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Full Length Research Paper

Anti-ulcer effects of ethanolic stem-bark extract and fractions of *lannea acida* on indomethacin-induced ulcer in rats

***Sarkiyayi, S., and Yusuf, U. A.**

Department of Biochemistry, School of Life Sciences, Modibbo Adama University of Technology, Yola, Adamawa State, Nigeria.
*Corresponding Author E-mail: sarkiyayi_shehu@yahoo.com

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The anti-ulcer effects of ethanolic stem-bark extract and fractions of *Lannea acida* on indomethacin-induced ulcer in experimental rats were investigated. Forty two (42) albino rats were grouped into seven different groups of six rats in each group. The quantitative and qualitative analyses of the phytochemicals of the fractions and extract were determined. Indomethacin was orally administered as a single dose of 30 mg/kg to induce ulcer in the rats. Ulcerated rats were orally treated with fractions and extracts of *lannea acida* at different doses and cimetidine (100 mg/kg) was used as reference drug once daily for 14 days. The rats were fasted for 24 h, on the last day of the treatment before the animals were sacrificed. At the end of the experiment, ulcer index, gastric secretions and antioxidant parameters were analyzed. Indomethacin administration caused significant increase in ($p<0.05$) ulcer index. There was significant reduction of ulcer index, volume of gastric juice, free, total acidity, malondialadehyde and pepsin activity when treated with extracts, and fractions compared with indomethacin untreated group. Administration of *Lannea acida* extract to ulcerated animals showed significant increases ($p<0.05$) of pH and superoxide dismutase (SOD) in the treated animals in a dose dependent style with the highest effect observed in rat administered with the highest dose. The results show the gastroprotective, ulcer and antioxidant effects of *Lannea acida* extract, which was revealed its ability to reverse the gastric juice secretion parameters and to scavenge free radical produced by MDA level. From the above findings, *Lannea acida* contain bioactive potential for treatment of indomethacin induced-ulcer.

Keywords: Anti-ulcer, *Lannea acida,* indomethacin, antioxidant and ulcer index

INTRODUCTION

Ulcers are deep lesions penetrating through the entire thickness of the gastrointestinal tract (GIT) mucosa and muscularis mucosa (Tortora *et al.*, 2006). Peptic ulcer disease (PUD) is common ailment that affects almost 50% of the world population (Najm, 2011). The normal stomach mucosa maintains a balance between protective and aggressive factors. This aggressive factors may include acid, pepsin, bile and *Helicobacter pylori,*

NSAIDS, lipid peroxidation that produce free radicals that affects the gastric mucus and bicarbonate secretion, prostaglandins (PG's), nitric oxide and antioxidant enzymes like catalase (CAT), superoxide dismutase (SOD) and glutathione levels (Marietta *et al*., 2010; Tripathi, 2008; Tulassay and Herszényi, 2010). Several naturals' drugs have been reported to possess anti-ulcer activity by virtue of their predominant effect on mucosal defensive factors (Sairam *et al*., 2001; Nwafor and Okoye, 2005). *Lannea acida* belongs to this class of therapeutic plants.

Since the dawn history, man has relied so much on medicinal plants for health and food needs because they are cheaper. The traditional use of medicinal plants for curing and preventing diseases/illnesses include the promotion of both physical, spiritual wellbeing among human beings. Medicinal plants are a source for a wide variety of natural antioxidants and are used for the treatment of diseases throughout the world (Rafieian-Kopaie and Baradaran, 2013). They possess different properties that enable them to cure these ailments such as antimicrobial, anti-cancer, anti-diabetic, anti-atherosclerosis, anti-ulcer, and even hepato-protective effects (Sharafati *et al*., 2011; Shirzad *et al*., 2012; Kazemi *et al*., 2010). It is in view of this that this research was conducted in order to find out the bioactive anti-ulcer properties of stem-bark extract of *Lannea acida.*

MATERIALS AND METHODS

Plant materials

The stem-bark of *Lannea acida* was collected at Sangere in Gire Local Government Area of Adamawa State, Nigeria. The study area lies on geographic location between latitude 8° N, 11° N and longitude 11.5°E, 13.5° E; (East Google Earth, 2016). The stem-bark was identified and authenticated in Plant Science Department, Modibbo Adama University of Technology, Yola Adamawa State.

Experimental animals

A total of seventy albino rats were obtained from National Veterinary Research Institute Vom, Jos, Nigeria. They were housed in polypropylene cages, and were given standard animal feed (Grower mash pellets, vital feed, Nigeria) and water *ad libitum* and maintained under laboratory conditions.

Preparation of ethanol extracts

The extract of the plant materials was obtained using cold maceration method described by (Umeh *et al*., 2005). The stem-bark was air-dried for three weeks. It was reduced to powdered form by grinding in pestle and mortar. Three hundred and sixty grams (360 g) of the powdered stem-barks were cold macerated in 3000 ml of absolute ethanol for 48 h with constant shaking and filtered using Whatman's filter paper No 1. It was then concentrated to dryness on water bath at 40°C and the crude extract was kept in a desiccators.

Fractionation of the ethanol extract of *lannea acida* stem-bark

The fractionation method described by **(**Abbot and Andrews, 1970; Ode *et al*., 2011) was used to separate the stem-bark extract into its component fractions.

Determination of qualitative phytochemicals screening of *Lannea acida*

Plant materials that were extracted using ethanol solutions were subjected to phytochemical screening to identify the constituents using standard procedures as described by Sofowora, (1993).

Determination of quantitative phytochemicals of *Lannea acida*

Plant materials that were extracted using ethanol solutions were subjected to phytochemical screening to quantify the constituents using standard procedures as described by Harborne (1973).

Estimation of flavonoids content

The total flavonoids content was measured using a modified colorimetric method. The appropriate amount of extract was added to a test-tube together with distilled water. Then 5% $NaNO_2$ was added after 5 min 10% $AlCl_3$ and after another 5 min 1 M NaOH followed by the addition of distilled water. The absorbance was measured against the blank at 510 nm after 15 min. The standard curve was prepared using different concentration of catechin. The flavonoids content was expressed as g catechin equivalents (CE) per 100 g of dry weight (dw) Vabkova and Neugebauerova, (2012).

Estimation of total alkaloids content

Total alkaloid was determined using Siddhuraju and Becker, (2003) method. One gram of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and it was covered and allowed to stand for 4 h. It was filtered and the extract was concentrated on a water bath to one quarter of the

original volume. Concentrated NH_4OH was added by drop wise to the extract until the precipitation was completed. The whole solution was allowed to settle and the precipitate was collected and washed with dilute NH_4OH and then filtered. The residue is the alkaloid, which was dried and weighed.

Determination of total phenols

The total phenol was estimated using the modified Folin-Ciocalteu photometric method. The appropriate amount of filtered ethanol stem-bark extract was oxidized with Folin-Ciocalteu's reagents and after 5 min the reaction was neutralized with saturated sodium carbonate. The solution was then immediately diluted to the volume of 50 ml with distilled water. The absorbance was measured at 750 nm after 90 min of incubation at room temperature against the blank. As the standard was used gallic acid. The total phenolic content is here expressed as g gallic acid equivalents per 100 g of dry weight Vabkova and Neugebauerova, (2012).

Determination of saponins content

Saponins content was estimated using the method of Harborne, (1973).Two gram of each sample was placed into a conical flask and 100 ml of 20 % aqueous ethanol was added. The sample was heated over a hot water bath for 4 h with continuous stirring at 55°C. The mixture was filtered and the residue re-extracted with another 200 ml 20 % ethanol. The combined extracts were reduced to 40 ml over water bath at 90°C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts was washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples was dried in the oven to a constant weight. Saponins content was calculated as percentage as follows.

Calculation: Percentage of Saponins = $\frac{\text{Weight of Residue (g)}}{\text{Weight of Extract Taken (g)}} \times 100$

Estimation of tannins content

Tannins content was estimated using the method of Harborne, (1973) as modified by Siddhuraju and Becker, (2003). To 100 mg of polyvinyl polypyrrolidone (PVPP) solution in a test tube, 1 ml distilled water and 1 ml of the sample extract was added respectively. The content was then vortexed and kept in the test tube at 4 °C for 4 h. This was centrifuged (5000 rpm for 5 min at room temperature) and the supernatant was collected. This supernatant has only simple phenolics other than tannins (The tannins have been precipitated along with the PVPP). The phenolics content of the supernatant was measured at 725 nm and expressed as the content of non-tannin phenolics (Tannic acid equivalent) on a dry matter basis. From the result and the result obtained for Total Phenol, the Tannin content of the sample was then calculated as follows:

Calculation*:* Tannin (%) = Total Phenolics (%) – Non-Tannin Phenolics (%)

Ulcer induction

Peptic ulceration was induced in the animals according to the procedure described by (Sayanti *et al*., 2007). Ulceration was induced in the animals with a single oral dose of indomethacin (30 mg/kg/body weight) using gastrointestinal tubes. They were deprived of food but had access to water 24 h prior to ulcer induction.

Experimental design

A total number of forty two (42) albino rats were used for the experiment. After acclimatization, the animals were randomly divided into seven equal groups of six animals per group and average weight taken and recorded for each group. The animals were ulcerated with single oral dose of indomethacin (30 mg/kg/body weight) on the first day of the experiment. The experimental rats were deprived of food but had access to water 24 hours prior to ulcer induction. The animals were grouped as follows: Group I was fed with normal diet and water and was kept as a normal control group. Group II were given indomethacin 30 mg/kg/body weight single dose served as negative control (ulcerated/ experimental control) while Group III animals were ulcerated with indomethacin (30 mg/kg/body weight) and treated with cimetidine (100 mg/kg/body weight) orally once daily as a standard control. Group IV and V were ulcerated with indomethacin (30 mg/kg/body weight) and subsequently treated with fractions I extracts of *Lannea acida* at different doses 200 and 400 mg/kg body weight. Group VI and VII were also ulcerated with indomethacin (30 mg/kg/body weight) and treated with 200 and 400 mg/kg/body weight of ethanolic extract of *Lannea acida.* Treatments with the reference drug and extract commenced four hours after indomethacin administration and lasted for two weeks.

Collection of gastric juice

On the last day of the treatment, the rats were subjected to an overnight fasting after which they were sacrificed using chloroform vapor as an anesthesia. The stomachs were removed, opened along the greater curvature,

rinsed with normal saline and the stomach content was drained into a centrifuge tube. The stomach content was collected and centrifuged at 3500 rpm for 10 minutes to collect the gastric juice which was used for parameters like volume of gastric juice, pH, pepsin activity (PA), free acidity (FA) and total acidity (TA) (Olaleye *et al*., 2008).

Determination of ulcer index

Ulcer index was measured by the method described by (Goya, 2002). The ulcerated lesion of the open stomach was measured with the help of a magnifying glass. The numbers of ulcers were counted. Mean ulcer score for each animal were expressed as ulcer index. Ulcer index was calculated.
Ulcer Index = 10/x

Where

$$x = \frac{\text{Total Mucosal Surface}}{\text{Total Ulcerated area}}$$

Homogenization process

Tissue from the glandular portion of stomach was excised and washed in the cold 1.15% potassium chloride solution. They were chopped into bits (0.5g) each and homogenized in four volumes of phosphate saline buffer (1:4 w/v; pH 7.4) using mortar and pestle. The resulting homogenate was centrifuged at 3500 rpm for 10 min. the stomach tissue homogenate was used to analyse for parameters like Malondialdehyde (MDA) and Superoxide dismutase (SOD). The glandular portion of stomach was prepared for Ulcer index determination.

Determination of biochemical parameters

Measurement of volume and pH of gastric juice

At the end of the experimental period, the rats were sacrificed and the stomachs were removed. The gastric content was collected and centrifuge for 10 min at 3000 rpm and the supernatant was separated. The volume and pH of centrifuged gastric juice were measured by graduated cylinder and digital *pH* meter according to the method described by (Muniappan and Sundararaj, 2003). The volume was expressed as mili litre (ml) (Parmar and Desai, 1993).

Determination of total and free acidity

The total and free acidity were determined by titrating with 0.01 NaOH using phenolphthalein and methyl orange (Trease and Evans,1992) modified by (Kulkarni, 2010).

Procedure

Pipette 1 ml of filtered gastric contents into small beaker, add 2 to 3 drops methyl orange and titrate with 0.01 N of NaOH until all traces of the red colour disappeared and the colour was yellowish orange. Note the volume of alkali added that indicated free acidity. Then added 2 drops of phenolphthalein and continued titration until a definite red tinge reappeared. Note the total volume of alkali added that indicated total acidity. The result of titration was expressed as ml of 0.1 N HCl per 100 ml of gastric contents. This was same as Meq/l.
Acidity was calculated by using the formular below:

$$\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Normality of NaOH} \times 100\ \text{mEq/L}}{0.1}$$

Determination of pepsin activity

The centrifuged gastric juice (0.1 ml) was added to 1 ml bovine albumin (0.5 % w/v in 0.01 N HCl; pH 2) and incubated for 20 min at 37°C. A duplicate background control tube (gastric juice blank), in which 1 ml albumin was replaced with 1 ml of 0.01 N HCl, was simultaneously run. Hydrolysis was stopped by adding 2 ml of 10% TCA. All of the tubes were heated in boiling water for 5 min, then, cooled. After denaturation of the protein by heating in a boiling water bath for 5 min, the precipitate was removed by centrifugation (9000 g for 10 min). A total of 1 ml of the supernatant was mixed with 0.4 ml of 2.5 N NaOH and 0.1 ml of the Folin- Ciocalteu reagent, and then the volume was adjusted to 10 ml using distilled water. Absorbance was measured at 700 nm. The peptic activity was calculated in terms of micrograms of tyrosine liberated per milliliter of gastric juice (Prino *et al*., 1971).

Determination of malondialdehyde level (MDA

Malondialdehyde Level activity was assay using the method developed by (Buege and Aust, (1978)]. Briefly,the homogenate (0.5 ml) was added to a solution containing 0.2 ml of 80 g/l sodium lauryl sulfate 1.5 ml of 200 g/l acetic acid, 1.5 ml of 8 g/l thiobarbituric acid (TBA), and 0.3 distilled water. The mixture was incubated at 98C for 1 hour. Upon cooling, 5 ml of n-butanol: pyridine (15:1) was added. The mixture was vortexed for 1 minute and centrifuged for 30 minutes at 4000 rpm. The absorbance of the supernatant was measured at 532 mn. The results were expressed as nanomoles MDA per gram wet tissues (nmoles/mg tissues).

Determination of superoxide dismutase (SOD)

Superoxide dismutase (SOD) activity was assayed using the method developed by (Das *et al*., 2000). Briefly, 1.4 ml homogenate of the reaction mixture was pipetted into a test tube. 100µl of the sample was added followed by pre incubation at 37°C for 5 min. 80 µl of riboflavin was added and the tubes were exposed for 10 min to 200 W Philips fluorescent lamps. The control tube contained equal amount of buffer instead of sample. The sample and its respective control were ran together. At the end of the exposure time, 1.0 ml of Greiss reagent was added to each tube and the absorbance of the color formed was measured at 543 nm.

RESULTS AND DISCUSSION

The ethanolic stem-bark extract of *Lannea acida* yielded three different fractions on fractionation. Both the extract and extract fractions possessed different phytochemical components such as Saponins, phenols, tannins, steroids alkaloids, flavonoids, other secondary metabolites (Table 1). This result obtained is in agreement with the report by (Azubuike *et al*., 2015; Onoshe *et al*., 2018). The result of quantitative analysis obtained shows that the major bioactive components could be alkaloids, flavonoids, saponins, phenols and tannins due to their high concentration compared to the rest. The extract fraction I recorded highest concentration of flavonoids and alkaloids compared to the rest (Table 2).

Flavonoids from the previous research reported by (Park *et al*., 2007, Nithya *et al*., 2016) revealed that it possess both anti-ulcer and antioxidant effects. This is due to its ability to protect the gastric wall mucosa against a number of ulcer agents through different mechanism of action, such as ability to scavenge free radicals, antioxidant properties, promote mucus production and inhibit the secretion of gastric acid (Nwagba *et al*., 2013).

Tannins obstruct ulcer generation due its ability to carry out protein precipitation and vasoconstriction potential. Their astringent action can help to precipitate microproteins on the ulcer site, there by forming a protective layer that inhibit the release of toxic substances and increase resistance to the action of proteolytic enzymes. This protect the beneath mucosa from destruction by ulcer index (Leonie *et al*., 2012; Prabhu *et al*., 2011).

Saponins is one of the bioactive compound that possess antiulcer potential, it achieve this by the production of protective mucus on the gastric mucusa wall; selectively inhibiting prostaglandins-F2 (PGF2) so as to protect the mucosa wall against the effect of acid secretions (Hassan *et al*., 2013).

Alkaloids play vital role in prevention and healing of gastric ulcer. Alkaloids stimulates neurons that signalizes for protection by inhibiting the acids secretion, thereby stimulating mucus secretions and elevating the gastric mucosal blood flow which aids in prevention and aids in healing of injury caused by aggressive agents both external and internal factors (Park *et al*., 2007).

Living organism utilizes oxygen in their day to day biological activities; however, due to its highly oxidizing nature it causes harmful effects. These harmful effects are caused by reactive oxygen species (ROS) or free radicals which later produce oxidative stress (Lushchak, 2014). Most plant extract that possess antiulcer effects achieved these by their ability to scavenge free radicals that promote ulcer healing (Dai and Ogle, 2005).

Administration of indomethacin caused significant increase in the ulcer index values (Table 3). The value reveals that indomethacin-induced gastric ulcer model was able to cause ulceration in the stomach region of the experimental rats (Nakamura *et al*., 2003). This result is in line with earlier reports by (Tolulope-Olaleye *et al*., 2013) who reported increased and formation of ulcer index when indomethacin was induced. It has been suggested that the major mechanism through which indomethacin cause ulcer (gastric mucosal damage) is through to be by inhibiting the release of protective factors such as cyclooxygenase-1 (COX1), prostaglandins (PGs), bicarbonate and mucus secretion; increasing the aggressive factor like gastric, pepsin and hydrogen ions; increasing reactive oxygen species (ROS) parameters and decreasing antioxidant parameters (Tulassay and Herszényi, 2010; Laine *et al*., 2008; Sostres *et al*., 2010; Suleyman *et al*., 2010). To regain the balance, different therapeutic agents including plant extracts and synthetic drugs were used (in experimental animals) to inhibit the gastric acid secretion or to boost and strengthen the mucosal defense mechanisms by increasing mucus secretion, enhancing prostaglandin synthesis. Cimetidine is one of the H_2-blockers that are widely used in the treatment peptic ulcer, inhibits secretion of gastric acid, play an important role in the reduction of ulcer index (Sostres *et al*., 2010). Treatment with both the ethanolic and fractions of stem-bark extract of *L. acida* and cimetidine at different concentrations significantly decreased ulcer index values, this values suggest that the extents of ulceration had reduced, this signifies recovery through increased in protective factors and decreased in the aggressive factors; couple with natural immune system activity (Nwagba *et al*., 2013). There was significant increase in volume of gastric juice, free acidity and total acidity of gastric juice and pepsin activity (Tables 5 and 6) on administration of 30 mg/kg body weight of indomethacin to the experimental rats. This may be due to introduction of reactive free radicals or inhibition of cyclooxygenase-1, inhibition of cyclooxygenase-1 lead to decreased in the prostaglandin level which in turns increased gastric acid secretion by histamine and results in an impaired gastro protection by mucin. This is in collaboration with the reports of (Bech *et al*., 2000; Biplab *et al*, 2011; Muhammed *et al*., 2012).

However, treatments of the experimental animal with

Table 1. Qualitative phytochemical screening of crude extract, Fraction I and Fraction II stem- bark extracts of *Lannea acida.*

Phytochemical	Crude Ethanolic extract	Fraction I	Fraction II
Saponins	+	+	+
Tannins	+	+	+
Alkaloids	+	+	+
Flavonoids	+	+	+
Terpenoids	+	-	-
Steroids	+	-	-
Phenols	+	+	+
Anthroquinone	+	+	-
Glycosides	-	-	-

Key: + = present and - = absent

Table 2. Quantitative phytochemical analysis of crude extract, Fraction I and Fraction II stem-bark extract of *Lannea acida.*

Phytochemical	Ethanolic Crude extract (%w/w)	Fraction I (%w/w)	Fraction II (%w/w)
Saponins	13.7	18.1	22.89
Tannins	9.3	9.70	7.51
Alkaloids	15.6	26.97	21.65
Flavonoids	24.2	26.97	22.88
Terpenoids	0.72	-	-
Steroids	1.56	-	-
Phenols	31.3	17.60	23.23
Anthroquinone	3.37	0.66	-

Table 3. Effects of ethanolic stem-bark extract of *Lannea acida* on ulcer index (mm^2) of indomethacin ulcerated rats.

Group	Treatment	Ulcer Index (mm^2)
I	Normal control	0.00 ± 0.00
II	Experimental control (indomethacin 30 mg/kg	0.77 ± 0.02^{bc}
III	Ulcerated rats treated with cimetidine 100 mg/kg	0.30 ± 0.03^{ab}
IV	Ulcerated rats treated with FI 200 mg/kg	0.48 ± 0.03^{bc}
V	Ulcerated rats treated with FI 400 mg/kg	0.36 ± 0.02^{ab}
VI	Ulcerated rats treated with ethanolic extract 200 mg/kg	0.62 ± 0.17^{b}
VII	Ulcerated rats treated with ethanolic extract 400 mg/kg	0.53 ± 0.19^{bc}

Values are expressed as Mean ± SEM (n=6).
[a]significantly lower ($p<0.05$) compared to values of experimental control (Indomethacin) group.
[b]significantly higher ($p<0.05$) compared to values of normal control group.
[c]significantly higher ($p<0.05$) compared to values of standard control (cimetidine) group.
FI = Fraction I extract.

(200 and 400 mg/kg body weight) fractions and extract of *Lannea acida* and cimetidine at 100 mg/kg body weight significantly decreased the volume of gastric juice, free and total acidity of gastric juice and pepsin activity (Tables 5 and 6) these drastic changed occurs due to the presence of flavonoids, saponins and phenols contained in the extracts (Park *et al*., 2007; Nithya *et al*., 2016; Biplab *et al*., 2011). The effect exhibited by the plant extracts was in agreement with the reports of (Dai and Ogle, 2005; Muhammed *et al*., 2006), that reveals increased in the above aforementioned parameters were reversed when treated with plant extracts. Treatment with ethanolic stem-bark extract of *Lannea acida* (fractions and crude extract) at different doses and cimetidine at (100 mg/kg body weight) yielded significant increases in pH of gastric juice (Table 4), this may be due to the following mechanisms of action exhibited by the plant extract: antioxidant, anti-secretory, gastroprotective attributes and ability to neutralized gastric acid. The increased in gastric juice pH is in connection with the increased in mucin content production (mucin is one of protective factor that repelled the actions of pepsin and gastric acid secretion and other external forces that is synthetic drugs and chemicals in the stomach) (Inas *et*

Table 4. Effects of ethanolic stem-bark extract of *lannea acida* on gastric pH of indomethacin ulcerated rats.

Group	Treatment	pH of Gastric Juice
I	Normal control	6.52 ± 0.06
II	Experimental control (Indomethacin 30 mg/kg	3.15 ± 0.62[b]
III	Ulcerated rats treated with Cimetidine 100 mg/kg	6.17 ± 0.67
IV	Ulcerated rats treated with FI 200 mg/kg	5.20 ± 0.11[b]
V	Ulcerated rats treated with FI 400 mg/kg	5.78 ± 0.06[b]
VI	Ulcerated rats treated with ethanolic extract 200 mg/kg	3.77 ± 0.10[b]
VII	Ulcerated rats treated with ethanolic extract 400 mg/kg	4.33 ± 0.11[b]

Values are expressed as Mean ± SEM (n=6).
[a]significantly higher (p<0.05) compared to values of experimental control (Indomethacin) group.
[b]significantly lower (p<0.05) compared to values of normal control group.

Table 5. Effects of ethanolic stem-bark extract of *Lannea acida* on gastric volume (ml), free acidity and total acidity of gastric juice (MEq/l) of indomethacin ulcerated rats.

Group	Treatment	Gastric Volume (ml)	Free Acidity (MEq/l)	Total Acidity (MEq/l)
I	Normal control	1.17 ± 0.02[ac]	11.17 ± 0.60[ace]	28.67 ± 0.76[ace]
II	Experimental control (indomethacin 30 mg/kg/b.w)	3.25 ± 0.04[bd]	38.33 ± 0.49[bd]	79.00 ± 0.73[bd]
III	Ulcerated rats treated with cimetidine 100 mg/kg/b.w	2.13 ± 0.05[ae]	17.00 ± 0.82[ae]	35.50 ± 0.89[ae]
IV	Ulcerated rats treated with FI 200 mg/kg/b.w	2.75 ± 0.04[ab]	26.33 ± 0.84[abe]	43.33 ± 1.12[abe]
V	Ulcerated rats treated with FI 400 mg/kg/b.w	2.34 ± 0.07[ab]	22.00 ± 0.58[abe]	40.83 ± 0.58[abe]
VI	Ulcerated rats treated with EE 200 mg/kg/b.w	2.95 ± 0.04[b]	33.83 ± 1.45[ab]	71.67 ± 0.95[ab]
VII	Ulcerated rats treated with EE 400 mg/kg/b.w	2.65 ± 0.06[ab]	29.33 ± 4.40[ab]	67.33 ± 1.17[ab]

Values are expressed as Mean ± SEM (n=6).
[a]significantly lower (p<0.05) compared to values of experimental control (indomethacin) group in each column.
[b]significantly higher (p<0.05) compared to values of normal control group in each column.
[c]significantly lower (p<0.05) compared to values of standard control (cimetidine) group in each column.
[d]significantly higher (p<0.05) compared to values of extract, fraction I and fraction II groups in each column.
[e]significantly lower (p<0.05) compared to values of extract groups in each column.
EE = Ethanolic extract; FI = Fraction I extract; b.w = body weight.

Table 6. Effects of ethanolic stem-bark extract of *Lannea acida* on pepsin activity of Gastric Juice (µg of tyrosine liberated/ml of gastric juice) of indomethacin ulcerated rats.

Group	Treatment	Pepsin activity (µg/ml)
I	Normal control	104.97 ± 1.53[ae]
II	Experimental control (indomethacin 30 mg/kg/b.w)	287.50 ± 3.75[bcd]
III	Ulcerated rats treated with cimetidine 100 mg/kg/b.w	115.00 ± 2.06[ae]
IV	Ulcerated rats treated with FI 200 mg/kg/b.w	148.67 ± 1.25[abc]
V	Ulcerated rats treated with FI 400 mg/kg/b.w	131.31 ± 1.06[ae]
VI	Ulcerated rats treated with EE 200 mg/kg/b.w	202.60 ± 3.20[bc]
VII	Ulcerated rats treated with EE 400 mg/kg/b.w	180.38 ± 4.41[abc]

Values are expressed as Mean ± SEM (n=6).
[a]significantly lower (p<0.05) compared to values of experimental control (indomethacin) group.
[b]significantly higher (p<0.05) compared to values of normal control group.
[c]significantly higher (p<0.05) compared to values of standard control (cimetidine) group.
[d]significantly higher (p<0.05) compared to values of extract, fraction I and fraction II groups.
[e]significantly lower (p<0.05) compared to values of extract groups.
EE = Ethanolic extract; FI = Fraction I extract; b.w = body weight.

al., 2011); activation of antioxidant potential of the plants and reactivation of natural antioxidant of the experimental animals.

Earlier administration of 30 mg/kg body weight of indomethacin to experimental animal leads to decreased in gastric wall mucus and pH of gastric juice. The value of the pH gives a clue on the concentration of hydrogen ions, which in turns revealed the presence or absence of ulcer in experimental rats. High gastric pH value indicates decreased in concentration of hydrogen ions this revealed

Table 7. Effects of ethanolic stem-bark extract of *Lannea acida* on lipid peroxidation (MDA Level) (n moles of MDA formed/mg protein) of indomethacin ulcerated rats.

Group	Treatment	Lipid Protein (MDA) nmoles/mg protein
I	Normal control	9.53 ± 0.17[ae]
II	Experimental control (Indomethacin 30 mg/kg/b.w)	58.55 ± 2.20[bcd]
III	Ulcerated rats treated with Cimetidine 100 mg/kg/b.w	13.32 ± 0.82[ae]
IV	Ulcerated rats treated with FI 200 mg/kg/b.w	24.53 ± 0.98[ae]
V	Ulcerated rats treated with FI 400 mg/kg/b.w	20.14 ± 0.50[ae]
VI	Ulcerated rats treated with EE 200 mg/kg/b.w	45.93 ± 1.27[bc]
VII	Ulcerated rats treated with EE 400 mg/kg/b.w	38.73 ± 0.58[bc]

Values are expressed as Mean ± SEM (n=6).
[a]significantly lower (p<0.05) compared to values of experimental control (Indomethacin) group.
[b]significantly higher (p<0.05) compared to values of normal control group.
[c]significantly higher (p<0.05) compared to values of standard control (cimetidine) group.
[d]significantly higher (p<0.05) compared to values of extract, fraction I and fraction II groups.
[e]significantly lower (p<0.05) compared to values of extract groups.
EE = Ethanolic extract; FI = Fraction I extract; b.w = body weight.

Table 8. Effects of ethanolic stem bark-extract of *Lannea acida* on Superoxide dismutase activity (U/mg protein) of indomethacin ulcerated rats.

Group	Treatment	SOD activity (U/mg Protein)
I	Normal control	59.82 ± 0.71[acde]
II	Experimental control (indomethacin 30 mg/kg/b.w)	9.24 ± 0.26[b]
III	Ulcerated rats treated with cimetidine 100 mg/kg/b.w	40.34 ± 0.42[ae]
IV	Ulcerated rats treated with FI 200 mg/kg/b.w	28.67 ± 0.37[ae]
V	Ulcerated rats treated with FI 400 mg/kg/b.w	35.80 ± 0.42[ae]
VI	Ulcerated rats treated with EE 200 mg/kg/b.w	11.47 ± 0.44[b]
VII	Ulcerated rats treated with EE 400 mg/kg/b.w	15.04 ± 0.27[b]

Values are expressed as Mean ± SEM (n=6).
[a]significantly higher (p<0.05) compared to values of experimental control (indomethacin) group.
[b]significantly lower (p<0.05) compared to values of normal control group.
[c]significantly higher (p<0.05) compared to values of standard control (cimetidine) group.
[d]significantly higher (p<0.05) compared to values of extract, fraction I and fraction II groups.
[e]significantly higher (p<0.05) compared to values of extract groups.
EE = Ethanolic extract; FI = Fraction I extract; b.w = body weight.

low secretion of gastric acid, whereas, low gastric pH indicates increased in concentration of hydrogen ions concentration, (Lullmann *et al*., 2000).

Induction of experimental rats with indomethacin increased their malonaldehyde (MDA) level (Table 7) hydroxyl ions which signified increased in reactive oxygen species (ROS) both free radicals and non-free radicals; and hydroxyl ions which are biochemical marker for the assessment of lipid peroxidation in cell membrane result in gastric mucosal damage. The reason for the production of lipid peroxidation when exposure to indomethacin was not well established but may be it happened during synthesis of prostangladins, (Satyanarayana and Chakrapani, 2008).

Living organisms are made up of cells or tissues. These are in a normal condition if there is a balanced between the rates of reactive oxygen species (free radicals) production and scavenging capacity of antioxidants. However, an alteration in the balance between the two factors results in oxidative stress which affects cellular functions leading to different diseases conditions such as ulcer (Sabiu *et al*., 2014).

Oral administration of ethanolic stem-bark extract of *Lannea acida* (200 and 400 mg/kg body weight of fractions and crude extract) and cimetidine (100 mg/kg body weight) daily for fourteen days significantly decreased the level peroxidation caused by indomethacin administration. The anti- ulcer effects observed due to administration *L.acida* could be because of the antioxidant potential it's possess and also the presence of bioactive compound called flavonoids (Park *et al*., 2007; Mota *et al*., 2009).

The level superoxide dismutase (SOD) decreased when indomethacin was administered to the experimental rats (Table 8), this occurred because of superoxide radicals presence. Superoxide dismutase is an antioxidant enzyme that scavenged the activity of superoxide radicals by converting it to less reactive component (O_2 + O_2 to H_2O_2 and O_2). However, superoxide radicals always counteract the activity of the

enzyme by render its inactive, this affects it activity (Satyanarayana and Chakrapani, 2008; Gega-Adebayo *et al*., 2013).

Administration of *L.acida* and cimetidine elevates the activity of the enzyme this may be because of flavonoids and phenols components found in *L.acida* extract. Flavonoids and phenols compound were found to possess antioxidant activities and gastroprotective which aid to scavenged free radicals. Similar findings by (Odabasoglu *et al*., 2006; Halici, *et al*.,) reported increase in MDA level and decreased in activity of SOD on administration of indomethacin on experimental rats.

Authors' declaration

The authors declare no conflict of interest.

Conclusion

In conclusion, the present study showed that administration of indomethacin causes gastric ulcer which was significantly reduced with the treatment of stem-bark extract of *Lannea acida* revealed by the anti-ulcer parameters analyzed. The anti-ulcer potential of the extract was dose dependent. The indices of this study demonstrated the reduction of gastric acid secretion and antioxidant effect, these revealed the anti-ulcer potential of the plant extract.

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