiment, but were allowed free access to clean water. Group 1 served as control and was treated with 20 mL/kg of normal saline. Groups 2, 3 and 4 received graded doses of 100 mg/kg, 200 mg/kg and 400 mg/kg of *A. senegalensis*, whereas group 5 was treated with standard drug (loperamide 4 mg/kg). One hour later, all the rats were challenged with 1 ml of castor oil orally. After 1 hour, each rat was anaesthetized with chloroform and sacrificed with the contents expelled into a measuring cylinder and the volume measured.

The percentage inhibition of intestinal content was calculated according to the formula of Bairagi et al. (2014).

$$\% \text{ inhibition} = 100 - \left( \frac{\text{ICE}}{\text{ICC}} \right) \times 100.$$

Where ICE = Mean volume intestinal content of each experimental group, ICC = Mean volume intestinal content of the control group.

## 2. 6. Antiulcer studies

### 2. 6. 1. Ethanol-induced gastric ulceration in rats

The method of Akuodor et al. (2012) was used for this study. Experimental rats were fasted for 24 hours but had access to water *ad libitum*. Thirty (30) Wistar rats were divided into 5 groups of 6 rats in each group. Group 1 (drug free) received 20 mL/kg of normal saline. Groups 2, 3 and 4 received 100, 200 and 400 mg/kg of the extract, respectively. While Group 5 received standard drug (Ranitidine 20 mg/kg). All the drugs were administered orally. After one hour, ulceration was induced by intragastric instillation of 1ml of 90 % ethanol. The next one hour, rats were sacrificed by Chloroform anaesthesia and their stomachs were removed, opened and examined for ulcerative lesions. Ulcer index was evaluated using the qualitative method for assessing the extent of experimental gastric ulcers. The percentage inhibition was calculated in relation to saline group according to the formula:

$$\% \text{ inhibition} = \text{UIt} / \text{UIs} \times 100$$

Where UIt and UIs correspond to ulcer index of treated and ulcer index of saline (control).

### 2. 6. 2. Acetylsalicylic acid-induced gastric ulceration in rats

The procedure as reported by Anosike and Ofoegbu, (2013) was adopted in this study with slight modification. Animals used were deprived of food for 48 hours but had access to water *ad libitum*. The Wister rats were divided into five groups of 6 in each group. Group 1 which served as control was treated with 20 mL/kg of normal saline, whereas *A. senegalensis* at concentrations of 100, 200 and 400 mg/kg were administered to groups 2, 3 and 4 respectively. The referenced drug (Ranitidine 20 mg/kg) was given to group 5. All administrations were carried out orally. At one hour, gastric lesions were induced with 150 mg/kg of aspirin to all the groups. After 5 hours, the rats were sacrificed by chloroform anaesthetized for ulcerative index.

### 2. 6. 3. Water immersion stress-induced ulceration in rats

This study was carried out according to the method described by Akuodor et al., (2013), Wistar rats used were fasted for 48 hours prior to the experiment but had water *ad libitum*. The animals were divided into 5 groups of 6 rats per cage. Groups 1 received 20 mL/kg normal saline as normal control, groups 2, 3 and 4 and 5 were given 100, 200 and 400 mg/kg of the ethanol leaf extract of *A. Senegalensis* respectively. Group 5 being the reference drug control, received 20 mg/kg ranitidine. One hour after treatment, stress ulcers were induced by forcing the rats to swim for one hour in a cylinder with 45 cm height and a diameter of 25 cm containing water to the height of 35cm maintained at  $50 \pm 1^{\circ}\text{C}$ . After swimming for one hour, the rats were removed, dried and injected intravenously via the tail vein 30 mg of Evans blue. Ten minutes later, all animals were sacrificed under chloroform anaesthesia and their stomachs removed. Formolsaline (2 % v/v) was then injected into the ligated stomachs for storage

overnight. After 24 hours, each stomach was opened along the greater curvature, washed in warm water and macroscopically and microscopically examined. The number of erosions was noted and severity recorded. Mean scores for each group were expressed as ulcer index (UI). From the data, the percentage inhibition of ulceration was determined.

### 2. 7. Statistical analysis

Results were expressed as means  $\pm$  SEM and analyzed with statistical package for social sciences (SPSS version 20) by using one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test. Difference in the mean  $P < 0.05$  was considered significant.

## 3 Results and Discussions

### 3. 1. Phytochemical analysis

It is important to know the chemical nature of plant products when their pharmacological responses are evaluated. Phytochemical evaluation of *A senegalensis* ethanol leaf extract showed the presence of these secondary metabolites; alkaloids, saponins, tannins, flavonoids, terpenoids, and anthraquinone, while phlobatannins and steroids were not detected.

### 3. 2. Acute toxicity tests

There were no lethality or toxic reactions observed at any of the doses administered. All the rats were healthy and active during and after the period of study. Hence, oral acute toxicity result was greater than 5000 mg/kg in rats.

Effect of ethanol leaf extract of *A. senegalensis* on castor oil induced diarrhoea in rats.

The leaf extract exhibited a significant ( $p < 0.05$  and  $p < 0.01$ ) dose - dependent inhibition of diarrhoea when compared with control. The leaf extract decreased both the frequency of defeacation in rats. The extract at the highest dose level compared favourably with the standard drug (Table 1).

### 3. 3. Effect of ethanol leaf extract of *A. senegalensis* on intestinal transit time in rats

The ethanol leaf extract showed a significant ( $p < 0.05$ ) dose-dependent reduction of intestinal transit time in all the groups when compared with control. The extract produced marked decrease in the propulsive movement and the intestinal length traveled by charcoal meal is almost comparable to that of atropine, standard drug (Table 2).

### 3. 4. The effect of *A. senegalensis* ethanol leaf extract on castor oil induced intestinal fluid accumulation in rats.

The ethanol leaf extract exerted significant ( $p < 0.05$  and  $p < 0.01$ ) anti-enteropooling activity in rats. The extract inhibited intestinal fluid volume when compared with control. Furthermore, the leaf extract compared favourably with the reference drug, loperamide (Table 3).

### 3. 5. The effect of ethanol leaf extract of *A. senegalensis* on acetylsalicylic acid induced ulceration in rats.

The leaf extract showed a significant dose dependent inhibition of ulceration in rats. The doses of *A. senegalensis* leaf extract administered (100 mg/kg, 200 mg/kg, and 400) exhibited 58 %, 69 %, and 78% ulcer protective activity respectively. The standard drug, ranitidine (20 mg/kg) exerted 80% protection (Table 4).

### 3. 6. The effect of the extract on ethanol induced gastric ulceration in rats.

The ethanol leaf extract of *A. senegalensis* extract significantly protected the rats against gastric mucosa damage by ethanol. The extract at different doses (100 mg/kg, 200 mg/kg and 400 mg/kg) possesses remarkable 57 %, 67 % and 81 % ulcer protective properties. However, ranitidine at 20 mg/kg showed 83 % ulcer protection (Table 5).

### 3. 7. Effect of the leaf extract on water immersion stress induced ulceration in rats.

Pre-treatment with *A. senegalensis* ethanol leaf extract for one hour before water immersion exhibited dose dependent ulcer protection of 59 %, 75 % and 86 % at 100 mg/kg, 200 mg/kg and 400 mg/kg respectively. The reference drug, ranitidine at 20 mg/kg showed protection level of 88 % (Table 6).

Table 1: The effect of *A. senegalensis* leaf on castor oil induced diarrhoea in rats

| Drug                   | Dose (mg/kg) | Mean frequency of diarrhoea | % Inhibition    |
|------------------------|--------------|-----------------------------|-----------------|
| Control                | 0.2mL        | 5.00 ± 0.37                 | -               |
| <i>A. senegalensis</i> | 100          | 1.67 ± 0.33                 | 67 <sup>a</sup> |
| <i>A. senegalensis</i> | 200          | 1.33 ± 0.21                 | 73 <sup>a</sup> |
| <i>A. senegalensis</i> | 400          | 0.67 ± 0.21                 | 90 <sup>b</sup> |
| Loperamide             | 4            | 0.33 ± 0.21                 | 93 <sup>b</sup> |

One-way ANOVA+Dunnet's post hoc (n=6). <sup>a</sup> Significantly different from control at p < 0.05.

<sup>b</sup> significantly different from control at p < 0.01.

Table 2: The effect of ethanol leaf extract of *A. senegalensis* on intestinal transit time in rats

| Drug                   | Dose (mg/kg) | Mean Intestinal Length(cm) | Mean distance traveled by marker (cm) | % Inhibition    |
|------------------------|--------------|----------------------------|---------------------------------------|-----------------|
| Control                | 0.2mL        | 94.50 ± 3.34               | 93.67 ± 3.53                          | -               |
| <i>A. senegalensis</i> | 100          | 83.00 ± 4.91               | 49.33 ± 3.37                          | 47 <sup>a</sup> |
| <i>A. senegalensis</i> | 200          | 92.00 ± 3.28               | 46.00 ± 4.90                          | 51 <sup>a</sup> |
| <i>A. senegalensis</i> | 400          | 95.50 ± 2.11               | 42.83 ± 2.09                          | 54 <sup>a</sup> |
| Atropine               | 5            | 106.00 ± 4.28              | 42.33 ± 2.01                          | 55 <sup>a</sup> |

One-way ANOVA+Dunnet's post hoc (n=6). <sup>a</sup> Significantly different from control at p < 0.05.

<sup>b</sup> significantly different from control at p < 0.01.

Table 3: The effect of ethanol leaf extract of *A. senegalensis* on castor oil induced enterpooling in rats

| Drug                   | Dose (mg/kg) | Mean volume of intestinal contents | % Inhibition    |
|------------------------|--------------|------------------------------------|-----------------|
| Control                | 0.2mL        | 4.33 ± 0.13                        | -               |
| <i>A. senegalensis</i> | 100          | 1.40 ± 0.15                        | 68 <sup>a</sup> |
| <i>A. senegalensis</i> | 200          | 1.07 ± 0.10                        | 75 <sup>a</sup> |
| <i>A. senegalensis</i> | 400          | 0.80 ± 0.09                        | 82 <sup>b</sup> |
| Loperamide             | 4            | 0.72 ± 0.07                        | 83 <sup>b</sup> |

One-way ANOVA+Dunnet's post hoc (n=6). <sup>a</sup> Significantly different from control at p < 0.05.

<sup>b</sup> significantly different from control at p < 0.01.

Table 4: The effect of Ethanol leaf extract of *A. senegalensis* on acetylsalicylic acid induced ulceration in rats

| Drug                   | Dose (mg/kg) | Ulcer index (UI) | % ulcer protection |
|------------------------|--------------|------------------|--------------------|
| Control                | 0.2mL        | 4.02 ± 0.34      | -                  |
| <i>A. senegalensis</i> | 100          | 1.67 ± 0.33      | 58 <sup>a</sup>    |
| <i>A. senegalensis</i> | 200          | 1.23 ± 0.25      | 69 <sup>a</sup>    |
| <i>A. senegalensis</i> | 400          | 1.00 ± 0.22      | 78 <sup>a</sup>    |
| Ranitidine             | 20           | 0.82 ± 0.26      | 80 <sup>b</sup>    |

One-way ANOVA+Dunnet's post hoc (n=6). <sup>a</sup> Significantly different from control at p < 0.05.

<sup>b</sup> significantly different from control at p < 0.01.

Table 5: The effect of ethanol leaf extract of *A. senegalensis* on ethanol-induced gastric ulceration in rats

| Drug                   | Dose (mg/kg) | Ulcer index (UI) | % ulcer protection |
|------------------------|--------------|------------------|--------------------|
| Control                | 0.2mL        | 4.23 ± 0.34      | -                  |
| <i>A. senegalensis</i> | 100          | 1.82 ± 0.31      | 57 <sup>a</sup>    |
| <i>A. senegalensis</i> | 200          | 1.40 ± 0.11      | 67 <sup>a</sup>    |
| <i>A. senegalensis</i> | 400          | 1.00 ± 0.22      | 81 <sup>b</sup>    |
| Ranitidine             | 20           | 0.52 ± 0.23      | 83 <sup>b</sup>    |

One-way ANOVA+Dunnet's post hoc (n=6). <sup>a</sup> Significantly different from control at p < 0.05.

<sup>b</sup> significantly different from control at p < 0.01.

Table 6: The effect of ethanol leaf extract of *A. senegalensis* on water immersion stress-induced ulceration in rats

| Drug                   | Dose (mg/kg) | Ulcer index (UI) | % ulcer protection |
|------------------------|--------------|------------------|--------------------|
| Control                | 0.2mL        | 4.32 ± 0.29      | -                  |
| <i>A. senegalensis</i> | 100          | 1.78 ± 0.31      | 59 <sup>a</sup>    |
| <i>A. senegalensis</i> | 200          | 1.07 ± 0.22      | 75 <sup>a</sup>    |
| <i>A. senegalensis</i> | 400          | 0.85 ± 0.25      | 86 <sup>b</sup>    |
| Ranitidine             | 20           | 0.52 ± 0.33      | 88 <sup>b</sup>    |

One-way ANOVA+Dunnet's post hoc (n=6). <sup>a</sup> Significantly different from control at p < 0.05.

<sup>b</sup> significantly different from control at p < 0.01.

The age long use of herbal medicines in the treatment of diarrheal disease is a common practice in many countries across the globe including Nigeria. Therefore, the need to substantiate or otherwise the folkloric claim of the leaf extract of *Annona senegalensis* as an antidiarrheal agent using different models of diarrhea cannot be overemphasized. The results



shows that there has been statistically significant reduction not only on the onset of diarrhea but also on its severity as revealed by the castor oil-induced diarrhea, gastrointestinal transit time and enteropooling in the present study.

Castor oil has been widely used in diarrhea studies because it is capable of causing the body through its metabolite, ricinoleic acid, to produce autocoids and prostaglandins which are known inducers of diarrhea in animals (Rahman et al., 2015). Castor oil is metabolized into ricinolic acid in the gut which causes irritation and inflammation in the intestinal mucosa, leading to the release of inflammatory mediators such as histamine and prostaglandins (Khalilur et al., 2015). The liberated prostaglandins then promote vasodilatation, mucus secretion and contraction of the smooth muscles in the small intestines. The prostaglandins, especially E group is known to cause diarrhoea in experimental animals and in humans. Hence, agents that inhibit prostaglandin biosynthesis are used in the treatment of castor oil induced diarrhoea (Brijesh et al., 2009).

However, the remarkable dose dependent decrease in all the tested diarrhoeal models in this study showed the efficacy of *A. senegalensis* ethanol leaf extract as an antidiarrhoeal agent. The leaf extract demonstrated significant activity in controlling the frequency of castor oil induced diarrhea, a feat that is comparable to the referenced drug, loperamide. This agent is one of the most efficacious and widely used antidiarrhoeal drugs (Balogun et al., 2011). Apart from regulating the gastrointestinal tract, it also slow down transit in the intestine and reduces colonic motility (Kaur et al., 2014).

In gastrointestinal transit time study, the leaf extract significantly decreased the distance travelled charcoal meal, showing that it could possess inhibitory effect on the excitatory neurotransmitters in the GIT thus leading to relaxation of the gut muscles and slowing down motility (Oyindamola et al., 2021). This assumption is further supported by the antispasmodic activity of the aqueous root and stem of this plant by antagonizing the actions of acetylcholine (Ahmed et al., 2020). Atropine at 5 mg/kg, slowed the propulsive movement in gastrointestinal transit test following its anticholinergic effect (Brown and Taylor, 1996). Tannins which is present in the leaf extract has been reported to inhibit gastrointestinal movement by decreasing the intracellular  $Ca^{2+}$  inward current or by activation of the calcium pumping system (Belemtougri et al., 2006), also by forming protein tannates, which make the intestinal mucosa more resistant and hence, decrease peristaltic movement (Ashok and Upadhyay, 2012).

The significant inhibition of the castor oil induced enteropooling in rats is an indication that *A. senegalensis* leaf extract produces relief in diarrhoea by spasmolytic activity *in vivo* and also anti – enteropooling effect (Saralaya et al., 2010). More so, *A. senegalensis* may have proved Geiger’s criteria for the classification of an agent as antidiarrheal (Aniagu et al., 2005). The antidiarrheal, antimotility and antienteropooling effects of the ethanol leaf extract of *Annona senegalensis* is in support of the findings of Ahmed et al. (2020).

Ethanol induces ulceration due to perturbations of superficial epithelial cells, especially the mucosal mast cells which causes liberation of vasoactive mediators like histamine and reactive oxygen species, resulting in gastric mucosa damage (Amandep et al., 2012). The effect of *A. Senegalensis* leaf extract in protecting the mucosal damage caused by ethanol is an indication of the extract response on both prostaglandins synthesis and free radical scavenging activities (Kalra et al., 2011). Moreover, an agent highly effective in challenging induction of gastric

lesions by ethanol may possess cytoprotective activity. Hence, could be one of the possible mechanisms through which *A. Senegalensis* significantly halted ethanol gastric lesions. The protective effect of the extract could be attributed to suppression of lipoxygenase activity (Amazu et al., 2015). Furthermore, it has been recorded that ethanol induced ulcers are not only inhibited by anti-secretory agents like ranitidine, but also agents that promote mucosal defensive factors (Essien et al., 2016).

Aspirin causes ulcer probably due to its activity on cyclo-oxygenase enzyme probably due to a decrease in prostaglandins synthesis and an improvement in acid secretion. The mucosa protective action of *A. Senegalensis* might result from stimulation of prostaglandin synthesis, since endogenous prostaglandins play essential role in gastroprotective activity (Akuodor et al., 2012). Aspirin induced ulcers are mediated through cell membrane destruction by free radicals synthesized from conversion of hydroperoxyl to hydroxyl fatty acids leading to cell damage (Sen et al., 2009). The leaf extract at different doses, exhibited a remarkable and dose dependent reduction in ulcer index. The significant activity of the extract further supports its gastroprotective potentials which may likely be mediated by prostaglandins.

Water immersion stress exposes the animal to both physiological and emotional stress. The ulcers induced by stress are due to autodigestion of gastric mucosal barrier, presence of hydrochloric acid (HCL) and free radicals production (Olaleye and Farombi, 2006). The ulcers are formed due to histamine release from hyper-secretion of acid and reduction in mucus production (Essiet et al., 2016). Stress may also cause an increase in gastrointestinal motility resulting in stomach folds which are more susceptible to damage on contact with acid (Adinortey et al., 2013). Administration of *A. Senegalensis* ethanol extract before subjecting the animal to stress, decreased the incidence and severity of stress induced gastric ulcer in rats. In this study, the presence of the leaf extract might have acted as a barrier between gastric mucosa and the excessive gastric acid production during stress which might have stimulated blood flow to the stomach and inhibit the ulcers caused by stress. The extract from *A. senegalensis* showed a high gastroprotective potential compared to ranitidine. Therefore, the reduction of the acidity of gastric juice by the leaf extract may also be due to its antihistaminic effect. These results are similar to those obtained by Konate et al. (2021). Ulcer index reduction of the leaf extract compared favorably with ranitidine, suggesting that the extract may follow ranitidine inhibitory mechanism.

The gastroprotective effect of *A. senegalensis* is believed to be by increasing the synthesis of endogenous prostaglandins, which in turn promote mucus secretion and enhance the mucosal barrier against the actions of various damaging agents (Akuodor et al., 2013). The observed protective activity of the extract may be attributed to the presence of phytoconstituents as earlier stated. These compounds most likely inhibit gastric mucosal injury by scavenging the stress - generated oxygen metabolites (Shokunbi and Odetola, 2008). Flavonoids have anti-inflammatory activity and protect the gastric mucosa against a variety of ulcerogenic agents in different mammalian species (Shetty et al., 2008). It is because of this that many studies have examined the anti-ulcerogenic activities of plants containing flavonoids. Plants containing flavonoids were found to be effective in preventing ulcerative lesions, mainly due to their antioxidant properties (Kaneria et al., 2012). The antioxidant activity of flavonoids has been of interest in recent time because of the strong evidence that oxidation processes are involved in

the mechanisms of several gastric disorders, including ulcerogenesis (Guan et al., 2013).

## 4 Conclusion

Conclusively, results obtained from this study indicate that the ethanol leaf extract of *A. senegalensis* possesses significant anti-diarrhoeal and ant-ulcer activities, thus justifying the wide spread use of this plant extract in traditional medicine for the treatment of gastrointestinal disorders.

## Acknowledgments

The author are grateful to Simon Eze Nwibo and Chibueze Nwonu for their technical assistance.

## Conflict of interests

The authors declared that there is no conflict of interests regarding publication of this paper.

## References

- Adinortey, M. B., Ansah, C., Galyuon, I., & Nyarko, A. (2013). In vivo models used for evaluation of potential antigastroduodenal ulcer agents. *Ulcers*, 2013. <http://dx.doi.org/10.1155/2013/796405>
- Ahmed, M., Arise, R. O., & Sudi, I. Y. (2020). Evaluation of the Antidiarrhoeal Activity of Aqueous Root and Stem Bark Extract of *Annona Senegalensis*. *International Journal of Life Sciences and Biotechnology*, 3(1), 1-17. <https://doi.org/10.38001/ijlsb.656112>
- Akuodor, G. C., Muazzam, I., Usman-Idris, M., Megwas, U., Akpan, J., Chilaka, K., Okoroafor, D., & Osunkwo, U. (2011). Evaluation of the antidiarrheal activity of methanol leaf extract of *Bombax buonopozense* in rats. *Ibnosina Journal of Medicine and Biomedical Sciences*, 3(01), 15-20. doi: 10.4103/1947-489X.210845
- Akuodor, G. C., Mbah, C. C., Essien, A. D., Akpan, J. L, Ezeokpo, B. C., & Iwuanyanwu, T. C. (2012). Ulcer protective and antidiarrhoeal effects of the aqueous stem bark extract of *Bridelia ferruginea* in rodents. *Pharmacologia*, 3, 591-597. doi: 10.17311/2012/591.597
- Akuodor G. C., Akpan J. L., Ezeunala M. N., Ajoku G. A., Essien A. D., Megwas A. U., Okoroafor D. O., Iwuanyanwu T. C., & Osunkwo U. A. (2012). Evaluation of anti-ulcer and

antimicrobial effects of *Verbena hastata* leaf extract. *African Journal Pharm Pharmacol*, 6(11), 778-782. doi: 10.5897/AJPP11.047

Akuodor, G. C., Essien, A. D., David-Oku, E., Chilaka, K. C., Akpan, J. L., & Ezeokpo, B. C., (2013). Ezeonwumelu JO. Gastroprotective effect of the aqueous leaf extract of *Guiera Senegalensis* in Albino rats. *Asian Pacific Journal of Trop. Medicine*, 6, 771 – 775. doi: 10.1016/S1995-7645(13)60136-4

Amandep, K., Robin, S., Ramica, S., & Sunil, K. (2012). Peptic ulcer: A review on etiology and pathogenesis. *International Research Journal of Pharmacy*, 3, 34-38.

Amazu, L. U., Anita, B. S., & Okokon J. E. (2015). Antiulcer activity of *S. monostachyus*. *Journal of Phytopharmacology*, 4, 97-101.

Anosike, C. A., & Ofoegbu, R. E. (2013). Anti-ulcerogenic activity of methanol extract of *Ceiba pentadra* stem bark on indomethacin and ethanolinduced ulcers in rats. *International Journal of Pharma Sciences*, 3(3), 223-228.

Aniagu, S. O., Binda, L. G., Nwinyi, F. C., Orisadipe, A., Amos, S., wambebe, C., & Gamaniel, K. (2005). Anti-diarrhoeal and ulcer-protective effects of the aqueous root extract of *Guiera senegalensis* in rodents. *Journal of Ethnopharmacology*, 97, 549-554. doi: 10.1016/j.jep.2005.01.009

Ashok, P. K., & Upadhyay, K. (2012) Tannins are astringent. *Journal of Pharmacognosy and Phytochemistry*, 1, (3), 45-50.

Bairagi, S. M., Aher, A. A., Nema, N., & Pathan, I. B. (2014). Evaluation of antidiarrhoeal activity of the leaves extract of *Ficus microcarpa* L. (Moraceae). *Journal of Marmara Pharmaceutical*, 18, 135-138. doi: 10.12991/mpj.2014187240

Bakare, R. I., Magbagbeola, O. A., Akinwande, A. I., Okunowo. O. W., & Green, M. (2011). Antidiarrhoea activity of aqueous leaf extract of *Momordica charactia* in rats. *Journal of Pharmacognosy Phytotherapy*, 3, 1-7. <https://doi.org/10.5897/JPP.9000010>

Balogun, S. O., Tanayen, J. K., Ajayi, A. M., Ibrahim, A., Oloro, O. J., Goji, A. D. T., Kiplagat, D. M., & Adzu, B. (2011). Preliminary evaluation of anti-diarrheal, ulcer protective and acute toxicity of ethanolic stem bark extract of *Ficus trichopoda* in experimental rodents. *Asian Journal of Medical Science*, 3, 37-42.

Belemtougri, R. G., Constantin, B., Cognard, C., Raymond, G., & Sawadogo, L. (2006). Effects of two medicinal plants, *Psidium juajava* L. (Myrtaceae) and *Diospyros mespiliformis* L. (Ebenaceae) leaf extracts on rats skeletal muscle cells in primary culture. *Journal of Zhejiang University Science*, 7, 56-63. doi: 10.1631/jzus.2006.B0056

- Brijesh, S., Daswani, P., Tetali, P., Antian, N., & Birdi, T. (2009). Studies on the antidiarrhoeal activity of *Aegle marmelos* unripe fruit: Validating its traditional usage. *BMC Complementary and Alternative Medicine*, 9, 47. doi:10.1186/1472-6882-9-47
- Brown, J. H., & Taylor, P. (1996). Muscarinic receptor agonists and antagonist. In: Hardman JG, Limbird LE, eds. Goodman and Gilman's The Pharmacological Basis of Therapeutics. 9th ed. New York: McGraw Hill.
- Essien, A. D., Essiet, G. A., Akuodor, G. C., Aja, D. O. J., Thoma, E. E, Nwadike, K. I., Nwachukwu, D. C., & Chilaka, K. C. (2016). Studies on gastrointestinal properties of ethanolic leaf extract of *Salacia lehmbachii* in Wistar rats. *African Journal of Pharmacy and Pharmacology*, 10, 451-457. <https://doi.org/10.5897/AJPP2016.4536>
- Essiet, G. A., Akuodor, G. C., Essien, A. D., Akpan, J. L., Asika, E. C., Nworie, E. M., & Chilaka, K. C. (2017). Protective effects of *Salacia lehmbachii* aqueous root bark extract against gastric ulcer in wistar rats. *Saudi Journal of Medical and Pharmaceutical Science*, 3, 306-309. doi: 10.21276/sjmp
- Essiet, G. A., Akuodor, G. C., Aja, D. O., Megwas, A. U., Ekenjoku, J. A., & Nworie, E. M. (2016). Antidiarrhoeal and antioxidant properties of ethanol leaf extract of *Pseudocedrela kotschyi*. *Journal of Applied Pharmaceutical Science*, 6, 107-110. doi: 10.7324/JAPS.2016.60318
- Ezugwu, C. O., & Odoh, U. E. (2003). Anticonvulsant activity of the root extract of *Annona senegalensis*. *Journal of Tropical Medicinal Plants*, 4, 51 – 55.
- Guan, L. H., Gong, D., Zhao, X., Cai, L., Yaun, B., Yang, M., Zhao, J., & Zou, Y. (2013). Genipin ameliorates age related resistance through inhibiting hepatic oxidative stress and mitochondrial dysfunction. *Experimental Gerontology*, 48, 1387-1394. doi: 10.1016/j.exger.2013.09.001
- Kalra, P., Sharma, S., & Kuma, R. S. (2011). Antiulcer effect of the methanolic extract of *Tamarindus indica* seeds in different experimental models. *Journal of Pharmacy and BioAllied Science*, 3, 236-241. doi: 10.4103/0975-7406.80778
- Kaneria, M., Kanani, B., & Chanda, S. (2012). Assessment of effect of hydroalcoholic and decoction methods on extraction of antioxidants from selected Idian medicinal plants. *Asian Pacific Journal of Tropical Biomedicine*, 2, 195-202. doi: 10.1016/S2221-1691(12)60041-0
- Kaur, M., Singh, A., & Kumar, B. (2014). Comparative antidiarrhoeal and antiulcer effect of the aqueous and ethanolic stem bark extracts of *Tinospora cordifolia* in rats. *Journal of Advanced Pharmaceutical Technology Research*, 5, 122-123. doi: 10.4103/2231-4040.137417

- Khalilur, R., Ashrat, U. C., Mohammed, T. I., Aniyuzzaman, C., Muhammad, E. U., & Chandra, D. S. (2015). Evaluation of Antidiarrhoeal Activity of methanolic Extract of *Maranta arundinacea* Linn. Leaves. *Advances in Pharmacological and Pharmaceutical sciences*, 1, 1-6. <https://doi.org/10.1155/2015/257057>
- Konate, K., Sanou, A., Raïssa, R. R., Aworet-Samseny, A., Benkhalti, F., Oksana Sytar, O., Brestic, M., Souza, A., & Dicko, M. H. (2021). Safety Profile, In Vitro Anti-Inflammatory Activity, and *in-vivo* Antiulcerogenic Potential of Root Barks from *Annona senegalensis* Pers. (Annonaceae). *Evidence-Based Complementary and Alternative Medicine*, 4441375. <https://doi.org/10.1155/2021/4441375>
- Mohammed, M. S., Khalid, H. S., Muddathr, A. E., EL-Tahir, K., Khan, A. A., Algadir, H. A., Osman, W. J., & Siddiqui, N. A. (2015). Effect of some plants' extracts used in Sudanese folkloric medicines on carrageenan induced inflammation. *Pakistan Journal of Pharmaceutical Science*, 28, 159-165.
- More, G., Tshikalang, E., Lall, N., Botha, F., & Meyer, J. J. M. (2008). Antimicrobial activity of medicinal plants against oral microorganisms. *Journal of Ethnopharmacology*, 119, 473-477. doi: 10.1016/j.jep.2008.07.001
- National Research Council. (2008). "Plant Name Details for *Annona Senegalensis*" in synopsis, *Plantarum*. 2, 95.1806.
- Nwonuma, C. O., Irokanulo, E. O., Jolaiya, A. E., & Ore, A. (2015). Effect of Aqueous Leaf Extract of *Annona senegalensis* on Selected Testicular Function Indices of Wistar Rats. *American Journal of Life Science*, 3(3), 203-212. doi: 10.11648/j.ajls.20150303.21
- National Institute on Health. (2011). *Guide for the Care and Use of Laboratory Animals*. 8th ed. Bethesda MD: National Institutes of Health. 82-83.
- Ogbodoyi, E. O., Abdulganiy, A. O., Adama, T. Z., & Okogun, J. I. (2007). In vivo trypanocidal activity of *Annona senegalensis* pers. Leaf extract against *Trypanosoma brucei brucei*, *Journal of Ethnopharmacology*, 112, 85-89. doi: 10.1016/j.jep.2007.02.015
- Okoli, C. O., Onyeto, C. A., B. P., Akpa, B. P., Ezike, A. C., Akah, P. A., & Okoye, T. C. (2010). "Neuropharmacological evaluation of *Annona senegalensis* leaves," *African Journal of Biotechnology*, 9, 8435-8444. <https://doi.org/10.5897/AJB10.033>
- Organization for Economic Co-operation and Development. (2002). *Guidelines for the Testing of Chemicals / Section 4: Health Effects Test No.423: Paris: Acute Oral Toxicity – Acute Toxic Class Method*. 2002

- Olaleye, O. E., & Farombi, E. O. (2006). Attenuation of indomethacin and HCL/ethanol-induced oxidative gastric mucosa damage in rats by kolaviron: A natural biflavonoid of Gracina kola seed. *Phytotherapy Research*, 20(1), 14-20. doi: 10.1002/ptr.1793
- Oyindamola, J. O., Akuodor, G. C., Obi M. I., Nwachukwu, E. O., Chilaka, K. C., & Irinmwiniwa, O. E. (2021). Comparative assessment of the anti-diarrheal effects of the ethanol leaf and stem extracts of Lantana camara in Wistar rats. *Magna Scientia advanced Research and Reviews*, 03(01), 035-045. doi: <https://doi.org/10.30574/msarr.2021.3.1.0059>
- Rahman, M. K., Chowdhury, M. A., Islam, M. T., Chowdhury, M. A., Uddin, M. E., & Sumi, C. D. (2015). Evaluation of antidiarrheal activity of methanolic extract of *Maranta arundinacea* Linn. leaves. *Advances Pharmacological and Pharmaceutical Sciences*, 257057. doi.org/10.1155/2015/257057
- Saralaya, M., Patel, P., Patel, M., Roy, S. P., & Patel, A. N. (2010). Anti-diarrheal activity of methanolic extract of Moringa oleifera Lam roots in experimental animal models. *International Journal of Pharmaceutical Research*, 2, 35-39.
- Sen, S., Chakraborty, R., De, B., & Mazumder, J. (2009). Plants and phytochemicals for pepticulcer: An overview. *Phcog Review*, 3, 270-9.
- Shetty, B. V., Arjuman, A., Jorapur, A., Samanth, R., & Yadau, S. K. (2008). Effect of extract of *Benincasa hispida* on oxidative stress in rats with indomethacin induced gastric ulcers. *Indian Journal of Physiology and Pharmacology*, 52, 178-182.
- Shokunbi, O. S., & Odetola, A. A. (2008). Gastroprotective and antioxidant activities of phallantus amaru extrats on absolute ethanol-induced ulcer in albino rats. *Journal of Medicinal Plant Research*, 2, 261-267.
- Sowemimo, A. A., Fakoya, F. A., Awopetu, I., Omobuwajo, O. R., & Adesaya, S. A. (2007). Toxicity and mutagenic activity of some Selected Nigerian plants. *Journal of Ethnopharmacology*, 113, 427– 432. doi: 10.1016/j.jep.2007.06.024
- Tarkang, P. A., Okalebo, F. A., Siminyu, J. D., Ngugi, W. N., Mwaura, A. M., Mugweru, J., Agbor, G. A., & Guantai, A. N. (2015). Pharmacological evidence for the folk use of Nefang: antipyretic, anti-inflammatory and antinociceptic activities of its constituents. *BMC Complementary and Alternative Medicine*, 15, 174. doi: 10.1186/s12906-015-0703-7
- World Health Organiyation. (1999). Loperamide: voluntary withdrawal from infant formulations. *WHO Drug inform.* 4, 73-74.