IJABR Vol. 12(1): 83 - 100 (2021)

Original article

GASTRO-PROTECTIVE AND ANTIOXIDANT EFFECTS OF AQUEOUS AND ETHANOL EXTRACTS OF *Commiphora africana* GUM RESIN (MYRRH)

***[1]Yahaya, A. M., [1]Mohammed, B. A., [1]Garba, R., [1]Hamzah, R. U., [1]Mahmoud, M. F. and [1]Abdulrahaman, K. A.**

[1]Department of Biochemistry, Federal University of Technology Minna – Nigeria.

Submitted: February, 2021; Accepted: April, 2021; Published: June, 2021

ABSTRACT

The gastro-protective and antioxidant effects of aqueous and ethanolic extracts of *Commiphora africana* gum resin (Myrrh) was determined in indomethacin (25mg/kg body weight) induced gastric damage Albino rats. The preliminary phytochemical screening of these extracts revealed the presence of flavonoids, tannins, cardiac glycosides, terpenes, saponins, alkaloids and reducing sugars. Acute toxicity study revealed that the extracts is safe up to 5000mg/kg body weight. Lesion in the stomach tissues of experimental rats pretreated with both extracts significantly ($P<0.05$) reduced. At doses of 250 and 500mg/kg body weight of the aqueous and ethanolic extracts the preventive index of (47.8, 53.1) % and (61.3, 74.3) % respectively as compared to 65.2% for positive control ranitidine. Highest gastro-protective effect 74.3% was shown by the ethanolic extract at a dose of 500mg/kg body weight. The Superoxide dismutase and Catalase activities were elevated with a significant decrease($P<0.05$) in Malondialdehyde levels upon administration of both extracts at doses of 250 and 500 mg/kg body weight in serum and stomach tissues of experimental animals, with the ethanolic extract been more effective at 500mg/kg body weight. In vitro antioxidant activity of both extracts via linoleic acid peroxidation assay revealed the inhibition of linoleic acid peroxidation by 57.4% and 64.9% respectively compared to the positive control (Ascorbic acid) with 54.1%. This research suggests that the aqueous and ethanolic extracts of *Commiphora africana* gum resin possess potent gastro-protective and antioxidant activities and could be developed into a drug for ulcer patients.

Keywords: Gastro-protective, Antioxidant, gum resin, ulcer, Indomethacin, Peroxidation.

***Corresponding author's email**: Yahaya.mohd@futminna.edu.ng, 07060762624

INTRODUCTION

Gastric ulcer is a major health hazard which occur as a result of discontinuity or breakage in gastric mucosa of the stomach. The pathogenesis of gastric ulcer is multifactorial ranging from imbalance between some endogenous aggressive factors such as hydrochloric acid, pepsin, refluxed bile, reactive oxygen species (ROS) and cytoprotective factors which include surface active phospholipids, prostaglandins, enzymatic and non-enzymatic antioxidants [1] to some diverse factors such as excessive use of non-steroidal anti-inflammatory drugs (NSAIDs), alcohol consumption, tobacco smoke, stress, pesticides and environmental pollutants. Other aggressive factors such as inadequate dietary habits, hereditary predisposition and infection by *Helicobacter pylori,* may also be responsible for development of gastric ulcer [2, 3].

Commiphora africana (*Commiphora*; gum bearing) which is generally called *African myrrh* belongs to the family *Burseraceae* and a group of plant called myrrh [4], occurring widely over North East Africa and sub-Saharan Africa in Angola, Botswana, Burkina Faso, Kenya, Mali, Niger, Somalia, South Africa, Sudan, and Zimbabwe. *Commiphora africana* is a shrub to small tree not more than 5m high, its branchlets often ending in spines, its bark is grey-green, peeling to reveal a shiny surface, red when damaged and then exuding bdellium (a clear, edible, aromatic gum) [5].

Commiphora africana has been found to possess several medicinal and industrial uses. The gum resin generally called "murr" in Arabic, "Dashi" in Hausa and "Myrrh" in English is the hardened, resinous exudates obtained from trees of certain Commiphora species [6]. It is used industrially in production of cosmetics and body lotion, also considered to be good insecticides, especially against termites [7]. The gum- resin is used medically in sealing and disinfecting wound; it is also applied as plaster and used for spasms [5]. The gum resin exudes naturally or from incisions made in the bark, which at first is yellowish color, but soon hardens in intense heat becomes darker, then it is harvested. There have been several investigations to show that the essential oils of Commiphora species are rich in furanosesquiterpenoids, and a total of around 20 different compounds of this type have been identified from plants in this genus [8]. The crude extracts of Commiphora gum have been found to possess anesthetic, antibacterial, antiulcerogenic and antifungal properties [9]. The fruits are used for treatment of typhoid fever and a remedy for stomach problems, the powdered bark is mixed with porridge to cure malaria with reports on the antimicrobial activity of *Commiphora africana* ethanolic leaf extracts [10].

Non-steroidal anti-inflammatory drugs (NSAIDs) been cheap, affordable and readily available are widely used in treatment of pain, fever and inflammation, however they have side effect which include gastric ulcer which is capable of inducing the upper gastrointestinal bleeding, internal bleeding, gastric outlet obstruction, inflammation of tissues that line the wall of the abdomen (pectoritis) and perforation of the stomach and intestines if left untreated [3].

In recent years, researches are been carried out on several natural products especially those derived from plant and has been used as alternative therapies in treatment of gastric ulcer and several ailments [11]. Several parts of *Commiphora africana* plant has been shown to have antiulcerogenic, anesthetic,

antibacterial, and antifungal properties [9]. However, most experiment were conducted on the extract of the leaf, root and bark of this tree with little or no research on the gastro protective and antioxidative activity of the myrrh (gum) of this particular *Commiphora* species (*Commiphora africana*). Thus, the research was carried out to determine the gastroprotective and antioxidant activities of Aqueous and Ethanolic extracts of gum resin of *Commiphora africana* in Indomethacin-induced gastric damage in albino rats.

MATERIALS AND METHODS

Plant Material

Commiphora africana gum resin was purchased at Kure market, Minna, Niger State on 13th of March 2013. Sample was identified at the Biological Science Department of Federal University of Technology (FUT), Minna. The solidified gum resin rinsed and dried at room temperature for a period of 2days before extraction.

Experimental Animals

A total of sixty-four (64) Wistar rats (weighing about 120-200g) of both sexes were used in this study. Experimental animals were obtained from the animal house of Department of Pharmacology and Clinical Pharmacy Ahmadu Bello University, Zaria. They were transported in well ventilated plastic cages to Department of Biochemistry, FUT Minna, Niger State where this study was carried out. Animals were used for experimental studies after acclimatization for a period of 2 weeks and throughout the period of experimental studies; the animals had unrestricted access to feed and water, they were fed on grower and tap water throughout this period.

Chemicals

All the chemicals used were of analytical grade, which include: Ethanol, Chloroform, Linoleic acid and Tween 20 produced by Amazon chemical limited (Lagos State), with Ascorbic acid, Ammonium thiocyanate, Ferrous chloride, Phosphate buffer, Adrenaline, Dichromate acetic acid reagent and Trichloroacetic acid (BDH, Britain).

Drugs

Indomethacin (25mg capsules) produced by Greenfield pharmaceutical limited (JIANG SU,China) and Ranitidine(Ranivan 150 mg) produced by CIPLA limited (Patalanga M.S, India) and Lansoparazole produced by Pharnas Lab PVC limited (Masat, India) were obtained at Opeyemi pharmacy, Ilorin Kwara state.

Extraction of Plant Sample

The solidified dried myrrh of *Commiphora africana* was collected and pounded in a mortar into powder. Aqueous and ethanol extract was then prepared by soaking 100g of the sample each in a beaker containing 500mLof ethanol and distilled water respectively for 2 days. It was then filtered using a filtered paper (Whatman size no. 1), the filtrate was evaporated to dryness in water bath at a temperature of 78^0C for ethanol extract and 100^0C for aqueous extract and black residue weighing 76.76g and 67.11g were obtained respectively. This was kept in an air tight bottle in a refrigerator for further analysis.

Phytochemical Screening

Phytochemical tests were carried out on the extracts using a standard procedure to identify the chemical use of most constituent as described by [12, 13].

Determination of Acute Toxicity LD_{50}

A total of 24 wistar rats (12 each for aqueous and ethanolic extract) was used to determine the acute toxicity of extracts. The rats were grouped into 4 groups of 3 animals each. Doses of extracts 100, 500, 1000 and 5000mg/kg body weight were administered orally to group 1, 2, 3 and 4 respectively. Animals were observed for 14days to check for behavioral changes in animals and mortality rate.

Experimental Design

Animals were acclimatized for a period of 2 weeks with free access to food and water. They were randomly divided into 8 groups of 5 rats (n=5). Animals were fasted for 12 hours and allowed free access to water except for the last hour before the experiment. Drugs, extracts and distilled water were administered orally to respective groups at a given dose per kg body weight of animals.

Group 1: 250mg AE+ IND 25mg, Group 2: 500mg AE + IND 25mg, Group 3: 250 mg EE+ IND 25mg, Group 4: 500mg EE+ IND 25mg, Group 5: Normal group, Group 6: IND 25mg, Group 7: IND 25mg + RAN 25mg, Group 8: IND 25mg + LAN 30mg.

NOTE: AE-Aqueous extract of *Commiphora africana* gum resin, EE-Ethanol extract *Commiphora africana* gum resin, IND-Indomethacin, LAN-Lansoprazole and RAN- Ranitidine

Oral Administration of Safe Dose of Extracts and Standard Drugs to Experimental Animals and Induction of Gastric Damage with Indomethacin (25mg/Kg Body).

The effects of aqueous and ethanol extract of *Commiphora Africana* (*Myrrh*) on indomethacin induced gastric damage were determined. The protective effects of extracts were determined compared with the H_2 receptor blocker, ranitidine (RAN) and the proton pump inhibitor, lasoprazole (LAN). Animals were divided into 8 groups each consisting of five rats. Two doses (250 and 500mg/kg body weight) of ethanol and aqueous extract respectively were prepared by dissolving them in distilled water respectively and RAN (25mg/kg body weight) and LAN (30mg/kg body weight) were administered orally to the assigned group of rats. After 5 minutes, IND (25mg/kg body weight) was administered orally to all the animals. After 8 hours, animals were sacrificed by high dose of chloroform followed by cervical dislocation.

Blood Sampling

Blood was collected in sterile sample bottles and allow to coagulate at room temperature for 30 minutes. Blood was then centrifuged at 3000rpm for 10minutes to obtain serum.

Assessment of Gastric Mucosal Lesion

After blood sampling, each stomach of rat was removed and open along a greater curvature. The stomach was washed with ice-cold saline and examined for macroscopical mucosal lesions. The gastric mucosal lesion was expressed in terms of ulcer index (UI) according to [14]. Which depends on the calculation of a lesion index by using 0-3 scoring system based on severity of each lesion. Severity factor was defined according to the length of the lesions.

Severity factors: 0=no lesions, 1=lesions < 1mm length, 2= lesions 2-4mm length and 3=lesions > 4mm length. Lesion scores for each rat was calculated as the number of lesions in the rat multiplied by the respective severity factor.

Ulcer index for each group was taken as the mean lesion score of the entire rat in the group. Preventive index (% inhibition)

of a given drug was calculated by the equation described by [15].

PI= UI of IND group- UI of pretreated group UI of IND group

Preparation of Stomach Tissue Homogenate

To prepare stomach tissue homogenate, the stomach of animals was washed in ice cold normal saline (0.9% NaCl) solution, blotted and weighed. The grounded tissue (each 0.5g) were then treated with 4.5mL of appropriate buffers, which have different pH for each biochemical assay. The resulting mixture was then homogenized and centrifuged at 1000g for 15 minutes then it was removed from the centrifuge and the supernatant was decanted and stored at -20^{0}C for further analysis.

Biochemical Investigation of Stomach Tissues and Serum

The following antioxidant enzyme activity; Catalase (CAT), Superoxide dismutase (SOD) and Lipid peroxidation assay (LPO) from the stomach tissues and serum of all rat groups were determined by spectrophotometry and colorimetry.

Determination of Superoxide Dismutase (Sod) Activity

The SOD activity of tissues of experimental animals were determined following the method described by [16]. The tissue homogenate obtained from experimental animals was centrifuged at 17000rpm for 20 minutes and 1mLof supernatant was diluted in 9mL of distilled water to make a one in ten dilutions. An aliquot of 0.2mL of the diluted tissue supernatant was added to 2.5mL of 0.05M phosphate buffer pH7.8 to equilibrate in the spectrophotometer and the reaction started by addition of 0.3mL of freshly prepared adrenaline to the mixture which was quickly mixed by inversion. The reference cuvette contained 2.5mL of phosphate buffer, 0.3mL substrate (adrenaline) and 0.2mL of distilled water. The increase in absorbance at 480nm was monitored every 30seconds for 150seconds. (Molar extinction= 4020$M^{-1}cm^{-1}$).

Calculation

SOD = Change in absorbance per minute x Total volume [17]

Molar extinction x Sample Volume

Determination of Catalase Activity

The Catalase activity of the tissue homogenate obtained from the experimental animals was determined following the method as described [18]. Catalase activity was assayed at 620nm and expressed as micromoles of H_2O_2 consumed/min/mg protein at 25 degrees Celsius. The reaction mixture (1.5mL) contained 1.0mL of 0.01M phosphate buffer (pH 7.0), 0.1mL of tissue homogenate and 0.4mL of 2M H_20_2. The reaction was stopped by the addition of 2.0mL of dichromate acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in ratio 3:1). (Molar extinction= 40$M^{-1}cm^{-1}$)

Calculation

CAT = Change in absorbance per minute X Total volume

Molar extinction x Sample Volume [18]

Lipid Peroxidation (LPO) Assay

Lipid hydroperoxides and aldehyde increase in concentration as a response to oxidative stress [19]. Malondialdehyde (MDA) an index of lipid peroxidation by TBARs was determined using the method of [20]. 1.0mL of supernatant was added to 2mLn of (1:1:1 ratio) TCA-TBA-HCl reagent (Thiobarbituric acid 0.37%, 0.24N

HCl and 15% TCA) Tricarboxylic acid-thiobarbituric acid-hydrochloric acid reagent boiled at 100^0C for 15 minutes, and allowed to cool. Flocculent materials were removed by centrifuging at 3000rpm for 10 minutes. The supernatant was removed and the absorbance read at 532nm against a blank. MDA was calculated using molar extinction for MDA TBA- complex of $1.56x10^5M^{-1}CM^{-1}$.

Calculation

$$MDA = \frac{\text{Absorbance X Total volume}}{\text{Molar extinction x Sample Volume}}$$

[20]

In-vitro Linoleic Acid Peroxidation Assay

Antioxidant activity of Aqueous and Ethanol extract of *Commiphora africana* gum resin extracts was determined using the method of the thiocyanate method [21]. The sample (1mg) in 1mL distilled water was mixed with 5mL linoleic acid emulsion (0.02M, pH 7.0) and %mL of phosphate buffer and the mixture were then homogenized. The reacting mixture was then incubated at 37^0C for 15 minutes. Aliquot of 0.1mL were taken at an interval of 3 minutes during incubation. The degree of oxidation was measured according to thiocyanate method by sequentially adding 4.5mL ethanol (75%), 0.1mL Ammonium thiocyanate (30%), 0.1mL sample solution and 0.1mL ferrous chloride (0.02M in 3.5% HCl). The mixture stood for 3 minutes and the peroxide value was then determined by reading the absorbance at 500nm using UV-VIS spectrophotometer. A control was performed with linoleic acid but without the extract. Ascorbic acid was prepared under the same conditions as those described above and used as positive control. Percentage inhibition of linoleic acid peroxide was calculated using the following equation as described by [21]:

$$I = \frac{\text{(1- Absorbance of sample at 500nm)}}{\text{Absorbance of control at 500nm}} \times 100$$

Statistical Analysis

The results are given as mean $\pm$ Standard deviation using the SPSS 16.0 software. Group comparisons were statistically analyzed using one- way analysis of variance (ANOVA) with multiple comparisons versus control group. Values of $P<0.05$ were taken as significant.

RESULTS

Acute Toxicity (LD_{50})

The doses of aqueous and ethanolic extracts administered to the rats did not show any toxic symptom of mortality or behavioral changes up to the dose level of 5000mg/kg body weight in the treated animals (i.e. LD_{50} >5000mg) for the period of 14 days and hence it was considered safe for other pharmacological screening.

Phytochemical Screening

Table 1 shows that the presence of phytochemicals of Aqueous and ethanolic extracts were common only for alkaloids, steroids, tannins, phenols and reducing sugars. However, anthranoids and Anthraquinones were completely absent in aqueous extract of *Commiphora africana* gum resin**.** High concentrations of alkaloids, saponins, terpenes, flavonoids, and phenols were observed in both Aqueous and ethanolic extracts of the gum resin. Cardiac glycosides appeared to be higher only in the ethanolic extract.

Table 1: Phytochemical Compositions of AE and EE of *Commiphora africana* Gum Resin.

Phytochemicals	AE	EE
Alkaloids	++	++
Anthranoids	-	+
Cardiac glycosides	+	++
Saponins	++	+++
Anthraquinones	-	+
Steroids	+	+
Terpenes	++	+++
Tannins	+	+
Flavonoids	++	+++
Phenols	++	++
Reducing Sugar	+	+

Key: AE-Aqueous extract, EE-ethanolic extract, +++High concentration, ++ moderate, + Trace, - Not detected

In vitro Linoleic Acid Peroxidation Assay

Table 2 shows the results of *in vitro* activity assays for aqueous and ethanolic extracts after incubation with linoleic acid emulsion expressed as percentage inhibition (%). The highest inhibition of linoleic acid peroxidation (64.9 %) was observed in the ethanolic extract of *Commiphora Africana* Gum resin, compared with ascorbic acid (54.1) that was chosen as the standard control.

Table 2: *In vitro* Linoleic Acid Peroxidation Assay of Aqueous and Ethanolic Extracts of *Commiphora Africana* Gum resin

Samples	Means of absorbance (500nm)	Inhibition of linoleic acid peroxidation (%)
AE	0.346±0.16*	57.4
EE	0.284±0.24*	64.9
Ascorbic acid	0.373±0.77*	54.1
Linoleic acid (Control)	0.813±0.31	-

Key: AE-Aqueous extract, EE-ethanolic extract. The measurements were calculated from five replicates. Ascorbic acid was examined as positive control. *Significant at $P<0.05$ as compared with control.

Table 3 shows the gastroprotective effect of aqueous and ethanolic extracts of *Commiphora africana* gum resin on stomach tissue of the experimental animals. The standard drug (LAN) at a dose of 25 mg/kg body weight, exhibited the highest % inhibition (83.5) of the ulceric stomach lesions, while the ethanolic extract (500 mg/kg body) shows a % inhibition (74.3) higher than that of the second standard drug (LAN) at a dose of 25 mg/kg body weight, and the aqueous extract (53.1) at the same dose.

Table 3: Gastroprotective Effects of Aqueous and Ethanolic Extracts of *Commiphora africana* Gum resin on Stomach Tissue of Indomethacin-Induced Gastric Damaged Rats

Groups	Dose mg/kg body weight	Ulcer area $(mm)^2$	% inhibition
IND + AE			
1	250	19.1 ± 2.09^a	47.8
2	500	17.2 ± 0.56^b	53.1
IND + EE			
3	250	14.1 ± 1.64^c	61.3
4	500	9.3 ± 1.81^d	74.3
NORMAL			
5	-	0.00±0.00	-
IND			
6	25	36.6±3.97	-
IND+RAN			
7	25	12.8±2.09	65.2
IND+LAN			
8	30	3.5±0.87	83.5

Key: AE-Aqueous extract of *Commiphora africana* gum resin, EE- Ethanol extract *Commiphora africana* gum resin, IND-Indomethacin, LAN- Lansoprazole and RAN- Ranitidine Results are expressed as mean±SD (n=4). a, b, c, d-Significantly different from IND group at $P<0.05$.

Table 4 shows the effects of aqueous and ethanolic extracts of *Commiphora africana* gum resin on the activities of superoxide dismutase (SOD) and catalase (CAT), in Serum of the induced-gastric damaged experimental animals. Higher activities of SOD (4.93±0.03) and CAT (9.56±0.05) were recorded for the normal group, compared with the induced-gastric damaged groups that were given 500 mg/kg body weight of the aqueous and ethanolic extracts (3.75±1.20 and 7.36±0.02 for SOD and CAT respectively). Lansoprazole (LAN) at a dose of 30 mg/kg body weight shows the highest activity of SOD (8.61±0.09), compared to Ranitidine (RAN). The lowest activity (1.20±0.07) was recorded for the negative control.

Table 4: Effects of Aqueous and Ethanolic Extracts of the Gum resin of *Commiphora africana* on Superoxide dismutase (SOD) and Catalase (CAT) Activities in Serum of Indomethacin-Induced Gastric Damaged Rats

Groups	Dose (mg/kg body weight)	SOD (mmol/min/mg tissue)	CAT (mmol/min/mg tissue)
IND + AE			
1	250	2.69±0.03[a]	2.57±0.02[a]
2	500	3.75±1.20[b]	2.64±0.07[b]
IND+ EE			
3	250	4.38±2.41[c]	4.64±0.08[c]
4	500	4.59±3.53[d]	7.36±0.02[d]
NORMAL			
5	-	4.93±0.03	9.56±0.05
IND			
6	25	2.35±2.03	1.20±0.07
IND+ RAN			
7	25	3.44 ±2.15	6.28±0.01
IND+LAN			
8	30	4.74 ±1.15	8.61±0.09

AE-Aqueous extract of *Commiphora africana* gum resin, EE- Ethanol extract *Commiphora africana* gum resin, IND-Indomethacin, LAN- Lansoprazole and RAN- Ranitidine

Results are expressed as mean±SD (n=4). a, b, c, d-Significantly different from IND group at $P<0.05$.

Table 5: The effects of aqueous and ethanolic extracts of *Commiphora africana* Gum resin on malondialdehyde (MDA) levels in serum of the experimental animals is shown in Table 5. Lowest levels of malondialdehyde were recorded for the normal and the treated (LAN) groups (1.03±0.31 and 1.08±0.51 respectively). The ethanolic extracts gave the lowest levels at both doses compared to the aqueous extract.

Table 5: Effects of Aqueous and Ethanolic Extracts of *Commiphora africana* Gum resin on Malondialdehyde (MDA) levels in serum of Indomethacin-Induced-Gastric Damaged Rats.

Groups	Dose (mg/kg bodyweight)	MDA(µmol MDA/mg tissue)
IND + AE		
1	250	3.76±0.38[a]
2	500	3.68±0.16[b]
IND+ EE		
3	250	2.38±0.51[c]
4	500	2.24±0.12[d]
NORMAL		
5	-	1.03±0.31
IND		
6	25	9.32±0.06
IND+ RAN		
7	25	3.55±0.23
IND+LAN		
8	30	1.08±0.51

Key: AE-Aqueous extract of *Commiphora africana* gum resin, EE- Ethanol extract *Commiphora africana* gum resin, IND-Indomethacin, LAN- Lansoprazole and RAN- Ranitidine. Results are expressed as mean±SD (n=4). a, b,c,d-Significantly different from IND group at $P<0.05$

Table 6 shows effects of the aqueous and ethanolic extracts *Commiphora africana* Gum resin on SOD and CAT activities in stomach tissues of the experimental animals. Highest activities of SOD (3.11±1.04) and CAT (3.95±0.18) were observed for the normal group. The ethanolic extract at a dose of 500 mg/kg body weight showed higher activities of SOD (2.18±2.15) and CAT (3.53±0.09) compared with the aqueous extract at the same dose, and the standard control (LAN) at a dose of 25 mg/kg body weight. However, the standard control (RAN) at a dose of 30 mg/kg body weight exhibited higher activities of SOD and CAT compared with the both extracts of *Commiphora africana* Gum resin.

Table 6: Effects of Aqueous and Ethanolic Extracts of *Commiphora africana* Gum resin on Superoxide dismutase (SOD) and Catalase (CAT) Activities in Stomach tissues of Indomethacin-Induced Gastric Damaged Rats

Groups	Dose (mg/kg body weight)	SOD (mmol/min/mg tissue)	CAT (mmol/min/mg tissue)
IND + AE			
1	250	1.36±1.15[a]	2.75±0.07[a]
2	500	1.62±1.91[b]	2.84±0.02[b]
IND+ EE			
3	250	1.97±1.30[c]	3.11±0.03[c]
4	500	2.18±2.15[d]	3.53±0.09[d]
NORMAL			
5	-	3.11±1.04	3.95±0.18
IND			
6	25	1.21±1.71	1.05±0.02
IND+ RAN			
7	25	1.89±1.30	2.87±0.06
IND+LAN			
8	30	2.54±1.07	3.61±0.01

Key: AE-Aqueous extract of *Commiphora africana* gum resin, EE- Ethanol extract *Commiphora africana* gum resin, IND-Indomethacin, LAN- Lansoprazole and RAN- Ranitidine. Results are expressed as mean±SD (n=4). a, b, c, d-Significantly different from IND group at $P<0.05$

Table 7 shows the effect of aqueous and ethanolic extracts of *Commiphora africana* gum resin on malondialdehyde (MDA) levels in stomach tissues of the experimental animals. The lowest values of MDA were recorded for the normal (1.25±0.09) and LAN (1.35±0.82) groups. The ethanolic extract at both doses, recorded lower levels of MDA when compared with the aqueous extract. The highest level of MDA was observed in the negative control.

Table 7: Effects of Aqueous and Ethanolic Extracts of the of *Commiphora africana* Gum resin on Malondialdehyde (MDA) levels in Stomach tissues of Indomethacin-Induced Gastric Damaged Rats

Groups	Dose (mg/kg body weight)	MDA(µmol MDA/mg tissue)
IND + AE		
1	250	3.80 ± 1.65^{a}
2	500	3.44 ± 0.26^{b}
IND+ EE		
3	250	3.10 ± 1.10^{c}
4	500	2.90 ± 1.49^{d}
NORMAL		
5	-	1.25 ± 0.09
IND		
6	25	7.75 ± 1.36
IND+ RAN		
7	25	3.47 ± 1.41
IND+ LAN		
8	30	1.35 ± 0.82

Key: AE-Aqueous extract of *Commiphora africana* gum resin, EE- Ethanol extract *Commiphora africana* gum resin, IND-Indomethacin, LAN- Lansoprazole and RAN- Ranitidine. Results are expressed as mean±SD (n=4). a,b,c,d-Significantly different from IND group at $P<0.05$

DISCUSSION

The use of non-steroidal anti-inflammatory drug (NSAIDs) is considered the major risk factor in gastric ulcer. The ulcerogenic activity of NSAIDs is related to their ability to inhibit endogenous prostaglandins (PG) synthesis because of inhibition of COX-1 and COX-2 therefore causing gastric mucosal damage as a result of inhibition of epithelial cell proliferation in the ulcer margin which is critical reepithelization of ulcer crater [22]. There has been considerable interest in finding natural antioxidant from plant materials to replace synthetic ones for effective management of therapeutic drug toxicity such as in gastric ulcer [23].

The Phytochemical screening performed in the present study demonstrated that the aqueous extract of *Commiphora africana* gum resin constitutes alkaloids, cardiac glycosides, saponins, terpenoids, tannins, flavonoids, phenols and reducing sugars. Whereas, the constituent present in the ethanol extract were the same as Aqueous extract except for two constituents found inclusive which are Anthranoids and Anthraquinones which are absent in the aqueous extract. Among these secondary compounds, Saponins, Terpenoids and flavonoids were found to be in high concentration in Aqueous and ethanolic extracts. These three secondary compounds are referred to as antiulcer compound [24]. This phytochemical composition of *Commiphora africana* gum extracts could explain the ulcer activity of the gum extract which was detected in this present study. Moreover, several plants

containing high amount of saponins have been shown to possess antiulcer activity [25] probably acting as activator of mucus membrane protective factors [26]. Terpenoids are widespread class of secondary compounds with several pharmacological activities including anti-inflammatory effect in rat paw edema model [27] and antiulcer activities [28]. Also, gastroprotective effect of flavonoids has been previously reported [29]. Free radical scavenging ability of flavonoids has been reported to protect the gastro intestinal tract from ulcerative and erosion lesion [30].

Indomethacin capsule, a representative of family NSAIDs that cause gastric ulcer and this effect is related to the ability of this agent to suppress prostaglandin synthesis. Therefore, making the gastric mucosa more susceptible to gastric damage. This leads to neutrophil infiltration and free radicals are released during the damage. In this present study Pretreatment of indomethacin induced gastric damage in albino rats with aqueous and ethanolic extracts significantly ($P<0.05$) reduced the gastric lesion of the stomach tissues with preventive index of 47.8%, 53.1%, 61.3% and 74.3% at a dose of 250 and 500mg/kg body weight of the aqueous and ethanolic extracts respectively in the experimental animals compared to positive control 65.2% for ranitidine. Highest gastroprotective effect 74.3% was shown by the ethanolic extract at a dose of 500mg/kg body weight this experimental result is in line with previously published by [31].

Acute toxicity via LD_{50} study revealed that the extracts were safe at 5000mg/kg body weight. In this present study, Catalase activity (CAT) and Superoxide dismutase activity (SOD) level were significantly decreased ($p<0.05$), whereas there was significant ($p<0.05$) increase in Malondialdehyde (MDA) activity of IND – Induced gastric rats stomach tissue and serum compared to the pretreated groups and healthy groups. Decrease in SOD and CAT activity could be due to presence of overproduction of free radical due to the damaged tissues. A high level of free radicals has been found to destroy cellular antioxidant enzymes and compromising the production of this first line defense enzymes, therefore reducing their level in the IND-induced untreated gastric damage albino rats [32]. This result is similar with previously published report and that NSAIDs cause increase in the concentration of mucosal H_20_2 and OH^- inhibiting gastric peroxidases [33]. Therefore, the concentration of H_20_2 in the tissues must be reduced immediately; the increasing level of H_20_2 may activate the CAT enzyme in gastric damage. In this study, SOD and CAT activities were significantly elevated ($p<0.05$) and Malondialdehyde (MDA) levels significantly decreased ($p<0.05$) by administration of doses (250 and 500mg) of aqueous and ethanol extracts of gum resin, suggesting that it has ability to restore this enzyme activity and it has been stated in literatures that during healing process, SOD and CAT levels are found to increase, this result is similar with previously published study [34]. The level MDA in Table 5 and 7 also indicate that there was significant increase ($p<0.05$) in lipid peroxidation in IND-induced rat tissues compared to other groups. Indomethacin is known to induce reactive oxygen metabolite in animal models which may contribute to mucosal injury [35]. These free radicals also damage the cellular antioxidant enzymes (CAT, SOD and others), acting as first line cellular defense against antioxidant injury.

This might lead to aggravated tissue damage during ulceration [36]. This present experimental result is in line with previous data (27, 29, 31). Indomethacin-induced gastric ulceration was accompanied with severe oxidative stress in gastric tissue causing damage to key biomolecules such as lipids. This was apparent from stimulated lipid oxidation leading to accumulation of Malondialdehyde.

As shown in this study, RAN and LAN treatment significantly reverted ($p<0.05$) the indomethacin –induced change in MDA levels along with significant increase ($p<0.05$) in CAT level suggest decreased lipid peroxidation and antioxidant activity of LAN and RAN. LAN and RAN are anti-secretory drugs, although they have different mode of action and they have often been reported to possess antioxidant and immunosuppressive action, which may be responsible for its anti-ulcerogenic activity [37, 38], although in this present study, LAN was found to be more effective than RAN. In Table 5 and 7, EE at 500mg dose provided a marked suppression of oxidative damage due to its excellent radical scavenging capacity, it brought MDA level close to normal level than RAN. In contrast to the MDA level in Table 5 and 7 LAN was more effective in suppressing oxidative damage compared to other groups. This study also shows that all extracts of *Commiphora africana* gum possess strong antioxidant activity (Table 2). Based on the present study, EE and AE possess an enhancing effect on mucosal antioxidant defense systems against oxidative damage in which EE at 500mg/kg body weight possess the highest gastroprotective and antioxidant activities.

CONCLUSION

This study showed that, the aqueous and ethanolic extracts of *Commiphora africana* gum resin possess potent gastroprotective and antioxidant activities. Therefore, the aqueous and ethanolic extracts of *Commiphora africana* gum resin are potential sources of new plant-based gastroprotective and antioxidant agents that may be considered as supplement to NSAIDs against gastric ulcer.

Acknowledgments

The authors are thankful to the Department of Biochemistry, Federal University of Technology Minna, for the laboratory space and also the expertise of the Laboratory Technologists during the bench work.

REFERENCES

1. Yeoman, M. D. and Naesdal, J. (2008). Ulcer Definition in NSAIDs Ulcer prevention trials. *Aliment Pharmacological Therapy*, 27(6):465-472.

2. Malyshenko, O. S., Beloborodova, E. T., Vavilov, A. M., Lomivorotova, G. V and Kasperskala, V. I (2005). Impact of Age and Type of Behaviour on the Courser of Ulcer disease. *Ter Arkh*, 77(2): 28-31.

3. Kim, J. M. (2008). Nsaids- induced gastroenterpathy. *Korean journal of Gastroenterology*, 52(3): 134-141.

4. Hanus, L.O., Rezanka, T., Dembitsky, V. M and Moussaleff, A. (2005). Myrrh Commiphora Chemistry. *Journal of Biomedical paper*, 149: 3-28.

5. Ezekiel, I., Mabrouk, M. A., Ayo, J. O., Goji, A. D.T., Okanachi, A. O., Mohammed, A and Tanko, Y. (2010). Study of Effect of Hydro-ethanolic Extract of *Commiphora africana* (Stem- bark) on Sleeping Time and Convulsion in Mice. *Asian Journal of Medical Sciences*, 2(3): 85-88.

6. Badria, F., Abou-Mohamed, G., El-Mowafy, A., Masoud, A. and Salama, O. (2001). Mirazid: A New Schistosomicidal Drug. *Pharmaceutical Biology (Lisse, Netherlands)*, 39(2): 127-131.

7. Gundidza, M., Mmbengwa, V., Sibambo, S. R., Magwa, M. L., Mushisha, O., Benhura, M. A., Gundidza, E. and Samie, A. (2011). Rheological moisture and ash content analysis of a gum resin from *Commiphora Africana. African Journal of Food Science*, 5(4): 188-193.

8. Massoud, A. M.; El-Ashmawy, I. M.; Nasr, M. A.; Salama, O. M. (2002). Mirazid; a new schistosomicidal agent derived from Myrrh: studies on its influence on male reproductive organs and bile flow. *Alexandria Journal of Pharmaceutical Sciences,* 16(2): 82-87.

9. Al-Mathal, E.M. and Fouad, M.A. (2004). *Commiphora africana* has been shown to have Antiulcerogenic, Anesthetic, Antibacterial and Antifungal Properties. *Journal of the Egyptian Society of Parasitology*, 34(2): 713-20.

10. Aliyu, R., Catsing, D. and Umar, H. S. (2002). Antimicrobial Activity and Phytochemical Screening of the Leaves of *Commiphora africana*. *West Africa Journal of Biological Science*, 13: 75-80.

11. Schmeda-Hirschmann, G and Yesilada, E. (2005). Traditional Medicine and Gastroprotective Crude Drugs. *Journal of Ethanopharmacology*, 100(1-2): 61-66.

12. Sofowora, A. (1993). Medicinal plants and Traditional Medicine in Africa. Spectrum books Ltd, Ibadan, Nigeria. Pp. 289.

13. Trease, G. E and Evans W.C (2002). Pharmacognosy, 15th Edn. Saunders; pp. 214-393.

14. Peskar, B. M., Ehrlich, K. and Peskar, B. A. (2002). Role of ATP-Sensitive Potassium Channels in Prostaglandin Mediated Gastro Protection in the Rat. *Journal of pharmacology and Experimental Therapeutics*, 301(3): 969-974.

15. Hano, J., Bugajski, J. And Danek, L. (1976). Effect of Adrenergic Blockage on Gastric Secretion Altered by Catecholamine in Rats. *Journal of Archives Immunology and Therapeutic Experimental (Warsz)*, 24(4): 507-524.

16. Mishra, H. P. and Fridovich, I. (1972). The Role of Superoxide Anion in the Autoxidation of Superoxide Dismutase. *Journal of Biological Chemistry,* 247(10): 3170-3175.

17. Sun, M. and Zigma, S. (1978). An Improved Spectrophotometric Assay of Superoxide Dismutase based on Epinephrine Antioxidation. *Journal of Analytical Biochemistry*, 90: 81-89.

18. Sinha, K. A. (1972). Colorimetric Assay of Catalase. *Journal of Analytical Biochemistry*. 47: 389-394.

19. Armstron, D. and Browne, R. (1994). The Analysis of Free radicals, lipid Peroxides, Antioxidant enzymes and Compounds Related to Oxidative Stress as Applied to the Clinical Chemistry laboratory. *Journal of free radical diagnostic medicine*, 366: 43-58.

20. Buege, J. A. and Aust, S. D. (1978). Microsomal Lipid Peroxidation. *Methods of Enzymology*, 52: 302-310.

21. Mitsuda, H., Yuasumoto, K., and Iwani, K. (1996). Antioxidation action of indole compounds during the autoxidation of linoleic acid. *Eiyoto shokuryo*, 19: 210-214.

22. Levi, S., Goodlad, R. A. and Lee, C.Y. (1990). Inhibitory Effect of NSAIDs on Mucosal Cell Proliferation Associated with Gastric Ulcer Healing. *Lancet*, 336(8719): 840-843.

23. Pratt, D. E. (1992). Natural Antioxidants from Plant material. In Huang, I.M., Ho, C.T., Lee, C. Y. Editors. Phenolic Compounds in Food and their Effect on Health. New York: *American Chemical Society,* 2(1): 54-72.

24. Lewis, D. A. and Hanson, P. J. (1991). Anti-ulcer drugs of plant origin*. Progress in Medicinal Chemistry,* 28: 201-31.

25. Morikawa, T., Li, N., Nagatomo, A., Matsuda, H., Li, X. And Yoshikawa, M. (2006). Triterpene and Saponins with Gastroprotective Effects from Tea Seed (the seed of *Camellia sinensis*). *Journal of Natural products*, 69(2): 185-190.

26. Saito, H., Lee, Y.M., Takaigi, K. and Kondo, N. (1977). Pharmacological Studies of *Panacis japonica*

Rhizoma. *Journal of chemical and Pharmaceutical Bulletin,* 25(5): 1017-1025.

27. Jung, H., Nam, J., Choi, J., Lee, K. and Park H. (2005). 19α-hydroxy ursane-type Triterpenoids Anti-nociceptive Anti-inflammatory Principles of the Root of *Rosa rugosa*. *Journal of Biological and Pharmaceutical Bulletin,* 28(1): 101-104.

28. Arrietta, J., Benitez, J., Flores, E., Castillo, C., and Navarette, A. (2003). Purification of gastroprotective triterpenoids from the stem bark of Amphipterigium Adstringens; role of prostaglandins, sulfhydryls, nitric oxide and capsaicin sensitive neurons. *Planta Medica*, 69(10): 905-909.

29. Reyes, M., Martin, C., Alarcon de la Lastra, C., Trujillo, J., Toro, M. V. and Ayuso, M. J. (1996). Antiulcerogenecity of the Flavonoids Fraction from *Enca andevalensis and Cabezuda Rivera*. *Journal of Physical Sciences*, 51(7-8): 563-569.

30. Borrelli, F. and Izzo, A. A. (2000). The Plant Kingdom as Source of Anti-Ulcer Remedies. *Journal of Phytotherapy Research*, 1418: 581-591.

31. Halici, M., Odabasoglu, F., Bayir, Y., Odabasoglu, F., Cakir, A., Aslan, A. and Suleyman, H. (2005). Effects of Water Extract of *Usenea longissima* on Antioxidant Enzyme Activity and Mucosal Damage caused by Indomethacin in Rats. *American Journal of Phytomedicine*, 12: 656-662.

32. Vandana, P. and Madhav, S. (2012). Anti-ulcer activity of Ipomoea batatas tuber (sweet potato). *Functional Food in Health and Disease Journal*, 2(3): 48-61.

33. Banajee, R. K. (1990). Non-Steroidal Anti-inflammatory Drugs Inhibit Gastric Peroxidase Activity. *ACTA Journal of Biochemistry and Biophysics,* 1034: 275-279.

34. Malairajan, P., Gopalakrishnan, G., Narasimhan, S. and Jessi Kala Veni., K. (2008). "Evaluation of the anti-ulcer activityof *polyalthia longifolia* (sonn) Thwaites in experimental rats". *Indian Journal of Pharmacology*, 40(3): 126-128.

35. Chattopadhyay, I., Bandypadhyay, V., Biswas K., Malty, P. and Banerjee, R.K. (2006). Indomethacin Inactivates Gastric Peroxidases to Induce Reactive Oxygen Mediated Gastric Mucosal Injury and Curcumarin protects it by Preventing Peroxidase Inactivation and Scarvenging reactive oxygen. *Journal of Free Radical Biology and Medicine*, 40(8): 1397-1408.

36. El-Missiry, M. A., El-Sayed, T. H. and Othman, A. I. (2001). Protection of Metal Complexes with SOD Mimetric Activity against Oxidative Gastric Injury Induced by Indomethacin and Ethanol in Rats. *Annals of Clinical Biochemistry*, 38: 694-700.

37. Lapenna, D., De-Giola, S., Mezzetti, A., Grossi, L., Festi, D., Marzio, l. and Cuccurullo, F. (1994). H_2 Receptor Antagonists are Scarvengers of Oxygen Radicals. *European Journal of Clinical Investigation,* 24(7): 476-481.

38. Ardestani, S. K., Jannlow, M. M., Kariminia, A. and Tavakoli, Z. (2004). Effect of Cimetidine and Ranitidine on Lipid Profile and Lipid Peroxidation in Gamma Irradiated Mice. *ACTA Journal of Medical Iran*, 42: 198-204.