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ANTIOXIDANT PROPERTIES OF AQUEOUS STEM BARK EXTRACT OF *ANOGEISSUS LEIOCARPUS* AGAINST ETHANOL-INDUCED GASTRIC ULCER IN RATS

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ABSTRACT
The Gastroprotective effect of aqueous stem bark extract of *Anogeissus leiocarpus* against ethanol induced gastric ulcer in albino rats was investigated. Eighty-six albino rats (weighing 160-250g) of both sexes were used in this study. The results of the qualitative analysis of phytochemical constituents of aqueous stem bark extract of Anogeissus leiocarpus shows the degree of abundance of these phytochemicals in mg/100g of the extract is as follows; 87.67 of flavonoids, 51.00 of alkaloids, 36.33 of saponins, 11.23 of tannin, and 15.53 of phenol. The oxidative analysis shows significant decrease in Malondialdehyde (MDA) at 100mg/kg, 200mg and 400mg/kg b.w as well as the (100mg/kg b.w) cimetidine group ($p<0.05$) when compared with the ulcer control group. Antioxidant studies shows a significant increase in Catalase at 100mg/kg, 200mg/kg and 400mg/kg b.w groups as well as 100mg/kg cimetidine group ($p<0.05$) when compared with the ulcer control group. There was a significant increase in Superoxide dismutase (SOD), at 400mg/kg b.w and 100mg/kg cimetidine groups when compared with the ulcer control group. In Glutathione Peroxidase (GPx), there was a significant increase in 400mg/kg b.w group ($p<0.05$) but there was no significance increase in 100 and 200mg/kg as well as (100mg/kg) cimetidine group ($p>0.05$) when compared with the ulcer control group. . The aqueous extract of *A. leiocarpus* was partially purified by column chromatography. Eluents with similar Rf values were pooled together into five fractions using thin layer chromatography (TLC). The qualitative (spectrophotometrically using DPPH) and quantitative antioxidant activity of the five pooled fractions were determined to using 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) spray to identify the fraction with highest activity on a TLC plate, an active spot turned from violet to yellow. Fraction A had higher DPPH percentage inhibition of (97.95%) and the lowest IC_{50} (20.88). In conclusion these findings suggest that aqueous stem bark extract of A. leiocarpus possesses antioxidant properties and dose-dependent gastroprotection, these justify the ethno medicinal use of the plant in the treatment and management of gastric ulcer.

Keywords: Antioxidant, ethanol, Ulcer, *Anogeissus leiocarpus*.

1.0 INTRODUCTION

Gastric ulcer is one of the major gastrointestinal disorders, which occurs due to an imbalance between the offensive (gastric acid secretion) and defensive (gastric mucosal integrity) factors (Laine *et al.,* 2008 and Shaker *et al.,* 2010). Predisposing factors of gastric ulcer include, stress, alcohol, cigarette smoking, non- steroidal anti-inflammatory drugs, *Helicobacter pylori* infection and chronic pancreatitis (Tariq *et al.,* 1986 and Mustafa *et al.,* 2015).

Ethanol is also known as a cause of gastric damage by altering protective factors, including decreasing mucus production and blood circulation within the mucosa (Boligon *et al.,* 2014) In addition, the gastric damage caused by ethanol may be due to the generation of reactive species, decreased cell proliferation, and an exacerbated inflammatory response (Amaral *et al.,* 2013).

Peptic ulcer is one of the most common diseases affecting mankind and the incidence has been estimated to range from 5 to 10% (Akimoto *et al.,* 1998 and Mahdy *et al.,* 2018). Africa has the highest pooled prevalence of *Helicobacter pylori* infection (70.1%; 95%CI, 62.6-77) (Hooi *et al.,* 2017). More than 100,000 cases per year were reported in Nigeria. In US alone, more than six million people are affected each year (Feinstein *et al.,* 2010).

Several orthodox pharmaceutical drugs such as histamine H_2-receptor antagonists, antacids, anticholinergic drugs and proton-pump inhibitors have been used in the management of peptic ulcers, but they bring about many adverse effects (Mahdy *et al.,* 2018). Presently, there has been growing interest in alternative therapies especially from plant sources due to their perceived lower side effects, ease of accessibility and affordability (Rates, 2001, Bassi *et al.,* 2014 and Strand *et al.,* 2017). Decoction from the plant has been used in folk medicine for the treatment of ulcers (Oluronti *et al*., 2012). There is need to validate these findings as a way to boost acceptability and hence usage of anti-ulcer therapy from stem bark of *Anogeissus leiocarpus*.

Anogeissus leiocarpus DC. Guill. & Perr. is a Combretaceae commonly called 'axlewood', locally referred to in Nigeria as '"Ayin", "Orin-odan" in Yoruba, "Marke" in Hausa and "Atara" in Igbo'. It is an evergreen tall tree found in savannah region of Tropical Africa, especially west and east Africa through tropical Southeast Asia (Steentoft, 1988; Odugbemi and Akinsulire, 2008). It is typically found growing at altitudes of 450 to 1900 m, and do grow on a range of soil types including compact clay soils (Vertisols) (Moctar and Sidi, 2007).

A. leiocarpus was reported to have high antimicrobial activities in many chemotherapeutic applications, hence its continued use in the treatment of bacterial infections (Mann *et al.,* 2009; Mann *et al.,* 2010; Mann, 2012). Castalagin isolated from these fractions showed trypanocidal activity on both, the human and domestic animal pathogens causing trypansomiasis (Shuaibu, 2008). It possesses wound healing activity in a dose-dependent manner and provides a scientific rationale for its traditional use in the management of wounds (Barku *et al.,* 2013). Studies suggest that the aqueous extract of *A. leiocarpus* could be used, with some degree of safety, by oral route (Agaie *et al.,* 2007; Olabanji *et al.,* 2007). It was demonstrated that *Anogeissus leiocarpus (DC.) Guill. & Perr.* and *Terminalia glaucescens Planch ex Benth*. had

significant antimicrobial properties including inhibition of the growth of *Helicobacter pylori* (Lawal *et al.,* 2016).

The objective of the present investigation was to assess, in an animal model, the antioxidative properties of aqueous stem bark extract of *Anogeissus leiocarpus* and to ascertain the possible protective effects of the extract as ulcer remedy as reported in earlier research on the extract. Gastroprotective effects were assessed using ulcer index and calculated protective index as well as histopathological examination. Ethanol-induced gastric ulcer in rats was carried out as a model for mimicking the PUD in humans.

2.0 MATERIALS AND METHODS

2.1 Experimental animals

Animals used in this study were purchased from the animal house of the Faculty of Pharmaceutical Science, Ahmadu Bello University, Zaria. Albino rats of both sexes weighing 160-250g were housed in environmentally controlled room (25 ± 2ºC, 12 h light/dark cycles) for three weeks to acclimatize which they were divided randomly into five groups of six animals each coded to prevent observer bias. The animals were fed with standard feed (Vital feeds LTD Kano) and water ad *libitum*.

2.2 Preparation of plant materials

The plant was obtained from Zaria city in March 2017. It was identified and authenticated in the herbarium unit of the Department of Botany, Faculty of life sciences, Ahmadu Bello University, Zaria, and Voucher number 900389 was deposited. Stem bark of *Anogeissus leiocarpus* was thoroughly washed and dried under the shade for 7 days. The stem bark was pulverized using pestle and mortar to fine powder (250g), placed in a mechanical shaker and exhaustively macerated in cold distilled water for 24 h. The mixture was allowed to settle and then filtered using a filter paper (Whatmann No 1). The filtrate was transferred into a petri-dish and concentrated in water bath at 40°C and was subsequently preserved as the extract. The extract obtained was weighed, and the percentage yield was calculated in term of air dried weight of the plant material as shown below:

$$Percentage\ yield = \frac{Weight\ of\ extract\ obtained}{Weight\ of\ initial\ sample} \times \frac{100}{1}$$

2.3 Quantitative phytochemical determinations of the Crude aqueous stem bark extract of *Anogeissus leiocarpus*

The detected phytochemical constituents were quantified as described:

Determination of Alkaloids (Harborne, 1973), Determination of Tannin (Van-Buren and Robinson), Determination of Flavonoids (Bohm and Kocipal- Abyazan, 1994), Determination of Saponins (Obadoni and Ochuko, 2001), Determination of phenols (Edeoga *et al.,* 2005)

2.4 The experimental protocol and ethanol-induced gastric lesions method

Group A–Normal control: Rats received only distilled water (0.5mL/100g body weight) (NC).

Group B–-Ulcer control treated with 70% ethanol: Rats received only 70% ethanol 1hr before sacrifice (0.5mL/100g body weight) (UC).

Group C–100mg/kg of the extract+ ethanol: Rat received 70% ethanol (0.5mL/100g body weight), 2 weeks after daily administration of aqueous stem bark extract of *A. leiocarpus* (100mg/kg body weight) (Al-100+ethanol).

Group D–200mg/kg of the extract+ ethanol: Rats received 70% ethanol (0.5mL/100gbodyweight), 2 weeks after daily administration of aqueous stem bark extract of *A. leiocarpus* (200mg/kg body weight) (Al-200+ethanol).

Group E –400mg/kg of the extract+ ethanol: Rats received 70% ethanol (0.5mL/100g body weight), 2 weeks after daily administration of aqueous stem bark extract of *A. leiocarpus* (400mg/kg body weight) (Al-400+ethanol).

Group F–cimetidine+ ethanol: Rats received 70% ethanol (0.5mL/100g body weight), 2 weeks after daily administration of cimetidine (100mg/kg body weight) (Cimetidine+ethanol).

Total number of animals in a group= 5

2.5 Gastric ulcer inducing dose of ethanol

To induce ulcers with ethanol, animals were fasted for 24–36 h following which absolute ethanol was administered at a dose of 1mL/200g body weight to each animal and after 1hr the animals were sacrificed. It is recommended that for every study, a preliminary assessment be done to determine the effective dose required for optimum induction of ulcers (Hollander *et al.,* 1985). An hour after the ethanol administration the animals were sacrificed by cervical dislocation followed by an abdominal incision. The stomach was removed and afterwards incised along the greater curvature. It was washed gently in running tap water and gastric mucosa spread on a filter paper for gastric lesions assessment. A 2x hand lens was used to locate the ulcers.

2.6 Biochemical assays

The level of protein in the homogenate was determined using the Biuret method. Oxidative stress markers namely; Catalase (CAT), superoxide dismutase (SOD), Glutathione peroxidase were determined using elabscience assay kit and Thiobarbituric acid reactive substances (TBARS) according to the method of Ohkawa *et al.*(1979).

2.7 Partial purification of crude stem bark aqueous extract of *Anogeissus leiocarpus* using column chromatography and thin layer chromatography

The crude extract was partially purified using Column chromatography and thin layer chromatography. Silica gel was used as a stationary phase. The slurry (the first eluent) was prepared by mixing silica gel 60-200 mesh and ethyl acetate. The bottom of the column was plugged with little cotton to prevent the adsorbent pass out, and then the silica gel suspension was poured into the column, set aside for 10 minutes and used. 4g of crude extract was mixed with silica gel to become more porous and subjected to column chromatography to separate the extract into its component fractions, methanol and ethyl acetate were added at various ratios (Ethyl acetate 100%, ethyl acetate: methanol 2:1, ethyl acetate: methanol 1:1, ethyl acetate: methanol 1:2 and methanol 100%). The column was eluted gradually with solvents of increasing polarity and effluents of 50mL was collected in labeled beakers and concentrated at room temperature. The flow rate was 5mL per minutes. Thin layer was used to monitor the column and the eluents were pooled together into five fractions. The column was eluted with solvents of increasing polarity.

2.7.1 Thin layer chromatography

Thin layer chromatography (TLC) provides partial separation of both organic and inorganic materials using thin-layered

chromatographic plates especially useful for checking the purity of fractions. Each fraction is applied on activated TLC plates with the help of capillary tube at a 1/2 inch apart from the lower edge of TLC plate, and plate is kept in a developing chamber containing suitable solvent system for specific time until the developing solvent reaches top of the upper edge of TLC plate. Plate is taken out from developing chamber, dried and solvent front was marked by lead pencil. Compound bands/spots visualized on TLC chromatogram were detected by visual detection, under UV light (254 nm), in iodine chamber and by using spray reagent (vanillin-sulfuric acid) for the presence of specific compounds. The visualized spots of the components in the chromatoplate were marked and the Rf value of each spot was calculated by the formula: Rf = distance travelled by the sample (cm)/distance travelled by the solvent (cm)

2.8 Quantitative antioxidant activity

Evaluation of the radical scavenging activity of each purified pooled fraction on the resolved TLC plate was carried out in quantitative terms only of strong, moderate, weak or no activity. The method reported by Braca *et al.* (2002) was followed. The TLC plates were sprayed with 0.2% (w/v) 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) in methanol and left. An active spot would turn from violet to yellow.

2.9 Qualitative antioxidant activity

This is the most widely reported method for determining the antioxidant activity of many plant drugs. DPPH is a stable free radical with violet colour. If free radicals have been scavenged, DPPH will change its colour from violet to pale yellow or colourless. This property allows visual monitoring at 517 nm. The 50% inhibitory concentration (IC_{50}) of each sample was calculated from the regression equation for each curve by substituting 50 for y and obtaining the unknown x in the equation $x = \frac{y+c}{m}$.

The method of Liyana- Pathiranan and Shahidi (2005) was used for the determination of scavenging activity of DPPH free radical in the extract solution. A solution of 0.135mM DPPH in ethanol was prepared and 1.0ml of this solution was mixed with 1.0ml of extract prepared in methanol containing 0.025-0.5mg of the plant extract and standard drug respectively. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 minutes. The absorbance of the mixture was measured spectrophotometrically at 517nm. The ability of the plant extract to scavenge DPPH radical was calculated by the equation:

$$DPPH\ radical\ scavenging\ activity = \left\{\frac{(Abs\ \text{control} - \text{Abs sample})}{(Abs\ control)}\right\} \times 100$$

Where; Abs control is the absorbance of DPPH radical+ methanol; Abs sample is the absorbance of DPPH radical+ sample extract or standard.

2.10 Statistical Analysis

The results were expressed as mean ±standard error of mean (SEM). Statistical comparisons were performed by one-way analysis of variance (ANOVA), followed by Dunnets post-hoc test. The data was analyzed by using Statistical Package for the Social Sciences (SPSS, version20.0). A P-value less than 0.05 were considered to be significantly different.

3.0 RESULTS

3.1 Quantitative analysis of phytochemical constituents of aqueous stem bark extract of Anogeissus leiocarpus.

The results of the qualitative analysis of phytochemical constituents of aqueous stem bark extract of *Anogeissus leiocarpus* shows the degree of abundance of these phytochemicals in mg/100g of the extract is as follows; 87.67 ± 0.09 of flavonoids, 51.00±0.12 of alkaloids, 36.33±0.09 of saponins and mg/g 11.23±0.15 of tannin, and 15.53±0.03 of total protein (Table 1).

3.2 Effect of aqueous stem bark extract of *Anogeissus leiocarpus* on antioxidative parameters in 70% ethanol induced ulcer in rats.

MDA level in all groups decreased significantly when compared with the ulcer control group. The catalase level in all of groups significantly increased when compared with ulcer control. There was no significant difference SOD level of groups administered 100 and 200 mg/kg when compared with ulcer control group. However there was a significant increase ($p<0.05$) in groups administered 400mg/kg extract and standard cimetidine at 100mg/kg when compared with ulcer control group. GPx level in groups administered 200 and 400mg/kg of extract showed a significant increase when compared with ulcer control group. However here was no significant difference in groups administered 100mg/kg extract and standard cimetidine at 100mg/kg when compared with ulcer control group (Table 2).

Table 1: Quantitative analysis of phytochemical constituents of the crude aqueous stem bark extract of *Anogeissus leiocarpus*

Flavonoids (mg/100g)	Alkaloids (mg/100g)	Saponins (mg/100g)	Tannins (mg/100g)	Phenols (mg/100g)
87.67±0.09	51.00±-0.12	36.33±0.09	11.23±0.15	15.53±0.03

Value are expressed as Mean ± SEM (n=3)

Table 2: Effect of aqueous stem bark extract of *Anogeissus leiocarpus* on some antioxidative enzymes and thiobarbituric acid reactive substances (TBARS) in ethanol- induced ulceration in albino rats (compared with positive (normal) control and negative (ethanol) control).

GROUP	MDA nmolml^{-1}prot	Catalase Umg^{-1}prot	SOD Umg^{-1} prot	GPx mUmg^{-1}prot
Normal Control	0.06±0.03^{b}	7.87±0.63^{b}	1.50±0.36^{b}	2.85±0.72^{b}
Ulcer Control	0.27±0.03^{a}	1.87±0.62^{a}	0.35±0.72^{a}	0.36±0.06^{a}
Al-100mg/kg + ethanol	0.11±0.00^{c}	6.56±0.58^{c}	0.72±0.08^{a}	1.32±0.31^{a}
Al-200mg/kg + ethanol	0.04±0.01^{d}	8.98±1.39^{d}	0.98±0.12^{a}	1.59±0.32^{c}
Al-400mg/kg + ethanol	0.05±0.03^{e}	12.25±1.30^{e}	1.20±0.08^{c}	2.33±0.29^{d}
Cimetidine-100mg/kg+ ethanol	0.10±0.03^{f}	8.51±1.23^{f}	1.01±0.10^{d}	1.05±0.27^{a}

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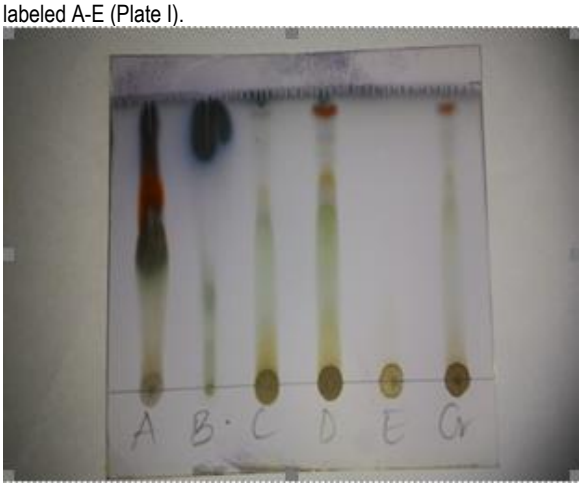
Data represent the Mean± SEM. (n=5)
Different superscripts along the column are significantly different at $p<0.05$ when compared with ulcer control

3.3 Column Chromatography the crude aqueous stem bark extract of *Anogeissus leiocarpus*.
Chromatogram of the five pooled fractions from column chromatography resolved using solvent system ethyl acetate: methanol 9:2 sprayed with p-anisaldehyde. The plates were labeled A-E (Plate I).



Fraction A (Beaker 1-4), Fraction B (Beaker 5-12), Fraction C (Beaker 13-22), Fraction D (Beaker 23-33), Fraction E (Beaker 34-50), Cr (Crude extract).

Plate I: TLC plate of partially purified pooled fractions of crude aqueous stem bark of *Anogeissus leiocarpus*.

3.3.1 Quantitative antioxidant activity of partially purified aqueous stem bark extract of *Anogeissus leiocarpus*.
The quantitative DPPH scavenging activities of the five pooled fractions using DPPH spray labeled A-E revealed fraction A to have the highest antioxidant activity. (Plate IX).

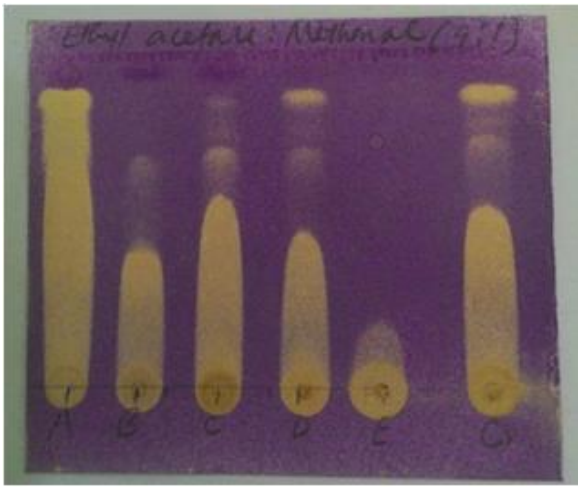


Plate II: TLC plate of the five partially purified pooled fractions of crude aqueous stem bark of *Anogeissus leiocarpus* resolved by ethyl acetate methanol 9:1 sprayed with DPPH solution

3.3.2 Qualitative antioxidant activity partially purified pooled fractions of crude aqueous stem bark of Anogeissus leiocarpus.
A scavenging activity in % inhibition and IC_{50} of the five pooled fractions from column chromatography is given in Figure 1 and table 4. Fraction A has an IC_{50} of 20.88, fraction B 53.89, fraction C 35.37, fraction C 35.37, fraction D 60.97, fraction E 197.95 and ascorbic acid 24.32. Fraction A has the highest percentage inhibition at various concentrations (52.60±0.10, 66.35±0.15, 92.20±0.10, 94.40±0.20, 97.95±0.35) as well as a lower IC_{50} (20.88) compared to Ascorbic acid with IC_{50} (24.32) and percentage inhibition (52.80±0.75, 63.40±0.75, 79.05±0.75, 91.35±0.15, 94.05±0.35).

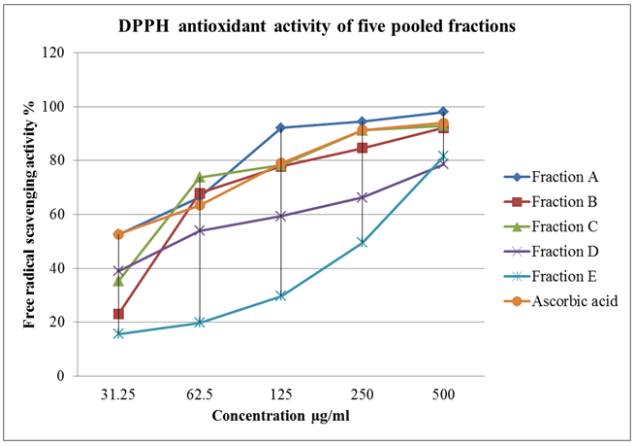


Figure 1: DPPH Free radical scavenging activity % and IC_{50} of the five pooled fractions

Table 3: IC_{50} of the five pooled fractions and ascorbic acid

CONC	IC_{50}
Ascorbic acid	24.32
Fraction A	20.88
Fraction B	53.89
Fraction C	35.37
Fraction D	60.97
Fraction E	197.95

4.0 DISCUSSION
The increased frequency of occurrence of gastric ulcers in humans, severe side effects and cost of some available synthetic drugs, arises the use of natural products an important alternative treatment (Bassi, *et al.,* 2014 and Strand *et al.,* 2017). In this sense

aqueous stem bark of *Anogeissus leiocarpus*, have proven to be advantageous in the treatment of various diseases in lab animals and patients (Shuaibu *et al.,* 2008, Atawodi *et al.,* 2011, Victor and Grace, 2013, Timothy *et al.,* 2015).

Plants are known to contain a variety of secondary metabolites. These secondary metabolites or bioactive compounds produce definite physiological actions on the human system. Many of these phytochemicals have been discovered and even isolated from a variety of medicinal plants. Unfortunately, however, not many of them have been exploited for clinical use (Ekwueme *et al.,* 2015). Phytochemical analysis of plants is predicated by the need for drug alternatives of plant origin, made imperative by the high cost of synthetic drugs as in the case of anti-ulcers. These secondary plant metabolites extractable by various solvents exhibit varied biochemical and pharmacological actions in animals when ingested (Nwogu *et al.,* 2008).

The results of the qualitative analysis of phytochemical constituents of aqueous stem bark extract of *Anogeissus leiocarpus* shows the degree of abundance of these phytochemicals in mg/100g of the extract is as follows; 87.67 of flavonoids, 51.00 of alkaloids, 36.33 of saponins, 11.23 of tannin, and 15.53 of phenol (Table 1). The result of the quantitative phytochemical analysis in this study varies from the result of Ahmad and Wudil were concentration of saponins (89.5 mg/100g) was found higher than the other phytochemicals (alkaloids-26.7 mg/100g, tannins-29.9 mg/100g, steroids-10.6 mg/100g, flavonoids-27.3 mg/100g, phenols-5.2 mg/100g, glycosides-1.7mg/100g) in the aqueous stem bark extract (Ahmad and Wudil, 2013). Flavonoids were found to be higher in concentration than other phytochemicals. The difference in the concentration of the phytochemical then those in literature might be due to geographical variations, nutrients, sunlight, irrigation, time of collection, age of plant among others.

The presence of flavonoids in the aqueous stem bark extract of *Anogeissus leiocarpus* could account for its use as an antinflammatory agent (Ekwueme *et al.,* 2015). It also means that the plant could be used to prevent damage caused by free radicals in the body (Dweck and Mitchell, 2002). Oxidative stress induced by ethanol was suppressed in the treatment group which proves the radical scavenging activity of the extract. Flavonoids exhibit dramatic effects on immune and inflammatory cells; these can be either immunosuppressant or immune stimulatory (Huang et al., 2010).

In this study ethanol induced oxidative stress in stomach tissue causes inhibition of antioxidant enzymes SOD, catalase (CAT) and glutathione peroxidase (GPX) has been corroborated and is directly involved in increased lipid peroxidation observed in ethanol- treated rats ulcer control group when compared with normal control group. Study carried out by Boligon et al. reported similar results relating to antioxidant enzyme activity (Boligon *et al.,* 2014). In addition lipid peroxidation measured as a biomarker as MDA caused by ethanol plays an important role in the pathogenesis of ethanol induced gastric lesions in gastric mucosa of rats. Pre-treatment with aqueous stem bark extract of *Anogeissus leiocarpus* exhibited antioxidant properties by decreasing the levels of MDA, suggesting its potential to protect against ethanol-induced lipid peroxidation in rats. Furthermore, aqueous stem bark extract of *Anogeissus leiocarpus* preserved the antioxidant activity of GPx, CAT, and SOD enzymes after ethanol administration, thus protecting the gastric mucosa (Table 2).

Aqueous stem bark extract of *Anogeissus leiocarpus* restored in a dose dependent manner the oxidative stress induced by ethanol. The antioxidant properties of *Anogeissus leiocarpus* were demonstrated by decreased levels of Malondialdehyde MDA and increase of antioxidant defenses (catalase, superoxide dismutase and Glutathione peroxidase). These protective effects described for the crude aqueous stem bark extract of *Anogeissus leiocarpus* could be associated with the presence of flavonoids, alkaloids, total phenols, tannins and saponins which was similarly reported in study by Shuaibu et al. where castalagin, flavogallonic acid, bislactone ellagic acids, flavonoids and phenolic diterpenes were found present (Shuaibu *et al.,* 2008). The free radical scavenging activity of the aqueous stem bark extract of *Anogeissus leiocarpus* might be considered as one of the possible mechanisms of its gastroprotective effects observed.

SOD represents the first line of defense against ROS by catalyzing the conversion of O_2—to oxygen and H_2O_2, the latter of which is catalyzed to H_2O by CAT or GPx (Nozik-Grayck *et al.,* 2005, Schrader and Fahimi, 2006 and Dayer *et al.,* 2008). The possibility of this protective effect being fostered by aqueous stem bark extract of *Anogeissus leiocarpus* is consistent with previous findings that *Anogeissus leiocarpus* engender a significant decrease in oxidative stress by increasing the antioxidant defense system and reducing the levels of lipid peroxidation in different pathologic conditions .(Atawodi *et al*., 2011).

The quantitative antioxidant activity of the partially purified pooled fraction using 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) spray as well as qualitative antioxidant activity using DPPH spectrophotometrically using ascorbic acid as a standard showed fraction A to possess the most activity. The DPPH test showed the reactivity of the test compound with a stable free radical. The DPPH gives a strong absorption band at 517 nm in visible region. The antioxidant compounds present in the extract convert DPPH radical to a more stable DPPH molecular product by donating an electron or a hydrogen atom. The colour change from deep purple of DPPH radical to light yellow of reduced form of DPPH allows the spectrophotometric determination of the antioxidant activity. The degree of reduction in absorbance measurement is an indication that the extract had a radical scavenging property hence its antioxidant power. The results are either expressed as IC_{50} that is the concentration of the antioxidant causing 50% DPPH scavenging or as % scavenging of DPPH at a fixed antioxidant concentration for all the samples.

The aqueous stem bark extract of *Anogeissus leiocarpus* exhibited a significant concentration dependent inhibition of DPPH activity with IC_{50} of 20.88 when compared with ascorbic acid IC_{50} 24.32 (Table 3). The lower the IC_{50} the higher the antioxidant potential of the sample. The extract had shown to be potent as compared with vitamin C with a maximum inhibition percentage of 97.95 % at 500 µg/ml and 94% for vitamin C at 500 µg/ml. The other fractions had higher IC_{50} and DPPH lower percentage inhibitions. This correlates with the research finding of Olutayo *et al.* (2011) where antioxidant activity of five traditionally used medicinal plants were evaluated including *Anogeissus leiocarpus*. Results of this study suggest that the plant extract contain phytochemical constituents that are capable of donating hydrogen to a free radical to scavenge the potential (antioxidant activities), which can counteract the oxidative stress/damage induced by ethanol on the gastric mucosa.

5.0 Conclusion

The results from the study shown that aqueous stem bark extract of *Anogeissus leiocarpus* (100, 200, 400mg/kg), demonstrated a

gastroprotective effect against ethanol-induced gastric ulceration in rats. The protection might be due to the antioxidant properties which are evident on their inductive effect on antioxidant enzymes (Superoxide dismutase, catalase and glutathione peroxidase) that make up endogenous scavengers of reactive oxygen species (ROS) evaluated in the study. Diminishing lipid peroxidation and improving defenses against the erosive lesions that characterize the development of gastric ulcer produced by ethanol.

Further research should be carried out on the anti-secretary activity of the plant. The bioactive components of the plant with anti-ulcer activity need to be isolated and characterized.

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