Antiulcerogenic effects of *Celosia trigyna* plant extracts on ethanol-induced gastric ulcer in adult Wistar rats

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ABSTRACT

Background and aim: Gastric ulcer is a chronic disease and serious health issue. *Celosia trigyna* is a medicinal plant used traditionally for wound healing. This study aimed to isolate the bioactive compounds from *Celosia trigyna* and to investigate the *in vitro* and *in vivo* anti-ulcerogenic effects of the extract*s* on ethanol-induced gastric ulcer on adult Wistar rats to determine their regenerative potential.
Experimental procedure: Seven groups (A − negative control, B − vehicle control, C, D, E, F and G − positive control, n = 5) of five adult Wistar rats received treatment for ethanol-induced gastric ulcer.
Results and conclusion: Phytochemical analysis led to the isolation of chondrillasterol, lutein, pheophytin a and chondrillasterol acetate. The *in vitro* results showed dichloromethane and hexane extracts to have maximum chymotrypsin inhibition relative to the standard (chymostatin) while *in vivo* results showed a significant increase in ulcer parameters of the vehicle control relative to groups treated with plant extracts (P < 0.05). Ulcer parameters and DNA density in groups treated with dichloromethane and hexane extracts were comparable to the negative control. Gross and histopathological findings confirmed gastric mucosa lesions in the vehicle control. There were mild ulcerations in groups treated with the ethyl acetate and methanol extracts with no observable ulcerations in the groups treated with dichloromethane and hexane extracts as the histoarchitectural outlines do not show any form of necrosis, distortion or cellular vacuolation. It was concluded that non-polar, hydrophobic compounds are able to remediate the degree of ulceration but not polar compounds.

1. Introduction

Gastric ulcer is a chronic disease that occurs when there is a breakage in the mucosa lining of the stomach. A physiological balance exists between aggressive factors and mucosa defence. When this balance is compromised in favour of aggressive factors, injury to the gastric mucosa occurs.[1] Some of the aggressive factors are *Helicobacter pylori*, nonsteroidal anti-inflammatory drugs, ethanol and genetic factors. Ethanol has a devastating effect on gastric mucosa as a result it has been used to induce gastric ulcer in animal models. Report has shown alcohol to compromise the integrity of gastric mucosa by aiding acid reflux into the subluminal layer of the mucosa and submucosa.[2] It may also act through a general mechanism affecting the release of hormones and the regulation of nerve function involved in acid secretion.[3] Due to an increase in the incidence of gastric ulcers and the side effects associated with some of the synthetic drugs being used for its treatment or management, there is need to find alternative solutions to this global problem.

Medicinal plant contains very active ingredients used for treating various diseases.[4] Tradomedical practitioners have provided insight into alternative treatments for gastric ulcers as many plant extracts have been very useful in their management. The leaf extracts of *Eruca sativa*to were found to exhibit anti-ulcer activity against ethanol-induced gastric mucosa injury in animal models.[5] Naru. (2014) also gave credence to the anti-ulcerogenic potential of the extracts from *Bauhinia purpurea* against ethanol-induced gastric mucosa injury in rats. The micro-anatomical potential of *Polygonum minus* aqueous leaf extracts on ethanol-induced gastric ulcers in rats was evaluated and it was concluded that the extract

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List of abbreviations			
^{13}C-NMR	carbon-13-nuclear magnetic resonance spectroscopy	GCMS	gas chromatography mass spectrometry
		H&E	hematoxylin and eosin
^{1}H-NMR	proton nuclear magnetic resonance spectroscopy	HRMS	high resolution mass spectrometry
2D NMR	two-dimensional nuclear magnetic resonance spectroscopy	M	mucosa
		ME	Muscularis externa
ANOVA	analysis of variance	MeOH	methanol
CC	column chromatography	MS	mass spectrometry
CDCl$_3$	deuterated chloroform	SD	standard deviation
DCM	dichloromethane	SM	submucosa
DNA	deoxyribonucleic acid	TLC	thin layer chromatography
DPX	distrene polystyrene and xylene	UI	ulcer index
EtOAc	ethyl-acetate	VVG	Verhoeff-van Gieson

protects against gastric ulceration by reduction of leucocyte infiltration and oedema.[1]

Celosia trigyna (*C. trigyna*) belongs to the plant family Amaranthaceae and is found in the Western and Northern part of Nigeria, South Africa, Southern Arabia and the Democratic Republic of Congo. Livestock and man often eat the leaves. Due to its affinity for water, it is often cultivated during the raining season. *C. trigyna* is used medicinally for the treatment of intestinal worms, chest pains, diarrhoea and mouth ulcers.[7] The leaves and roots are used to treat wounds and skin problems.[8] The leaves are reported to be rich in calcium, phosphorus, iron, proteins, and vitamins A, C and E.[9] Research on *Celosia* species such as *Celosia argentea* reported improved wound healing in a rat burn model.[10] Despite the medicinal value of *C. trigyna*, its healing and anti-ulcerogenic potential has not been explored.

Therefore, the aim of this study was to determine the anti-ulcerogenic potential of the leaf extracts of *C. trigyna* in animal models following ethanol-induced gastric ulcers to add to current knowledge on alternative treatment of gastric ulcers using plant extracts. The major secondary metabolites present in the active extracts were also isolated and identified.

2. Materials and methods

2.1. Plant materials and reagents

The leaves from *Celosia trigyna* were collected from the northern and western regions of Nigeria. The plant was authenticated by a taxonomist in the Botany Department, Obafemi Awolowo University, Ile-Ife. A voucher specimen (reference number: IFE- 17466) was deposited at the herbarium. Tris-HCl buffer (60 μL, 0.9 units), N-succinyl phenyl-alanine-P-nitroanilide substrate, chymotrypsin, chymostatin and omeprazole were supplied by Sigma Aldrich (St. Louis, MO, USA).

2.2. Extraction

The leaves of *C. trigyna* were air-dried and ground using a crushing machine (Daiki Rika Kogyo Co-ltd, Japan). Thereafter, the ground sample (1.0 kg) was sequentially extracted with hexane, dichloromethane (DCM), ethyl acetate (EtOAc) and methanol (MeOH) by continuous shaking on an orbital shaker for 48 h each. The extracts were filtered, in turn, and the filtrate concentrated using a rotary evaporator (Butchi Rotary Evaporator, New Jersey) and stored in the refrigerator at 4 °C until required.

2.3. Isolation and characterisation

Isolation of the compounds was done by column chromatography using Merck silica gel 60 (0.040−0.063 mm) while aluminium sheets (Merck silica gel 60, 20 × 20 cm F254) were used for thin layer chromatography (TLC). The hexane and DCM extracts of the leaves were combined due to similar TLC profiles (Rf factor), while the TLC profile of the EtOAc and MeOH extracts showed no polar compound that could be isolated in sufficient quantity. Characterisation of isolates was done using spectroscopic techniques (nuclear magnetic resonance (NMR, 400 MHz) using ^{1}H NMR, ^{13}C NMR and 2D NMR) in deuterated chloroform (CDCl$_3$) or methanol (MeOD), ultraviolet visible spectroscopy (UV−Vis), Fourier-transform infrared spectroscopy (FT-IR) and gas chromatography−mass spectrometry (GCMS). The combined extract (28.8 g) was loaded onto a silica gel column using a gradient elution system of hexane: EtOAc starting with 100% hexane that was stepped by 10%−100% EtOAc. Eight fractions of 100 mL were collected at each eluent step. Fraction 16 afforded compound **A1**, a dark green amorphous solid (100 mg), fractions 23 afforded compound **A2**, a white solid (1.4 mg), fraction 36 afforded compound **A3**, a white solid (50 mg) while fraction 37−40 were purified further to give compound **A4**, a yellow solid (40 mg).

2.4. Animal care

Animal care was in accordance with the National Institute of Health guide for the care and use of laboratory animals. Ethical clearance was obtained from the Ethical Committee of the Institute of Public Health, Nigeria before commencement of the research (IPHOAU/12/1010). The rats were procured, housed in plastic cages and acclimatized for one week before the commencement of research at the Animal Holdings of the Department of Anatomy and Cell Biology, Obafemi Awolowo University, Ile-Ife, Nigeria. They were kept at room temperature, fed a standard animal diet and allowed free access to clean water.

2.5. Extract preparation for in vivo anti-ulcer assay

The dried extracts (hexane, DCM, EtOAc and MeOH) were reconstituted at a dose of 50 mg per kg body weight for the four extracts. Omeprazole was the reference anti-ulcer drug of choice for this study. It was obtained from the Health Centre, Obafemi Awolowo University, Nigeria. The drug was constituted and administered orally at a dose of 10 mg per kg body weight.[11]

2.6. In vitro anti-ulcer assay (α-chymotrypsin assay)

The chymotrypsin inhibition assay was performed on the extracts according to the method as described by Saleem et al. using the standard drug, chymostatin as the positive control. The α-chymotrypsin inhibitory activity (%) and IC_{50} values (concentration at which 50% enzyme catalysed reaction occurs) were calculated for all the extracts and standard drug.

2.7. Study design

Thirty-five adult female Wistar rats, weighing between 141 and 222 g were used for the study. The rats were randomly assigned into seven groups (A-G) of five animals each. Group A (negative control) was given double distilled water (5 mL kg^{-1}) and 1 mL double distilled water per kg body weight, every day. Group B (vehicle control) was given 90% ethanol (5 mL kg^{-1}) and 1 mL of 20% tween 80 per kg body weight, every day. Groups C— F were given 90% ethanol (5 mL kg^{-1}) and the respective plant extracts (50 mg per kg body weight in 20% tween 80, every day). The plant extracts were: group C — MeOH, group D — DCM, group E —EtOAc and group F - hexane. Group G (positive control) was given 90% ethanol (5 mL kg^{-1}) and omeprazole (10 mg per kg body weight every day).

2.8. Ulcer induction

Ethanol (90%, 5 mL kg^{-1}) was orally administered to the test groups (B − G) after a 24 h fast. Gastric ulceration occurred after 1 h of ethanol administration.[13] After 24 h, treatment commenced on rats in the test groups (B − G) as stated above, repeatedly for 14 days.

2.9. Sacrifice of animals

All the rats were sacrificed under slight chloroform anaesthesia 24 h after the last administration. Midline incisions were made and stomachs were excised, open along the lesser curvature and washed in normal saline. The stomachs of the rats were spread on a dissecting board for gross photography using a high resolution camera at a fixed distance of 10 cm from the tissue. Images captured were imported into imageJ software (sponsored by the National Institute of Health, USA) for measurement and determination of ulcer parameters.

2.10. Total ulcer and mucosa area

The total ulcer area was determined by first calibrating the imageJ software with a known distance which was in millimetre (mm) using an e-ruler. Thereafter, the stomach mucosa images of each animal in each group was imported to imageJ and with the help of free hand tools, the ulcerated areas were mapped, the data generated, and the total ulcer area for each animal was calculated.

2.11. Ulcer score

The ulcer score was determined by generating a grid line on the imported image. Thereafter, the mean ulcer score for each group was generated.

The ulcer index (UI) was calculated by adopting the formula:

Ulcer index = [Ulcerated area] / [Total mucosa surface area] x100

The curative ratio was calculated using the formula:

Curative ratio = [UI control] − [UI treated] / [UI control]

2.12. Histopathological analysis

Small portions of the gastric tissue were fixed in 10% formol saline for histopathological analysis. Slides were stained for hematoxylin and eosin (H&E) for histoarchitectural outline and Verhoeff-van Gieson (VVG) stain for collagen fibre demonstration according to Bancroft and Gamble.[14]

2.13. Histochemical analysis

Demonstration for the presence of DNA was carried out using the Feulgen staining technique as described by Bancroft and Gamble.[14] Quantification of DNA through staining intensity was done using imageJ software which included counting of the stained DNA.

2.14. Statistical analysis

Data was analysed using IBM Statistics for the Social Sciences (PASW) Statistics, version 24, (IBM Cooperation, Cornell, NY, USA) with Duncan multiple range test option and expressed as mean ± SEM. Statistical significance between the groups was determined by one-way analysis of variance (ANOVA); P < 0.05 was considered statistically significant except where otherwise stated.

3. Results and discussion

3.1. Isolation of compounds from Celosia trigyna

The combined hexane and DCM extracts yielded four compounds, namely, a pigment pheophytin a (**A1**), two sterols (chondrillasterol acetate (**A2**), chondrillasterol (**A3**)) and a carotenoid, lutein (**A4**). The physical and spectral data of these compounds were verified with that of literature.

3.2. In vitro anti-ulcer activity (α-chymotrypsin assay)

The result showed the DCM (83.2%) and hexane (81.3%) extracts from Celosia trigyna to exhibit maximum chymotrypsin inhibitory activity with IC_{50} values of 12.46 and 14.93 μg mL^{-1}, respectively, which was comparable to the standard drug, chymostatin (Table 1). The EtOAc and MeOH extracts also showed moderate to good inhibitory activity. The percentage inhibitory activity of the isolated compounds is shown in Fig. 1. Lutein showed highest chymotrypsin inhibition (73.9%) which was closely followed by chondrillasterol acetate (72.4%), chondrillasterol (71.3%) and pheophytin a (68.6%).

Table 1
α-Chymotrypsin activity (Mean ± SEM, n = 5) of different extracts from Celosia trigyna.

Test Samples	Inhibition (%)	IC_{50} (μg mL^{-1})
Hexane extract	81.3 ± 0.5^c	14.93 ± 0.2
Dichloromethane extract	83.2 ± 0.2^c	12.46 ± 1.3
Ethyl acetate extract	68.2 ± 0.3^a	54.94 ± 0.14
Methanol extract	75.0 ± 0.6^b	42.33 ± 0.11
Chymostatin	89.1 ± 0.2^d	7.51 ± 0.12

Different superscript letters (a-d) signify significantly different means while means with the same letters are not significantly different (P < 0.05, Turkey's post hoc comparisons).
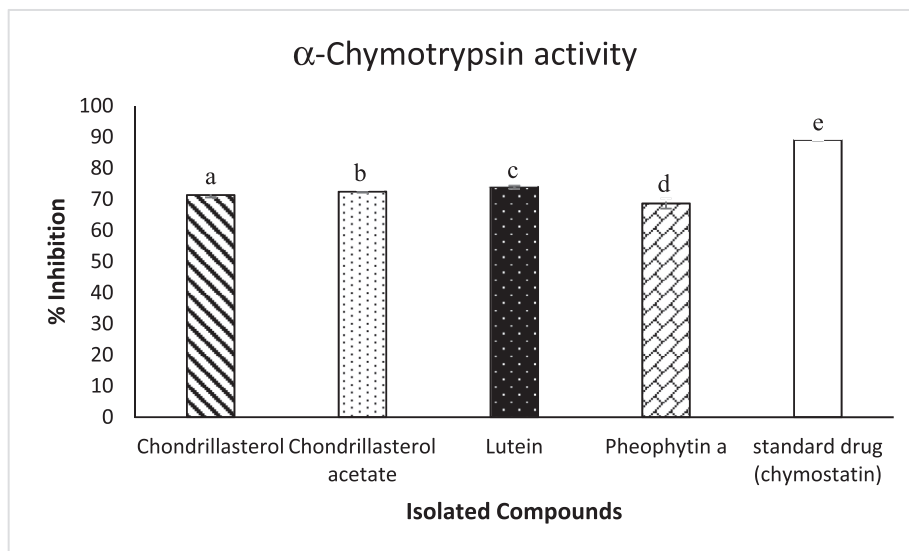
Fig. 1. α-Chymotrypsin activity in four isolated compounds. Values are given as mean ± SEM for % inhibition of α-Chymotrypsin activity in each group. Different letters (a–e) above the bars signify significantly different means while means with the same letters are not significantly different (P = 0.001, Turkey's post hoc comparisons).

3.3. Macroscopic analysis of ulcer lesions

The result showed the ulcer lesion to be significantly higher (P < 0.05) in group B (vehicle control) compared to group A (negative control). There was a significant reduction in the total ulcerated area in the groups treated with *C. trigyna* (groups C − F) compared to those treated with omeprazole (group G, positive control) (Fig. 2 and Table 2). Hexane and DCM extracts of *C. trigyna* showed better regenerative potential (curative ratio) that is comparable to the positive control (Fig. 2 and Table 2). The ulcer index and total ulcer score was significantly higher (P < 0.05) in the vehicle control compared to the other groups. Groups treated with hexane and DCM did not have any ulcer index and score similar to groups treated with omeprazole and unlike the groups treated with
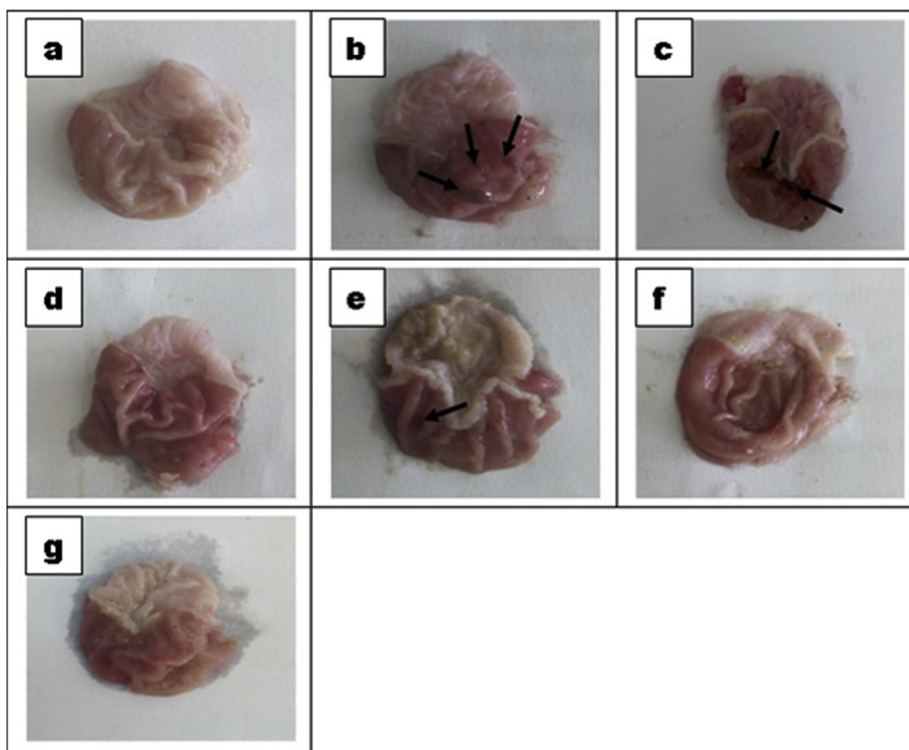

Fig. 2. Macroscopic view of the stomachs in (a) group A - negative control (absence of hemorrhagic patches) (b) group B - vehicle control (numerous hemorrhagic patches that characterize the entire mucosa) (arrow) (c) group C- treated with methanol extract (hemorrhagic patches (arrow) (d) group D - treated with dichloromethane extract (absence of hemorrhagic patches) (e) group E−treated with ethyl acetate extract (tenderness in the mucosa and scanty hemorrhagic patches) (arrow) (F) group F − treated with hexane extract (absence of hemorrhagic patches) (G) group G − positive control (absence of hemorrhagic patches).

Table 2
Ulcer parameters (Mean ± SEM, n = 5) in all animal groups.

Groups	Total ulcerated area (mm²)	Total mucosa surface area (mm²)	Ulcer index (%)	Total ulcer score (lesion/900 mm²)	Curative ratio (%)
A	0.00 ± 0.00ᵃ	1745.99 ± 29.95ᵃ	0.00 ± 0.00ᵃ	0.00 ± 0.00ᵃ	-
B	315.53 ± 38.93ᵇ	2591.55 ± 27.69ᵈᵉ	12.15 ± 1.46ᵇ	4.80 ± 0.37ᶜ	0.00
C	36.68 ± 19.35ᵃ	1968.52 ± 16.67ᵇ	1.88 ± 0.99ᵃ	1.80 ± 0.80ᵇ	84.53
D	0.00 ± 0.00ᵃ	2445.36 ± 53.23ᵈ	0.00 ± 0.00ᵃ	0.00 ± 0.00ᵃ	100.00
E	7.40 ± 1.96ᵃ	2240.52 ± 83.08ᶜ	0.33 ± 0.09ᵃ	1.40 ± 0.24ᵇ	97.28
F	0.00 ± 0.00ᵃ	2633.21 ± 103.39ᵉ	0.00 ± 0.00ᵃ	0.00 ± 0.00ᵃ	100.00
G	0.00 ± 0.00ᵃ	1687.87 ± 47.13ᵃ	0.00 ± 0.00ᵃ	0.00 ± 0.00ᵃ	100.00

Group A (negative control), group B (vehicle control), group C − treated with MeOH extract, group D − treated with DCM extract, group E −treated with EtOAc extract, group F − treated with hexane extract and group G (positive control) − treated with omeprazole.
Different superscript letters (a-e) signify significantly different means while means with the same letters are not significantly different (P < 0.05, Turkey's post hoc comparisons).

MeOH and EtOAc extracts (Fig. 2 and Table 2).

3.4. Histopathological analyses

The histopathological result showed evidence of ulceration in the vehicle control compared to the negative control. There was epithelial discontinuation and mucosa damage characterised by submucosal oedema, and leucocyte infiltration (Fig. 3). There was no evidence of ulceration in groups treated with the DCM and hexane extracts as the histoarchitectural outlines do not show any form of necrosis, distortion or cellular vacuolation (Fig. 3). This observation was comparable with the negative and positive controls. However, there was evidence of epithelial recovery processes

in groups treated with the MeOH and EtOAc extracts. There was depletion of collagen fibres in the vehicle control compared to the negative control. There was evidence of collagen deposition within the submucosae and mucosae of *C. trigyna* and positive control. The collagen deposition in groups treated with the DCM and hexane extracts were comparable to the negative control (Fig. 4).

3.5. Histochemical analysis

The DNA density was quantified as shown in Fig. 5. There was a significant (P < 0.001) decrease in DNA density in the gastric mucosa of the positive control compared to the negative control. The DNA density of groups treated with the DCM and hexane extracts
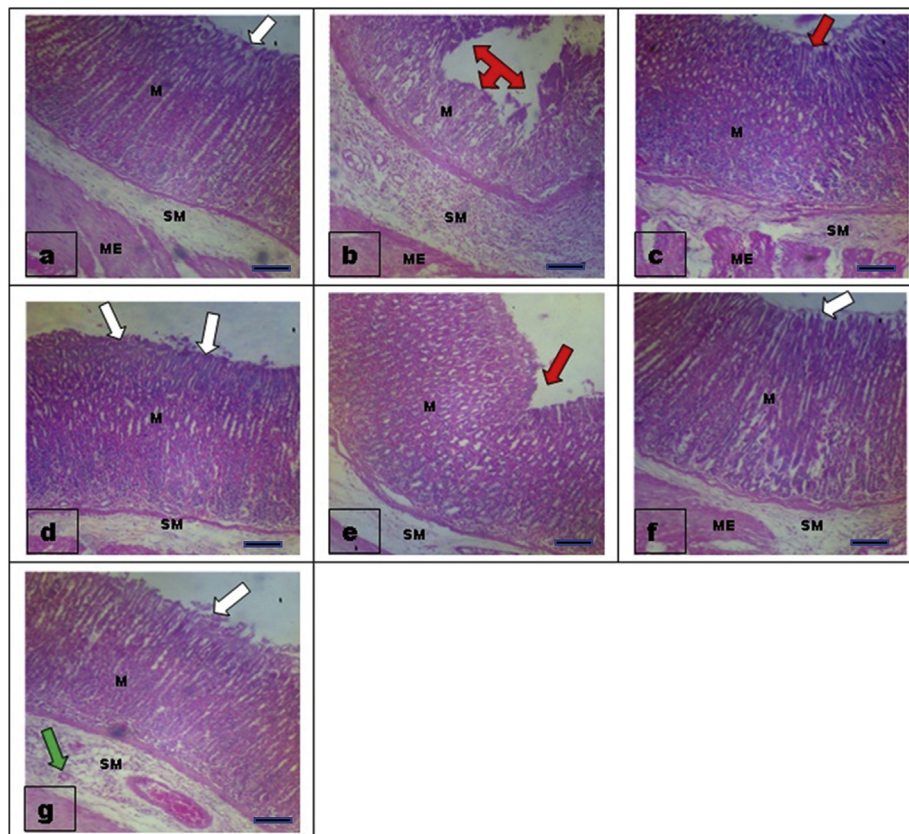

Fig. 3. Photomicrographs of the stomachs in (a) group A-negative control (intact mucosa (M)) arrow showing the epithelium (b) group B -vehicle control (discontinuation of the epithelium - arrow) and the disruption of the mucosa (M) (c) group C- treated with methanol extract (slight ulceration) (arrow) (d) group D - treated with dichloromethane extract (intact epithelium) (arrow) (e) group E−treated with ethyl acetate extract (epithelial discontinuation) (arrow) (f) group F - treated with hexane extract (intact mucosa (M) and epithelium) (arrow) (G) Group G − positive control (intact mucosa (M) and epithelium) (arrow). H & E Mag: 100 × ; Scale bar: 5 mm.
Red arrow − point of ulceration; white arrow − intact epithelium; green arrow − blood vessel; M − mucosa; SM − submucosa; ME − muscularis externa.
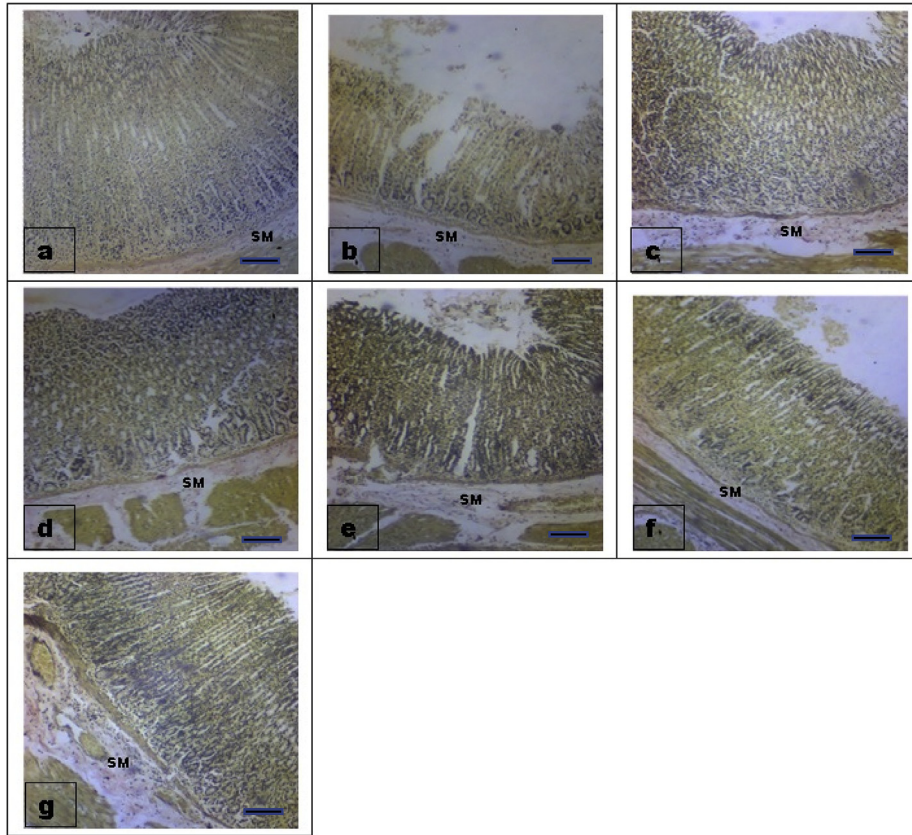
Fig. 4. Photomicrographs of the stomachs in (a) Group A-negative control (intense distribution of collagen fibers within the submucosa (SM)) (b) Group B - vehicle control (scanty distribution of collagen fibers within the submucosa (SM)) (c) Group C- treated with methanol extract (gradual restoration of collagen fibers within the submucosa (SM)) (d) Group D - treated with dichloromethane extract (distribution of collagen fibers within the submucosa (SM)similar to group A) (e) Group E–treated with ethylacetate extract (gradual restoration of collagen fibers within the submucosa (SM)) (F) Group F –treated with hexane extract (distribution of collagen fibers within the submucosa (SM) similar to group A) (G) Group G –positive control (distribution of collagen fibers within the submucosa (SM) similar to groups A, D and F). VVG. Mag: 100 × ; Scale bar: 5 mm. Collagen fibres – stain pink.
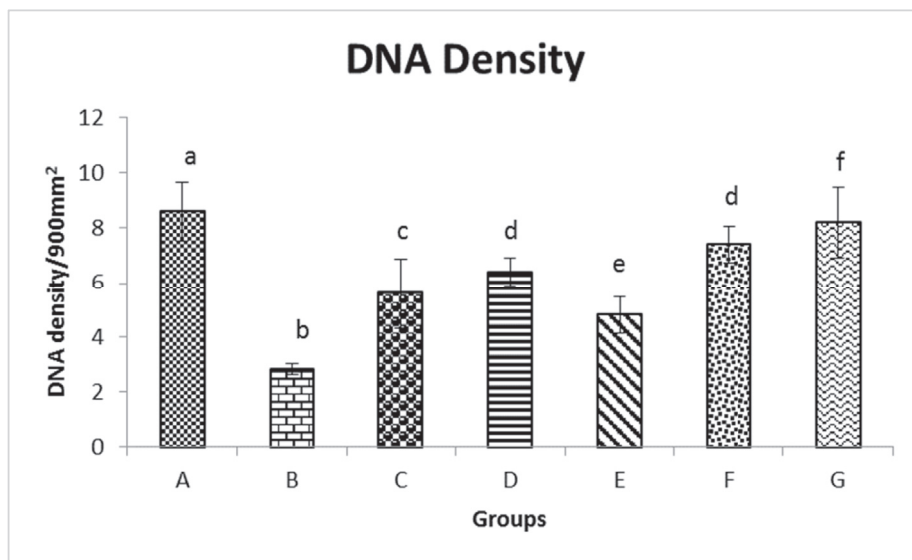

Fig. 5. DNA density. Values are given as mean ± SEM for quantification of DNA density/900 mm^2 using Feulgen stain in each group. Different letters (a–f) above the bars signify significantly different means while means with the same letters are not significantly different (P = 0.001, Turkey's post hoc comparisons).

was significantly higher than those treated with the MeOH and EtOAc extracts (Fig. 5).

Gastric ulcer, a form of peptic ulcer is frequent in clinical practice[1] but the aetiology of most cases of gastric ulcer is shrouded in mystery. It has, however, been documented that gastric ulcer may occur due to imbalances between the aggressive factors on one hand and the maintenance of mucosa integrity through endogenous defence mechanisms on the other.

The structural data of isolated compounds in this study was compared with that of literature. Compound **A1**, a dark green amorphous pigment, was identified as pheophytin a (molecular weight 893.5530 and molecular formula $C_{55}H_{74}N_4O_5$).[15,16] Pheophytin a is a chlorophyll molecule lacking a central Mg^{2+} ion. It can be produced from chlorophyll by treatment with a weak acid, producing a dark bluish waxy pigment. Compound **A2**, a white powder, was identified as chondrillasterol acetate (stigmasta-7,22-dien-3β-yl-acetate, molecular weight 454.7275 and molecular formula $C_{31}H_{50}O_2$).[17] Compound **A3**, a white solid, was identified as chondrillasterol (stigmasta-7,22-dien-3β-ol, molecular weight 397.3148 and molecular formula $C_{29}H_{48}O$).[18,19] Compound **A4**, a yellow solid, was identified as lutein (molecular weight 568.9 and molecular formula $C_{40}H_{56}O_2$).[20]

The α-chymotrypsin enzyme enables the cleavage of peptide bonds by hydrolysis reaction which occurs slowly in the absence of a catalyst. The four extracts (DCM, hexane, MeOH and EtOAc) of *C. trigyna* showed inhibitory activity against α-chymotrypsin. The maximum inhibitory activity was achieved by DCM and hexane extracts which was comparable to chymostatin (positive control). Study in the field of epidemiology has proved that colon, prostate and breast cancers are very rare in vegetarian populations.[12] This observation was traced to the ability of some edible plants to inhibit proteases such as chymotrypsin enzyme. In the gastrointestinal tract, activation of protease-activated receptors by proteases can induce pro-inflammatory responses[21] that are common in ulcer manifestation. Flausino et al. related anti-ulcerogenic effects of *Casearia sylvestris* extract to protease inhibition and that the isolated compounds are possibly responsible.[22] The isolated compounds showed the percentage inhibition of chymotrypsin to be in increasing order of lutein > chondrillasterol acetate > chondrillasterol > pheophytin (Fig. 1).

The result from this study showed ethanol to induce an ulcer in the experimental groups. Evidence from ulcer parameters also shows the DCM and hexane extracts of *C. trigyna* to provide better remediation against ethanol-induced gastric ulcer than the MeOH and EtOAc extracts. The total ulcer area, total ulcer score and ulcer index in groups treated with the DCM and hexane extracts were comparable to the negative control and group treated with omeprazole. Also, groups treated with DCM and hexane extracts showed no trace of gastric ulceration and, as such, demonstrated similar anti-ulcerogenic potential as omeprazole. This observation may be due to the presence of the four compounds in these extracts.

Carotenoids, such as lutein, is one of the most common natural pigments found in plants and they are responsible for many attractive colours found in plant leaves, fruits, and flowers.[20] Epidemiological studies revealed a correlation between increased consumption of a diet rich in carotenoids with a reduction in the risk of degenerative disorders, such as cancer, cardiovascular disease, ophthalmological diseases and ulcer.[23,24] The protective effect of carotenoids have been associated with their antioxidant activity thereby defending the cells and tissues from oxidative damage.[25] Carotenoids were also shown to be potent antioxidants that enhanced cytoprotection in peptic ulcers and were efficacious in the healing of gastric ulcers.[26] The enhancement of the effective healing of gastric ulcers, as observed by the DCM and hexane extracts in this study, could be due to the antioxidant properties of lutein (the compound with the highest inhibitory activity) present in the extracts.[20]

In addition to the efficacious healing activity by the lutein, the presence of pheophytin a in the extracts can also contribute to the effectiveness of the extracts as discovered by Hsu et al. (2013). He reported pheophytins to have good antioxidant property and also possess ability to prevent oxidative DNA damage and lipid peroxidation, when the antioxidant properties of chlorophylls and pheophytins were compared.[27] Pheophytin a can also reduce ulcer formation or promote healing process, being an agent capable of inhibiting reactive oxygen species.[28] The significant increase in the DNA density of groups treated with plant extracts when compared to the vehicle control suggests an ongoing regenerative process. Increase in DNA density is evidence of DNA replication with cell division, a process that culminates into tissue regeneration.[29] Healing occurs after cellular damage is caused by necrotizing agents such as ethanol.

The mechanism by which the DCM and hexane extracts brought about DNA turnover could be due to the synergic effects of chondrillasterol, lutein, chondrillasterol acetate and pheophytin a isolated in these extracts.[30] Histopathological examination showed epithelial discontinuation, mucosa damage, submucosa oedema, and leucocyte infiltration in the positive control. This observation may be due to oxidative stress.[6] Oxidative stress in ethanol toxicity precipitates neutrophil infiltration which is inhibitory to the healing process of gastric ulcers.[6,31] Following treatment with *C. trigyna* extracts, the degree of ulceration was drastically reduced in groups treated with the MeOH and EtOAc extracts but totally ameliorated in groups treated with the DCM and hexane extracts as evident in the ulcer score and photomicrographs. Sufficient vascularization of the connective tissue enhances the migration of myofibroblasts beneath the epithelium to form the ulcer bed in gastric ulcers.[32] This eventually leads to the production of collagen fibrils and other extracellular matrices to form ulcer scars. This could explain the increase in collagen fibres in groups treated with extracts from *C. trigyna* compared to the untreated group. Increased collagen deposition observed in the hexane and DCM extracts may be due to the presence of the secondary metabolites.

4. Conclusion

In conclusion, this study indicates *C. trigyna* to be efficacious in the management of gastric ulcers. The anti-ulcerogenic properties exhibited by the plant extracts are due to the presence of secondary metabolites. Better remediation of gastric ulcers by the DCM and hexane extracts are due to synergic effects of phytocompounds and the enhancing effects of the major secondary metabolites (lutein, pheophytin a and the sterols). The anti-ulcerogenic effects of the DCM and hexane extracts were comparable to omeprazole.

Declaration of competing interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtcme.2019.11.004.

References

1. Wasman S, Mahmood A, Salehhuddin H, Zahra A, Salmah I. Cytoprotective activities of Polygonum minus aqueous leaf extract on ethanol-induced gastric ulcer in rats. *J Med Plants Res*. 2010;4:2658—2665.
2. Oh TY, Yeo M, Han SU, et al. Synergism of Helicobacter pylori infection and stress on the augmentation of gastric mucosal damage and its prevention with α-tocopherol. *Free Radic Biol Med*. 2005;38:1447—1457.
3. Chari S, Teyssen S, Singer M. Alcohol and gastric acid secretion in humans. *Gut*. 1993;34:843—847.
4. Varadharaj V, Muniyappan J. Phytochemical and phytotherapeutic properties of Celosia species-A review. *Int J Pharm Phytochem Res*. 2017;9:820—825.
5. Saleh MM, Qader SW, Thaker AA. Gastroprotective activity of Eruca sativa leaf extract on ethanol-induced gastric mucosal injury in Rattus norvegicus. *Jordan J Biol Sci*. 2016;9:47—52.
6. Naru FE. Effects of Bauhinia purpurea leaf extract against ethanol-induced gastric mucosal ulcer in rats. *Int J Histol Cytol*. 2014;1:93—98.
7. Okujagu TF, Etatuvie SO, Eze I, Jimoh B, Nweke C, Mbaoji C. *Medicinal plants of Nigeria; south-west Nigeria*. In: *Collaboration with Lagos State Traditional Medicine Board and Botany Department*. vol. 1. University of Lagos; 2006:20.
8. Mabona U, Van Vuuren S. Southern African medicinal plants used to treat skin diseases. *South Afr J Bot*. 2013;87:175—193.
9. Okwu DE, Josiah C. Evaluation of the chemical composition of two Nigerian medicinal plants. *Afr J Biotechnol*. 2006;5:357—361.
10. Priya KS, Arumugam G, Rathinam B, Wells A, Babu M. Celosia argentea Linn. leaf extract improves wound healing in a rat burn wound model. *Wound Repair Regen*. 2004;12:618—625.
11. Kwon H-W, Kim D-J. Protective effects of BK-1202 on the indomethacin-induced gastric ulcer in rats. *J Korean Med Sci*. 2015;36:42—55.
12. Saleem H, Ahmad I, Ashraf M, Gill MSA, Nadeem MF, Shahid MN. In vitro studies on anti-diabetic and anti-ulcer potentials of jatropha gossypifolia (Euphorbiaceae). *Trop J Pharm Res*. 2016;15:121—125.
13. Ortaç D, Cemek M, Karaca T, Büyükokuroğlu ME, Özdemir ZÖ, Kocaman AT. In vivo anti-ulcerogenic effect of okra (Abelmoschus esculentus) on ethanol-induced acute gastric mucosal lesions. *Pharm Biol*. 2018;56:165—175.
14. Bancroft JD, Gamble G. In: Bancroft, Gamble, eds. *Theory and Practice of Histological Techniques*. sixth ed. Philadelphia, USA: Churchill Livingstone/Elsevier; 2008:83—104.
15. Klimov VV. Discovery of pheophytin function in the photosynthetic energy conversion as the primary electron acceptor of Photosystem II. *Photosynth Res*. 2003;76:247—253.
16. Wang T, Jonsdottir R, Ólafsdóttir G. Total phenolic compounds, radical scavenging and metal chelation of extracts from Icelandic seaweeds. *Food Chem*. 2009;116:240—248.
17. Cameron A, Ewen M, Auton M, Abegunde D. *The World Medicines Situation 2011: Medicines Prices, Availability and Affordability*. Geneva: WHO; 2011.
18. Rasoanaivo LH, Wadouachi A, Andriamampianina T, et al. Triterpenes and steroids from the stem bark of Gambeya boiviniana Pierre. *J Pharmacogn Phytochem*. 2014;3:68—72.
19. Mai NT, Van Kiem P, Thu VK. Sterols from stems of momordica cochinchinesis (Lour.) spreng. *Vietnam J Chem*. 2017;55:606.
20. El-Raey MA, Ibrahim GE, Eldahshan OA. Lycopene and lutein; A review for their Chemistry and medicinal uses. *J Pharmacogn Phytochem*. 2013;2:245—254.
21. Vergnolle N. Protease inhibition as new therapeutic strategy for GI diseases. *Gut*. 2016;65:1215—1224.
22. Flausino Jr O, Abissi BM, Vieira Jr GM, Santos AG, Silva DH, Cavalheiro A. Protease inhibition activity of extracts from Salicaceae species from Brazilian Cerrado and Atlantic Rain Forest and of an enriched fraction of clerodane diterpenes (casearins). *Rev Bras Farmacogn*. 2009;19:755—758.
23. Mayne ST. Beta-carotene, carotenoids, and disease prevention in humans. *FASEB J*. 1996;10:690—701.
24. Sindhu ER, Kuttan R. Carotenoid lutein protects rats from gastric ulcer induced by ethanol. *J Basic Clin Physiol Pharmacol*. 2012;23:33—37.
25. Stahl W, Sies H. Antioxidant activity of carotenoids. *Mol Asp Med*. 2003;24:345—351.
26. de Lira Mota KS, Dias GEN, Pinto MEF, et al. Flavonoids with gastroprotective activity. *Mol*. 2009;14:979—1012.
27. Hsu CY, Chao PY, Hu SP, Yang CM. The antioxidant and free radical scavenging activities of chlorophylls and pheophytins. *Food Nutr Sci*. 2013;4:1—8.
28. Ray A, Chaudhuri SR, Majumdar B, Bandyopadhyay SK. Antioxidant activity of ethanol extract of rhizome of Picrorhiza kurroa on indomethacin induced gastric ulcer during healing. *Indian J Clin Biochem*. 2002;17:44—51.
29. Stower H. Regeneration: key role for polyploidization in wound healing. *Nat Rev Genet*. 2013;14:825.
30. Majumdar M. *Evaluation of Tectona Grandis Leaves for Wound Healing Activity*. RGUHS; 2005.
31. Cheng C, Koo M. Effects of Centella asiatica on ethanol induced gastric mucosal lesions in rats. *Life Sci*. 2000;67:2647—2653.
32. Fornai M, Colucci R, Antonioli L, Ghisu N, Tuccori M, Blandizzi C. Effects of pantoprazole on ulcer healing delay associated with NSAID treatment. *N-S Arch Pharmacol*. 2009;379:305—313.