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Gastroprotective and antioxidant potentials of ethanolic stem bark extract of *Margaritaria discoidea* (Euphorbiaceae) in rats

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ABSTRACT

Ethnopharmacological relevance: Decoctions prepared from the bark of *Margaritaria discoidea* (Baill.) G. L. Webster (Euphorbiaceae) are used in Nigeria and other parts of West Africa in the treatment of wounds and ulcers. The study was conducted to investigate the gastric ulcer protective effect of ethanolic stem bark extract of *M. discoidea* in rats.

Materials and methods: Antiulcer assays were performed using ethanol, indomethacin and pylorus ligation-induced ulcer models at the dose of 50, 100 and 150 mg/kg, p.o. The antioxidant effect of the extract was evaluated *in vitro* and by studying its effect on antioxidant enzymes (superoxide dismutase, catalase, and reduced glutathione) and lipid peroxidation in the stomach tissue of rats in ethanol-induced model. Solvent fractions (hexane, dichloromethane, ethyl acetate, butanol and aqueous) from the crude extract were investigated for antiulcerogenic effects in ethanol-induced ulcer model at the dose of 150 mg/kg. GC–MS analysis of the active hexane fraction was also carried out.

Results: The extract significantly ($P < 0.05$) reduced gastric lesion in ethanol and indomethacin-induced ulcer models. The extract had significant influence on *in vivo* antioxidant status in ethanol-induced model. In pylorus ligation-induced model, only the dose of 150 mg/kg showed significant reduction (88.89%, $P < 0.05$) of ulcer lesions. There was no significant reduction in the gastric juice volume and total acidity. The solvent fractions showed ulcer inhibition in varying degrees but significance ($P < 0.01$) was only observed in the hexane fraction. Ethyl esters of palmitic and linoleic acids were found as the major compounds in the GC–MS analysis of the hexane fraction.

Conclusion: Our results suggest that *M. discoidea* possesses gastroprotective activity possibly mediated through antioxidant mechanism. The data obtained in this study provide some support to the traditional use of *M. discoidea* in the treatment of gastric ulcer.

1. Introduction

Gastric ulcer is the most common form of peptic ulcer and the most predominant of the gastrointestinal diseases (Falcao et al., 2008; Lakshmi et al., 2010). It is a chronic and recurrent disease, with multi-etiopathological factors. Stress, smoking, *Helicobacter pylori* infection and ingestion of non-steroidal anti-inflammatory drugs (NSAID) augment the gastric ulcer incidences (Vonkeman et al., 2007). Free radicals, particularly reactive oxygen species (ROS) have also been implicated in the mechanism of acute and chronic ulceration in the gastric mucosa (Sathish et al., 2011). An approach to manage gastric ulcer disease, therefore, is through the scavenging of ROS and the stimulation of the endogenous antioxidant enzymes in the stomach, in addition to the other approaches such as, the inhibition of gastric H^+K^+-ATPase and the elimination of *H. pylori* using antibiotics (Nartey et al., 2012).

The current trend of research is the investigation of medicines of plant origin because medicinal plants enjoy wide acceptability by the population and serve as cheaper alternatives to orthodox medicine (Akah and Nwabie, 1994), especially in the developing countries. In line with this, the potential gastroprotective and antioxidant properties of *M. discoidea* were investigated.

M. discoidea (Baill.) G. L. Webster (Euphorbiaceae) is a tree which can grow up to 30 m tall depending on its location. The stem is usually straight with rough, flaking bark which is greyish–brown on top and reddish beneath. It is widely distributed in Africa region where it is being used for treatment of various ailments. In Nigeria and Ghana, the decoction of the bark is used in the treatment of wounds and ulcers while in Malawi, the

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powdered bark extract is applied to swellings and inflammation for quick relief (Irvine, 1961; Burkill, 1994).

Previous pharmacological studies of *M. discoidea* have shown that the plant displays acaricidal (Kaaya et al., 1995), anti-inflammatory and analgesic (Adedapo et al., 2009), filaricidal (Cho-Ngwa et al., 2010) and cytotoxic (Johnson-Ajinwo et al., 2015) activities. Securinega alkaloids such as phyllochrysine and securinine (Mensah et al., 1988; Weenen et al., 1990; Fehler, 2000), betulinic acid (Calixto et al., 1998), hydroxylgenkwanin-8-C-[α-rhamno-pyranosyl-(1→6)]-β-glucopyranoside (margadiscoside), genkwanin-6-C-[α-rhamnopyranosyl-(1→6)]-β-glucopyranoside, kaempferol-3-O-α-rhamnopyranosyl-(1→2)-β-glucopyranoside-7-O-α-rhamnopyranoside and kaempferol-3-O-α-rhamnopyranosyl-(1→2)-[α-rhamno-pyranosyl-(1→6)]-β-glucopyranoside-7-O-α-rhamnopyranoside (Ekuadzi et al., 2014) have been reported isolated from the plant. No report was found in the literature that demonstrates the gastroprotective property of the plant. Therefore, the present study was carried out to evaluate the gastroprotective potential of the ethanolic extract of *M. discoidea.*

2. Materials and methods

2.1. Plant material and extract preparation

The stem bark of *M. discoidea* was collected in Ikire (7.35 latitude, 4.18 longitude), Osun state in south-western Nigeria in the month of February, 2013. The specimen was authenticated by Mr. T. K. Odewo at the Herbarium of the Department of Botany, Faculty of Science, University of Lagos, with a voucher specimen number, LUH 5552.

The stem bark was cleaned, cut into small pieces and dried in the oven at 40 °C. The dried material was powdered using a laboratory mechanical grinder. The powdered material (900 g) was macerated twice with absolute ethanol (2.5 L) for 48 h, at room temperature. The extract was filtered and evaporated to dryness in a water bath at 40 °C. The extract obtained was dark brown in color and the percentage yield was 5.98% (w/w).

2.2. Fractionation of the crude extract

The extract (30 g) was dissolved in 80 ml of water and fractionated by successive solvent extraction with n-hexane (2 ml × 200 ml), dichloromethane (2 ml × 750 ml), ethyl acetate (2 ml × 750 ml) and n-butanol saturated with water (2 ml × 750 ml) in a separating funnel. Each extract as well as remaining aqueous phase after solvent extractions was evaporated to dryness to yield hexane (HEX, 0.33 g), dichloromethane (DCM, 7.10 g), ethylacetate (EtOAc, 1.92 g), butanol (BuOH, 1.92 g) and aqueous (AQU, 3.23 g) extracts, respectively.

2.3. Phytochemical screening

Preliminary phytochemical screening of the crude extract was carried out using established procedures (Harborne, 1998; Trease and Evans, 2002). Quantification of polyphenolic classes including the estimation of total phenolics (Wolfe and Liu, 2003), flavonoids (Ordonez et al., 2006) and proanthocyanidins (Sun et al., 1998) was carried out.

2.4. GC–MS analysis of the active hexane fraction

Agilent 6890N GC system furnished with an auto sampler (Agilent 7683 injector series) was coupled to a 5973 Network mass selective detector (GC–MS) (based on a quadruple mass separator) was used to run the hexane fraction of the plant. A J&W Scientific HP-5MS silica fused capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness) was used with helium as the carrier gas at a constant flow rate of 1.0 ml/min. Splitless injection of 2 μl of the sample was automatically done by an injector (injector 7683 series) on the instrument from a syringe 10 μl. The oven temperature range was set at 70 °C and ramped at 4 °C/min to 250 °C. The injector temperature was set at 250 °C and detector temperature 280 °C. Mass spectra were taken at 70 eV with a mass range of m/z 40–500. Identification of the components was achieved by comparison of the retention time and mass spectra of each separated peak with the databank of the instrument, NIST 2005 library and the literature.

2.5. Animals

Healthy male Wistar albino rats weighing between (100–150 g) and male Swiss albino mice (20–30 g) were used in this study. Animals were housed in polypropylene cages at controlled conditions of a light and dark cycle (12 h/12 h) and a temperature of 22 ± 2 °C. They were given pellet feed (Vital feed, UAC PLC, Nigeria) and water *ad libitum*. The experiments were performed after getting necessary approval from the Institutional Animal Ethical Committee (CM/COM/08/VOL.XXV) of University of Lagos and governed by the United States National Academy of Sciences Guide for the Care and Use of Laboratory Animals (2011).

2.6. Acute toxicity test

Acute toxicity of the ethanolic extract of *M. discoidea* was determined in male Swiss albino mice according to the method of Hilaly et al. (2004) with slight modifications. Mice fasted for 18 h were randomly divided into five groups of six mice per group. Graded doses of the extract (400, 800, 1600, 3200 and 5000 mg/kg) were separately administered by gavage using a suitable canula to the mice in each of the groups and the control group received distilled water (10 ml/kg). All animals were then observed for toxic symptoms and mortality for 24 h and then over a period of 7 days.

2.7. Gastroprotective activity

2.7.1. Effect of M. discoidea extract on ethanol-induced ulcer

The experiment was performed with slight modifications of the method reported by Kim et al. (2008). Rats were divided into five groups ($n=7$) and fasted for 24 h prior to oral administration of vehicle (water, 10 ml/kg), misoprostol (0.1 mg/kg) or extract (50, 100 and 150 mg/kg). One hour later, absolute ethanol (1 ml) was orally administered to the rats for the induction of gastric ulcer. Animals were sacrificed 1 h later after ethanol administration; their stomachs were removed and longitudinally excised along the greater curvature and rinsed thoroughly in normal saline. This was followed by macroscopic examination of the gastric mucosal for ulcer lesions. The number, length and severity of the ulcers were noted and scored on an arbitrary 0–6 point scale (Galati et al., 2001). The % of ulcer inhibition was calculated in relation to the ulcer index as follows: *UI*=Total ulcer score/No of ulcerated animals

% of inhibition $=(A_0-A_1)/A_0\times 100$ where A_0=ulcer index of control and A_1=ulcer index of treated group.

This model was also used for the screening of the fractions at the dose of 150 mg/kg. This dose gave the best activity in all the three models used in this study.

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