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*Full Length Research Paper*

# Anti-ulcerogenic evaluation of the fractions of the ethanol layer of the chloroform-ethanol extract of the leaves of *Dacryodes edulis*

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**In traditional medicine, the leaves of *Dacryodes edulis* are utilised in the treatment of gastric ulcer and hence, the chloroform and ethanol layers of the chloroform-ethanol extract of the leaves of *D. edulis* were evaluated for their anti-oxidant vitamin contents and proximate compositions. Based on preliminary investigations, the more promising layer (ethanol layer) was further fractionated and the effects of the fractions on ulcer index and curative ratio of diclofenac-ulcerated Wistar rats were determined using standard methods. The anti-oxidant vitamin contents of the chloroform and ethanol layers showed the following: vitamins A (1.83 ± 0.08 and 1.79 ± 0.08 µg/100 g), C (0.62 ± 0.05 and 0.79 ± 0.03 mg/100 g) and E (1.17 ± 0.07 and 1.04 ± 0.05 mg/100 g) respectively. The proximate compositions of the chloroform and ethanol layers were: moisture (3.15 ± 0.11 and 4.08 ± 0.18%), crude fibre (5.41 ± 0.29 and 5.29 ± 0.27%), ash (5.09 ± 0.27 and 4.12 ± 0.18%), crude fats (4.43 ± 0.13 and 3.98 ± 0.12%), crude proteins (17.24 ± 0.43 and 17.80 ± 0.29%) and carbohydrates (64.68 ± 1.40 and 64.73 ± 1.35%) respectively. Each of the chloroform and ethanol layers of the chloroform-ethanol extract was found to be safe at a dose as high as 5000 mg/kg body weight (b.w). At the two doses (100 and 200 mg/kg b.w), the n-hexane, chloroform, ethyl acetate and methanol fractions of the ethanol layer dose-dependently decreased and increased the ulcer indices and curative ratios respectively of the rats in the test groups when compared with those of the rats in the ulcer-untreated group albeit these effects were not significant (p>0.05) except those of the methanol fraction at the dose of 200 mg/kg b.w. Results of the methanol fraction (200 mg/kg b.w only) were comparable to those of the standard anti-ulcer drug, ranitidine at the dose of 150 mg/kg b.w. The data of this study indicate that the methanol fraction of the ethanol layer of the chloroform-ethanol extract of the leaves of *D. edulis* possess remarkable anti-ulcerogenic effect and might serve as source of novel drugs for ulcers in future.**

**Key words:** *Dacryodes edulis*, anti-oxidant vitamins, proximate composition, ulcer index and curative ratio.

## INTRODUCTION

A peptic ulcer is a sore on the lining of the stomach or duodenum, the beginning of the small intestine. Less commonly, a peptic ulcer may develop just above the stomach in the oesophagus, the tube that connects the mouth to the stomach (Verma et al., 2011). Peptic ulcer affects a large proportion of the world's population and is

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**Figure 1.** A young *D. edulis* tree showing the stem, the leaves and the fruits.

induced by several factors including stress, smoking, nutritional deficiencies and ingestion of non-steroidal anti-inflammatory drugs (Devi et al., 2011). A number of drugs are available for the treatment of peptic ulcers in current medicine as histamine II ($H_2$) receptor antagonist, proton pump inhibitors or cytoprotective agents such as sucralfate inter alia but clinical evaluation of these drugs indicates high incidences of relapse, side effects (arrhythmias, gynaecomastia, impotence, arthralgia, hypergastrinaemia and haemopoeitic changes) and drug interactions (Ahmad et al., 2013). Several studies have shown the beneficial effects of medicinal plants on ulcer (Wandale et al., 2012; Ahmad et al., 2013). Many natural products and modern synthetic drugs have been used to treat gastric ulcer but so far, a complete cure has not been discovered. The exploration of new anti-ulcer drugs has therefore, continued to be a field of active research.

*Dacryodes edulis* (Figure 1) is of the family, Burseraceae. It is a versatile plant in African ethnomedicine as its various parts are utilised in the treatment of array of ailments. Its bark has long been used to cicatrise wound in Gabon (Walker and Silans, 1961). In this case, the bark is pulped and then applied directly to the wound. In Democratic Republic of Congo, the plant is employed in the treatment of diverse diseases. The decoction of the bark is taken orally to treat leprosy. It is also used as a gargle and mouth-wash to treat tonsillitis. The bark is comminuted with meleguetta pepper to cure dysentery, anaemia, spitting blood and as an emmenagogue; when mixed with palm oil, it is applied topically to relieve pains, debility, stiffness and skin diseases (Bouquet, 1969). The leaves are chewed with kolanut as an anti-emetic. The leaf sap is used as an ear drop to treat ear trouble while the leaf decoction is prepared to produce vapour that treats fever and headache (Bouet, 1980). In Congo Brazzaville, the leaves are boiled with those of *Lanata camara*, *Cymbopogon citratus* and *Persea americana* in water to form a decoction for treating malaria. A steam bath can also be taken from the decoction to treat the same

ailment. Boiling the leaves with those of *P. americana* alone can be used to treat headache, antalgic and cephalgy (Diafouka, 1997). Recently, Jiofack et al. (2010) reported that the leaves are made into plaster to treat snakebite in southwest Cameroon. The bark resin is used in Nigeria to treat parasitic skin diseases and jiggers (Dalziel, 1937; Hutchinson et al., 1963). When applied in lotions and creams, the resin smoothens and protects the skin (Ekpa, 1993). The aroma of the resin when liberated through burning is believed to ward off evil spirit in Nigeria (Sofowora, 2008). The leaves are often crushed and the juice released to treat generalised skin diseases such as scabies, ringworm, rashes and wound while the stem or stem twigs are employed as chewing sticks for oral hygiene (Igoli et al., 2005; Ajibesin et al., 2008). The leaves boiled singly or with lemon grass or pulp oil are employed in the treatment of peptic ulcer (Omonhinmin, 2014). In this study, the anti-ulcerogenic properties of the fractions of the ethanol layer of the chloroform-ethanol extract of the leaves of *D. edulis* are reported.

## MATERIALS AND METHODS

Fresh leaves of *D. edulis* were plucked from their tree at Government Reserved Area (GRA), Nsukka Local Government Area of Enugu State, Nigeria. The leaves were identified by Mr. Alfred Ozioko of Bioresource Development and Conservation Programme (BDCP) Research Centre, Nsukka.

### Extraction procedure for the chloroform-ethanol extract

A known weight (1200 g) of the pulverised *D. edulis* leaves was macerated in 5 volumes (w/v) of chloroform-ethanol (2:1) at room temperature for 24 h. The mixture was filtered with Whatman No 1 filter paper. The filtrate of the macerate was vigorously shaken with 20% distilled water to obtain two layers. The upper layer (ethanol layer) was separated from the lower layer (chloroform layer). The ethanol and the chloroform layers were concentrated in a rotary evaporator, air-dried, weighed and stored in the refrigerator.

### Vacuum liquid chromatography (VLC) of the ethanol layer

Twenty-five grams (25 g) of the ethanol layer was purified by vacuum liquid chromatography using silica gel (230-400 mesh, 3.0 x 30 cm, 500 g) as the stationary phase and eluted with n-hexane (1500 ml), chloroform (3500 ml), ethyl acetate (2500 ml) and methanol (2500 ml) to obtain the n-hexane, chloroform, ethyl acetate and methanol fractions respectively.

### Anti-oxidant vitamin analyses

The concentrations of vitamins A, C and E were determined for the chloroform and ethanol layers of the chloroform-ethanol extract of the leaves of *D. edulis* using the methods described by Pearson (1976).

### Proximate analyses

Percentage composition of moisture, crude fibre, ash, crude fats, crude proteins and carbohydrates were determined for the chloroform and ethanol layers of the chloroform-ethanol extract of the leaves of *D. edulis* using the methods described by AOAC (1990).

### Animals

Adult male Wistar rats of between 8 and 12 weeks old with an average weight of 125 ± 25 g and albino mice weighing 30 ± 5 g were obtained from the Animal house of the Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka. The rats were acclimatised for one week under a standard environmental condition with a 12 h- light and dark cycle and maintained on a regular feed and water *ad libitum*. The Principles of Laboratory Animal Care were adhered to. The University Animal Research Ethical Committee approved the experimental protocol.

### Chemicals and reagents

The chemicals used for this study were of analytical grade. They included the following: diclofenac (Evans Medical Plc., Nigeria), ranitidine (Evans Medical Plc., Nigeria), absolute ethanol, chloroform, acetone, diethyl ether, chloroform-acetic anhydride, TCA-chloroform, oxalic acid, boric acid, methyl red, ferric chloride, α-α-dipyridine, concentrated and dilute $H_2SO_4$, loctanol, sodium sulphate, KOH, NaOH and Indolephenol reagent (Sigma-Aldrich, Inc., St. Louis, USA).

### Acute toxicity study

The acute toxicity and lethality ($LD_{50}$) of the chloroform and ethanol layers of the chloroform-ethanol extract were determined using mice according to slightly modified method of Lorke (1983).

### Gastric ulcer index and curative ratio

The methods described by Rezq and Elmallh (2010) were employed in these studies.

### Statistical analysis

The data obtained were subjected to one-way analysis of variance (ANOVA). Significant differences are observed at $p<0.05$. The results are expressed as means ± standard errors of means (SEM). The analysis was done using the computer software known as Statistical Product and Service Solutions (SPSS), version 18.

## RESULTS

### Anti-oxidant vitamin contents of the chloroform and ethanol layers of the chloroform-ethanol extract of the leaves of *D. edulis*

Table 1 shows that the ethanol layer contained the higher concentration of vitamin C (0.79 ± 0.03 mg/100 g) while the chloroform layer contained the higher concentrations of vitamins A (1.83 ± 0.08 µg/g) and E (1.17 ± 0.07 mg/100 g).

**Table 1.** Anti-oxidant vitamin contents of the chloroform and ethanol layers of the chloroform-ethanol extract of the leaves of *D. edulis.*

| Anti-oxidant vitamins | Chloroform layer | Ethanol layer |
|---|---|---|
| A (µg/100 g) | 1.83 ± 0.08 | 1.79 ± 0.08 |
| C (mg/100 g) | 0.62 ± 0.05 | 0.79 ± 0.03 |
| E (mg/100 g) | 1.17 ± 0.07 | 1.04 ± 0.05 |

Values are expressed as means of three determinations ± SEM.

**Table 2.** Proximate compositions of the chloroform and ethanol layers of the chloroform-ethanol extract of the leaves of *D. edulis.*

| Proximate constituents | Compositions (%) | |
|---|---|---|
| | Chloroform layer | Ethanol layer |
| Moisture | 3.15 ± 0.11 | 4.08 ± 0.18 |
| Crude fibre | 5.41 ± 0.29 | 5.29 ± 0.27 |
| Ash | 5.09 ± 0.27 | 4.12 ± 0.18 |
| Crude fats | 4.43 ± 0.13 | 3.98 ± 0.12 |
| Crude proteins | 17.24 ± 0.43 | 17.80 ± 0.29 |
| Carbohydrates | 64.68 ± 1.40 | 64.73 ± 1.35 |

Values are expressed as means of three determinations ± SEM

**Proximate compositions of the chloroform and ethanol layers of the chloroform-ethanol extract of the leaves of *D. edulis***

The ethanol layer contained higher percentage of crude proteins (17.80 ± 0.29%) while the chloroform layer contained higher percentages of crude fibre (5.41 ± 0.29%), ash (5.09 ± 0.27%) and crude fats (4.43 ± 0.13%) as shown in Table 2.

**The acute toxicity and lethality ($LD_{50}$) of the chloroform and ethanol layers of the chloroform-ethanol extract of the leaves of *D. edulis***

There was no lethality or any sign of toxicity in the four groups of mice that received 10, 100 and 1000 mg/kg body weight of the chloroform and ethanol layers and 5 ml/kg body weight of the vehicle respectively at the end of the first phase of the study. At the end of the second phase of the study, no death was recorded in the groups of mice that received 1600, 2900 and 5000 mg/kg body weight of the chloroform and ethanol layers within 24 h of administration.

**Effects of the graded doses of the n-hexane, chloroform, ethyl acetate and methanol fractions of the ethanol layer of the chloroform-ethanol extract of the leaves of *D. edulis* on ulcer index**

The ulcer index of the rats in the ulcer-untreated group (group 2) was significantly ($p<0.05$) higher (6.02 ± 0.31 mm) when compared to the value (0.00 ± 0.00 mm) obtained for the rats in the normal control group (group 1) which received 5 ml/kg body weight of normal saline only. The ulcer indices of the rats in groups 3 (1.02 ± 0.06 mm) and 11 (2.89 ± 0.15 mm) were significantly ($p<0.05$) lower than that of the rats in group 2 (6.02 ± 0.31 mm). There were however, no significant ($p>0.05$) differences between the ulcer indices of the rats in groups 4 (5.91 ± 0.30 mm), 5 (5.68 ± 0.29 mm), 6 (5.22 ± 0.30 mm), 7 (4.01 ± 0.30 mm), 8 (5.79 ± 0.29 mm), 9 (5.33 ± 0.31 mm) and 10 (4.15 ± 0.32 mm) and the value obtained for the rats in group 2 (6.02 ± 0.31 mm) as shown in Figure 2.

**Effects of the graded doses of the n-hexane, chloroform, ethyl acetate and methanol fractions of the ethanol layer of chloroform-ethanol extract of the leaves of *D. edulis* on curative ratio**

Table 3 shows that the curative ratios of the rats in groups 3 (83.06 ± 7.05%) and 11 (51.99 ± 5.38%) were significantly ($p<0.05$) higher than that of the rats in group 2 (0.00 ± 0.00%). There were however, no significant ($p>0.05$) differences between the curative ratios of the rats in groups 4 (1.83 ± 0.22%), 5 (5.65 ± 0.54%), 6 (13.29 ± 1.37%), 7 (33.39 ± 3.03%), 8 (3.82 ± 0.39%) 9 (11.46 ± 1.28%) and 10 (30.40 ± 2.95%) and that of the rats in group 2 (0.00 ± 0.00%).

## DISCUSSION

The amounts of the anti-oxidant vitamins in the chloroform and ethanol layers of the chloroform-ethanol extract of the leaves of *D. edulis* were: vitamins A (1.83 ± 0.08 and 1.79 ± 0.08 µg/100 g), C (0.62 ± 0.05 and 0.79 ± 0.03 mg/100 g) and E (1.17 ± 0.07 and 1.04 ± 0.05 mg/100 g) respectively. This means that the vitamins A and E present in the chloroform and ethanol layers of the chloroform-ethanol extract of the leaves of *D. edulis* were more in the chloroform layer than in the ethanol layer whereas vitamin C was more in the ethanol layer than in the chloroform layer. The anti-ulcerogenic properties of the fractions as seen in the present study might be, in part, due to the amount of the anti-oxidant vitamins in the ethanol layer of the chloroform-ethanol extract of the leaves of *D. edulis.* While the optimal nutrient intake to promote wound healing is unknown, increased needs for carbohydrates, proteins, zinc and vitamins A, C and E have been documented (Scholl and Langkamp, 2001). Vitamin C aids in wound healing by increasing collagen synthesis, neutrophil function and angiogenesis. Collagen production also serves to produce a barrier to pathogens (Scholl and Langkamp, 2001).

Proximate compositions of the chloroform and ethanol layers of the chloroform-ethanol extract of the leaves of *D. edulis* were: moisture (3.15 ± 0.11 and 4.08 ± 0.18%),

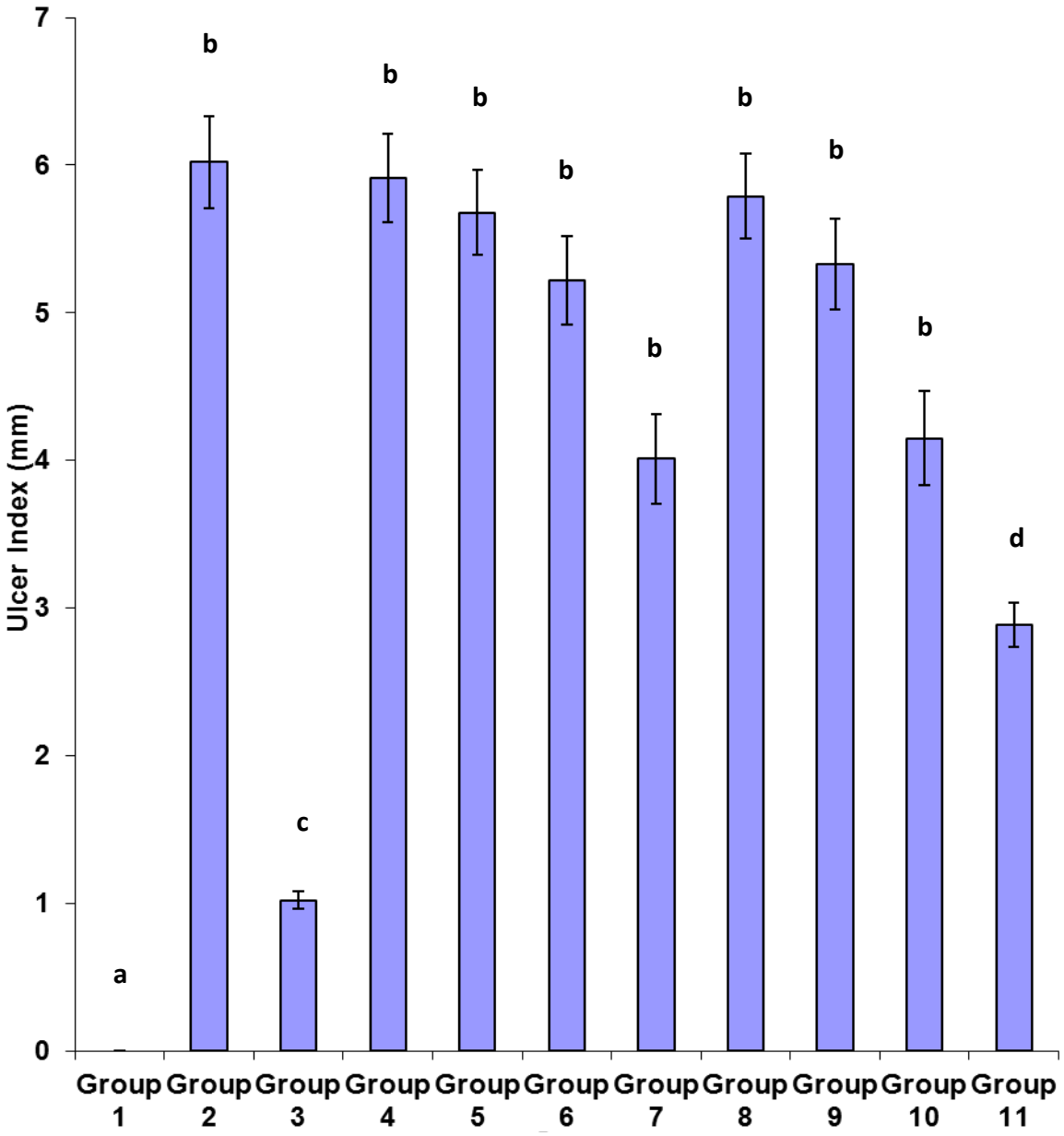



**Figure 2.** Effects of the n-hexane, chloroform, ethyl acetate and methanol fractions of the chloroform-ethanol extract of the leaves of *D. edulis* on ulcer index [values for groups with different alphabets are significantly ($p<0.05$) different]. Group 1: 5 ml/kg body weight (b.w) of normal saline only (Normal control); Group 2: 5 ml/kg b.w of normal saline + 200 mg/kg b.w of diclofenac (Positive control); Group 3: 150 mg/kg b.w of ranitidine + 200 mg/kg b.w of diclofenac (Standard control); Group 4: 100 mg/kg b.w of the n-hexane fraction + 200 mg/kg b.w of diclofenac; Group 5: 200 mg/kg b.w of the n-hexane fraction + 200 mg/kg b.w of diclofenac; Group 6: 100 mg/kg b.w of the chloroform fraction + 200 mg/kg b.w of diclofenac; Group 7: 200 mg/kg b.w of the chloroform fraction + 200 mg/kg b.w of diclofenac; Group 8: 100 mg/kg b.w of the ethyl acetate fraction + 200 mg/kg b.w of diclofenac; Group 9: 200 mg/kg b.w of the ethyl acetate fraction + 200 mg/kg b.w of diclofenac; Group 10: 100 mg/kg b.w of the methanol fraction + 200 mg/kg b.w of diclofenac; Group 11: 200 mg/kg b.w of the methanol fraction + 200 mg/kg b.w of diclofenac.

crude fibre (5.41 ± 0.29 and 5.29 ± 0.27%), ash (5.09 ± 0.27 and 4.12 ± 0.18%), crude fats (4.43 ± 0.13 and 3.98 ± 0.12%), crude proteins (17.24 ± 0.43 and 17.80 ± 0.29%) and carbohydrates (64.68 ± 1.40 and 64.73 ± 1.35%), respectively. This implies that the crude proteins and carbohydrates present in the chloroform and ethanol layers of the chloroform-ethanol extract of the leaves of *D. edulis* were more in the ethanol layer than in the chloroform layer. The anti-ulcer properties of the fractions as evidenced in this study could also be, in part, due to the presence and/or amounts of some of the proximate constituents in the ethanol layer of the chloroform-ethanol

**Table 3.** Effects of the graded doses of the n-hexane, chloroform, ethyl acetate and methanol fractions of the ethanol layer of the chloroform-ethanol extract of the leaves of *D. edulis* on curative ratio.

| Groups | Curative ratios (%) |
|---|---|
| 1 | $100.00 \pm 0.00^{a}$ |
| 2 | $0.00 \pm 0.00^{b}$ |
| 3 | $83.06 \pm 7.05^{c}$ |
| 4 | $1.83 \pm 0.22^{b}$ |
| 5 | $5.65 \pm 0.54^{b}$ |
| 6 | $13.29 \pm 1.37^{b}$ |
| 7 | $33.39 \pm 3.03^{b}$ |
| 8 | $3.82 \pm 0.39^{b}$ |
| 9 | $11.46 \pm 1.28^{b}$ |
| 10 | $30.40 \pm 2.95^{b}$ |
| 11 | $51.99 \pm 5.38^{d}$ |

Values for groups with different superscript letters are significantly ($p<0.05$) different.

extract of the leaves of *D. edulis*. Carbohydrates and proteins are important in the wound healing process or recovery from ulcers (Crowe and Brockbank, 2009). While proteins are required for all stages of the wound healing process including fibroblast proliferation, collagen synthesis, angiogenesis and immune function, adequate intake of carbohydrates prevents the utilisation of the proteins as energy substrates (Crowe and Brockbank, 2009).

Acute toxicity test on the chloroform and ethanol layers of the chloroform-ethanol extract of the leaves of *D. edulis* using mice showed that each of the layers administered to the mice at a dose as high as 5000 mg/kg body weight by oral route within twenty-four hours of observation, had no fatal effect on the mice which indicates that the chloroform and ethanol layers have low toxicity at high doses when administered by oral route.

Evaluation of the effects of the fractions of the ethanol layer of the chloroform-ethanol extract of the leaves of *D. edulis* on gastric ulcer experimentally induced with diclofenac in rats showed that, they dose-dependently decreased and increased the ulcer indices and curative ratios respectively of the treated rats with the effects of the 200 mg/kg body weight of the methanol fraction being the most remarkable. These indicate that the leaves of *D. edulis* contain anti-ulcerogenic agents. The observations that the fractions of the ethanol layer decreased and increased the ulcer indices and curative ratios respectively of the treated rats might be attributed to the anti-oxidant vitamin content and proximate composition of the ethanol layer of the chloroform-ethanol extract of the leaves of *D. edulis* as shown by the results of their analyses. Also, the finding that diclofenac induced ulcer in all the treated rats is in accord with the reports of Verma et al. (2011), Santos et al. (2012), Wandale et al. (2012) and Khan and Khan (2013). The mechanism by which diclofenac and other NSAIDs cause injury to the gastric mucosa is mainly due to the inhibition of cyclooxygenase and suppression of prostaglandin-mediated effects on mucosal protection. Besides, it has been proposed that neutrophil and oxygen radical-dependent microvascular injuries may be important processes that lead to mucosal damage in response to NSAID administration (Wallace, 2000). These agents cause the activation of neutrophils and their adherence to the vascular endothelium, hence, blocking capillaries and reducing local gastric blood flow. NSAIDs inhibit COX and thereby reduce the intrinsic ability of the mucosa to resist injury induced by endogenous and exogenous aggressors (Wandale et al., 2012). Administration of exogenous prostaglandins has also been shown to decrease or prevent gastric damage induced by NSAIDs (Wallace, 2001). Therefore, the decreases and increases in the ulcer indices and curative ratios respectively of the rats treated with the fractions of the ethanol layer of the chloroform-ethanol extract of the leaves of *D. edulis* in this study are in parts, indications of the anti-ulcerogenic properties of the leaves of *D. edulis*.

In conclusion, the findings of this study lend credence to the use of the leaves of *D. edulis* in traditional medicine for the treatment of gastric ulcer.

## Conflict of Interests

The authors have not declared any conflict of interests.

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