Evaluation of Methanol Stem-bark Extract of *Ximenia americana* Linn (Olacaceae) for Phytoconstituents and Gastroprotection in Rats

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**Background:** The Hausa/Fulonis in northern Nigeria and other tribal-communities use different parts of *Ximenia americana* for several ailments such as malaria, infectious diseases, fever and constipation with the stem-bark used for gastric ulcers. The plant is one of eight species of Olacaceae family that grows mostly in the tropical countries and has common names as sour or monkey plum, known in the Northern part of Nigeria as ‘Tsada’, in Eastern part as ‘Anya Nwona’ and in Western part as ‘Igo’. The study was an attempt to validate the purported ethnomedicinal use of the stem-bark of the plant in gastric ulcer.

**Objective:** This study investigated the phytochemical constituents of stem-bark extract and its gastro-protective potential.

**Methods:** Lorke’s acute toxicity-test for oral median lethal-dose (LD₅₀) estimation and preliminary phytochemical screening were conducted. The antiulcer effect was evaluated on indomethacin and ethanol induced ulcer-models using Wistar rats in two sets of 5-groups of 5 each, starved for 24h. Oral body-weight normal saline(1ml/kg), standard-drug (100mg/kg cimetidine or misoprostol) for the respective models and extract-doses (250, 500 and 1000mg/kg) administered for 30 minutes prior to body-weight 6h indomethacin ulceration or 1h absolute ethanol(1ml) were used. Lesions and larger-diameter (≥3mm) ulcer-spots in isolated-stomachs of euthanized-rats were counted.

**Results:** The extract contained alkaloids, anthraquinones, carbohydrates, flavonoids, saponins, steroidal-glycosides, tannins and terpenoids and significantly (p≤0.05) and dose-dependently reduced mean ulcer-spots as with cimetidine and misoprostol. Reduction was significant only for 500 and 1000mg/kg extract-doses. Nine severe ulcer-spots in normal control rats against 3 in 250 and 500mg/kg groups and none in the 1000mg/kg or cimetidine occurred with indomethacin ulcerogen. Severe ulcer-spots were not found with ethanol-ulcerogen even in normal-control rats.

**Conclusion:** This study therefore, supported the folkloric use of *Ximenia americana* stem-bark in ulcer management.

**Keywords:** *Ximenia americana*, gastric-ulcer, indomethacin, ethanol, anti-ulcer, gastroprotection

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1. **Introduction**

Plants and herbs are now increasingly being used to maintain health and cure many ailments of both mankind and animals (WHO, 2013). In addition to essential nutrients, food plants also contain natural secondary metabolites often referred to as phytochemicals or phytoalexins (Hasler and Blumberg, 1999). The
medicinal benefits and toxicity potentials of plants are related to these active chemical contents and which are often not established alongside their essential nutrients. These natural organic constituents which vary in type and amount with the various parts of a plant are usually identified by phytochemical screening (Ekor, 2013). Discovering the constituents responsible for the medicinal effect of each plant part helps in selecting the part with most of the constituents for optimal medicinal use and/or effect of the plant.

Peptic ulcers are parietal painful sores that occur in the epithelial lining of the upper gut (stomach, oesophagus, or small intestine) due to excess gastric acid secretion (Abbas and Kumar, 2010). Stomach ulcer is more harmful and if improperly treated often results in life-threatening complications of medical emergency and hospitalisations including abnormal bleeding, perforative holes in the walls of stomach and duodenum and/or gastric outlet obstruction that may require gastric surgery (Bardhan and Royston, 2014). This plant had been evaluated for anti-trypansosomal activity (Maikai et al, 2008), anti-microbial effect (Ogunleye and Ibitoye, 2003) and analgesic activity (Siddaiah et al, 2009; Soro et al, 2009). The present study is aimed at establishing the phytochemical constituents of the methanol stem-bark extract of the plant and its efficacy in gastric ulcer.

2. Methods

2.1 Collection and identification of plant material

The whole *Ximenia americana* plant collected in June, 2014 from Tashan-yari area in Makarfi Local Government Area of Kaduna State (June, 2015) was identified and authenticated by a taxonomist, Mallam Sanusi of the Department of Biological Sciences, Ahmadu Bello University, Zaria by comparing it with an existing voucher number 099.

2.2 Experimental animals

Adult male Wistar rats (150-200 g) obtained from the Animal House Facility of the Department of Pharmacology and Therapeutics, Faculty of Pharmaceutial Sciences, Ahmadu Bello University, Zaria were used for the study. The animals were allowed to acclimatise to laboratory conditions with food and water provided *ad-libitum* except when fasting is required.

All experiments were carried out in compliance with the institution's Ethics in Animal Handling in accordance with the regulation for the Care and Use of Laboratory animals as accepted internationally (NIH, rev 1996).

2.3 Drugs and chemical reagents

All drugs and chemicals used in this study were of analytical grade and include: Misoprostol (Cytotec Pfizer Inc. IL, USA); Cimetidine (Pauco Pauco Pharmaceutical Ind. Nig Ltd.); Indomethacin (M/S Aphantee Pharm. Nig. Ltd); Ethanol and methanol, formaldehyde, Chloroform (BDH Chemical Ltd. Poole England). All other chemicals used were also products of BDH, England; Mayer and Baker, England and Merck, Darmstand, Germany.

2.4 Preparation of plant extract

The stem bark of *Ximenia americana* were scraped off the stems of the plant and dried under shade until a constant weight was obtained and then crushed into powder. Soxhlet extraction method was used whereby the obtained powdered material (759 g) was packed into a filter thimble underneath a conical flask containing the solvent (methanol) and which on heating evaporated and condensed through the condenser into the thimble for 72 h extraction. The mixture was then filtered to obtain the filtrate which was concentrated to dryness using a rotary evaporator over water bath at 55 °C to obtain the dark-brown methanol extract which was used for the study.

2.5 Determination of phytochemicals

The qualitative phytochemical analyses were carried out according to the methods of Trease and Evans (2002) and the constituents investigated for include alkaloids, anthraquinone carbohydrates, tannins, steroids, terpenoids, saponins, steroidal glycosides.

2.6 Assessment of mucosal cytoprotective effect of *X. americana* stem bark extract for ulcer inhibition in rats

The method of indomethacin ulceration of Djahanguiri (1969) was used. Twenty five (25) rats deprived of food for 24 h were divided into five (5) groups of 5 rats each and the various groups were treated per body weight for 30 minutes with normal saline (1 ml/kg), cimetidine (100 mg/kg) and extract doses of 250, 500 and 1000 mg/kg. Indomethacin (100 mg/kg) was administered to all the groups of rats for 6 h ulcer-induction after which the rats were anaesthetised with chloroform and the abdomen incised to isolate the stomach into 2% v/v formal saline overnight. The stomachs were then cut open along the greater curvature and spread out on filter papers to count the total number of lesions as well as the number of ulcer spots of larger diameter ≥3 mm.

In another group of experiment, the model of ethanol-induced ulcers of Robert (1979) was also used. Twenty five (25) rats deprived of food for 24 h were divided into five (5) groups of 5 rats per group and treated per body weight for 30 minutes with normal saline (1 ml/kg), misoprostol (100 mg/kg) and extract doses of 250, 500 and 1000 mg/kg. Absolute ethanol (1 ml) was then administered to all the rats in each group for ulcer induction of 1 h after which the rats were sacrificed under chloroform anaesthesia and the abdomen incised to isolate the stomachs which were then washed and spread open on filter papers to count the total number of lesions as well as the number of ulcer spots of larger diameter ≥ 3 mm.

2.7 Statistical analyses

Data obtained were presented as tables. The difference between means was analysed using one way analysis of variance (ANOVA). Dunnett post-hoc test was used to obtain Statistical significant differences at p≤0.05.
3. Results

The preliminary phytochemical screening of the methanol stem-bark extract of *Ximenia americana* revealed the presence of alkaloids, anthraquinones, carbohydrates, flavonoids, saponins, steroidal glycosides, tannins and terpenoids.

**Activity against indomethacin-induced ulcers**

The extract significantly and dose-dependently reduced the mean ulcer spots in similar manner as the standard agent cimetidine. The reduction was significant only for the two higher doses of the extract (500 and 1000 mg/kg). However, the ulcer lesions were more prevented in the rat groups of cimetidine which showed mean ulcer spots of 0.6 ± 0.4 as against 1.80 ± 0.9 of the 1000 mg/kg extract dose. Only 3 severe ulcer spots for each of the two lower extract dose groups against 9 severe ulcer spots for the normal saline control group occurred with indomethacin ulcerogen, with no severe ulcer spots in both the cimetidine and 1000 mg/kg extract treated rat groups (Table 1).

**Activity against ethanol-induced ulcers**

The ethanol-induced ulcer lesions were also reduced in the extract treated groups and similarly, the reduction in the ulcer lesions was significant in rats of the two higher dose levels of the extract as with indomethacin-induced ulcer and both doses prevented the occurrence of lesions to same extent. The standard antiulcer drug (misoprostol) used for this study also inhibited the ulcer spots as expected. There were no severe ulcers (≥ 3mm spots) found in any of the groups including the normal saline control group (Table 2).

### Table 1: Effect of *X. americana* stem-bark extract on indomethacin-induced mucosal lesions in rat

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Mean ± SEM of ulcer spots</th>
<th>No. of ulcer spots ≥ 3mm (index of severity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N/S (1 ml)</td>
<td>9.20 ± 1.40</td>
<td>9</td>
</tr>
<tr>
<td>EXT 250 mg</td>
<td>6.60 ± 1.07</td>
<td>3</td>
</tr>
<tr>
<td>EXT 500 mg</td>
<td>4.60 ± 0.51*</td>
<td>3</td>
</tr>
<tr>
<td>EXT1000 mg</td>
<td>1.80 ± 0.86*</td>
<td>0</td>
</tr>
<tr>
<td>Cimetidine 100 mg</td>
<td>0.60 ± 0.40*</td>
<td>0</td>
</tr>
</tbody>
</table>

Data presented as mean±SEM; Statistical tool: one way ANOVA and * = p≤0.05 (Dunnett post-hoc test); Ext=Extract; N/S= normal saline; n=5.

### Table 2: Effect of *X. americana* stem-bark extract on ethanol-induced mucosal lesions in rat

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Mean ± SEM of ulcer spots</th>
<th>No. of ulcer spots ≥ 3mm (index of severity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N/S (1 ml)</td>
<td>7.60 ± 0.86</td>
<td>0</td>
</tr>
<tr>
<td>EXT250mg</td>
<td>5.20 ± 0.58</td>
<td>0</td>
</tr>
<tr>
<td>EXT500mg</td>
<td>2.40 ± 0.24*</td>
<td>0</td>
</tr>
<tr>
<td>EXT1000mg</td>
<td>2.40 ± 0.40*</td>
<td>0</td>
</tr>
<tr>
<td>Misoprostol 100mg</td>
<td>0.80 ± 0.50*</td>
<td>0</td>
</tr>
</tbody>
</table>

Data presented as mean±SEM; Statistical tool: one way ANOVA and * = p≤0.05 (Dunnett post-hoc test); Ext=Extract; N/S= normal saline; n=5.

### Discussion

In this study, the preliminary qualitative phytochemical screening showed that the methanol stem bark extract of this plant contained alkaloids, anthraquinones, flavonoids, saponins, tannins, terpenoids, steroidal glycosides and carbohydrates. This finding however, is consistent with other works reported with other parts of the plant in the literature (Maikai et al, 2005; Barro et al, 2008). Some of these constituents reported to possess anti-ulcer effects include flavonoids and other phenolic compounds which prevents gastric mucosal lesions and thus provides the cytoprotective type of antiulcer effect (Zayachikivska et al, 2005; Barro et al, 2008). Saponins, tannins and terpenoids also possess certain properties related to anti-ulcer effect (Borelli and Isso, 2000, Sofidiya et al, 2012). Thus, there may be need for quantitative phytochemical analysis for the individual components of antiulcer activity of this extract.
The antiulcer effect of the extract assessed in ulcerogenic rats of indomethacin and absolute ethanol-induced models in which the cytoprotective barrier mechanisms of the mucosal linings are disrupted to cause overproduction of acid fluids with ulcers, showed that the extract at doses of 500 and 1000 mg/kg significantly (p<0.05) and dose-dependently reduced the mean ulcer spots as with the standard drugs, cimetidine and misoprostol. However, it was only at the 1000 mg/kg extract dose group of the indomethacin ulcer model that severe ulcer lesions did not occur as also with cimetidine standard antiulcer drug. Each of the two lower extract doses showed three (3) severe ulcer spots against nine (9) for the normal saline control group. The fewer number of severe ulcer spots in the two lower extract test group and none in the 1000mg/kg group for indomethacin induced ulcer suggests protection. This study showed that the higher the dose of the extract, the more the protection of ulcer, but there may be concerns of possible dose-related toxic effects.

The absence of severe ulcers in any of the rat groups of ethanol-induced ulceration including the normal saline control group seemed to suggest that it is a milder ulcerative agent than indomethacin which produced severe ulcer lesions in the rats of the lower extract doses of 250 and 500 mg/kg and also of the normal saline control group. Indomethacin causes accumulation of cyclic AMP from blockade of prostaglandin production (Matsui et al, 2011). This might have caused an increased attack of the gastric acid on the barriers of the mucosal wall. Attack from excess gastric acid from any mechanism could be in the form of inhibition of GIT mucin production as with the mucinase activity of H. pylori or increased gastric emptying of the stomach with consequent incomplete neutralisation of the acid (HCl) that results in irritation and/or inflammation of the mucosa (Pounder et al, 1978). Thus, in addition to boosting production of prostaglandins that would stop excess acid production, potential antiulcer agents could enhance mucosal blood flow functions and/or secretion of gastric mucous and bicarbonate which constitute the defensive barrier for resisting acid attack (cytoprotection) and/or maintaining integrity of the mucosal wall (Cohen, 1987). Agent for ulcer therapy could therefore be of cytoprotectant for increased acid resistance or acidity inhibitory to prevent acid accumulation.

5.0 Conclusion

Antiulcer or gastroprotective effects of the stem-bark extract of Ximenia americana were observed, and may have been a composite effect of its various phytochemicals acting through one or more mechanisms.

Conflict of Interest declaration

The authors declare no conflict of interest.

Acknowledgements

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Matsui H, Shimokawa O, Kaneko T, Nagano Y, Rai K and Hyodo S (2011). This might have caused an increased attack of the gastric acid on the barriers of the mucosal wall. Attack from excess gastric acid from any mechanism could be in the form of inhibition of GIT mucin production as with the mucinase activity of H. pylori or increased gastric emptying of the stomach with consequent incomplete neutralisation of the acid (HCl) that results in irritation and/or inflammation of the mucosa (Pounder et al, 1978). Thus, in addition to boosting production of prostaglandins that would stop excess acid production, potential antiulcer agents could enhance mucosal blood flow functions and/or secretion of gastric mucous and bicarbonate which constitute the defensive barrier for resisting acid attack (cytoprotection) and/or maintaining integrity of the mucosal wall (Cohen, 1987). Agent for ulcer therapy could therefore be of cytoprotectant for increased acid resistance or acidity inhibitory to prevent acid accumulation.

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