Original Research Article

Evaluation of Antiulcer Activity of *Commiphora africana* (A. Rich) Engl. (Burseraceae) Stem-bark Extracts Using Ethanol Induced Ulcer Model in Albino Rats

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ABSTRACT

This study was designed to evaluate the antiulcer activity of *Commiphora africana* stem-bark extracts using ethanol induction model in laboratory rats and to identify phytochemical constituents of the extracts responsible for the observed activity. *C. africana* stem-bark was extracted with n-hexane, ethyl acetate and methanol gradient wise in a soxhlet apparatus at 50°C. The extracts were subjected to qualitative phytochemical analysis, toxicity experiment and anti-ulcer evaluation using ethanol – induced gastric ulcer in laboratory rats. A standard anti-ulcer agent, omeprazole was used as reference standard. The data were analyzed by one-way Analysis of Variance with significant level at (p<0.05). The percentage yield from the gradient extraction of *C. africana* stem-bark showed methanol to be the highest. Thin Layer Chromatographic analysis visualized with specific reagents confirmed the presence of steroids/triterpenes, phenolic compounds and flavonoids in the stem-bark of *C. africana*. LD₅₀ was above 5000 mg/kg and did not cause mortality in all the tested rats. Ethanol triggered severe gastric ulcers with mean ulcer index (12.8 ± 0.97 mm) and pretreatment with *C. africana* stem-bark n-hexane, ethyl acetate and methanol extracts at (250, 500 and 1000 mg/kg) and Omeprazole (20 mg/kg) produced a significant (p<0.05) dose dependent anti-ulcer activity with increase in percentage ulcer inhibition of (9.38%, 43.75% and 57.81%) for CAHE, (59.38%, 68.75% and 85.94%) for CAEE and (65.63%, 73.44% and 90.63%) for CAME respectively. Omeprazole had 68.75% ulcer inhibition. This study demonstrated that *C. africana* has anti-ulcer potential and it justified the traditional uses of the stem-bark in ulcer treatment.

Keywords
*Commiphora africana*, TLC, Acute toxicity, Antiulcer activity, Ethanol.

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Introduction

Peptic ulcer is one of the most common, chronic gastrointestinal disorders in this modern era. Now it has become a common global health problem affecting a large number of people world-wide and also still a major cause of morbidity and mortality (Chan, 2002; Soll, 1998). It is now considered to be one of the modern age epidemics affecting nearly 10% of world population (Zapata-Colindres et al., 2006).
About 6,000 die of ulcer related complication. Peptic ulcer causes significant morbidity which is mainly related to pain and hospitalization for complication (Buger et al., 2000). Many studies indicate that plant products are potential agents for healing ulcers and largely preferred because of the absence of unwanted side effects and their effectiveness (Jhasnsi et al., 2010).

*Commiphora africana* (A. Rich) Engl. is a species in the family Burseraceae. The plant is called “dashi” in Hausa, “badadi” in Fulf dulde and “kabi” in Kanuri languages of Nigeria (Dalziel, 1937; Keay, 1989). It is a bush shrub or small tree mainly found in the savannah woodland and drier parts of tropical Africa (Ekarika et al., 2012). It is traditionally used for the treatment of a number of ailments including the treatment of typhoid, wound healing, relieve pain, dysentery, heart burn, snake-bites, as anti-malaria, as a plaster and spasms (Lewis and Elvin-Lewis, 1977, Hanus et al., 2005). The ethanolic leaf Extract was found to have lipid profile activity in laboratory rat and antimicrobial activity (Adebayo et al., 2006; Goji et al., 2009). The stem-bark was reported to have hypoglycaemia effect in laboratory animals (Aliyu et al., 2002). Traditionally, the powdered stem-bark of the plant taken with banana has been used for the treatment of ulcer (Mal. Zakir Abdulhamid, Personal communication). This study was designed to evaluate the antiulcer activity of *C. africana* the stem bark and to identify phytochemical constituents of the extracts responsible for the observed activity.

**Materials and Methods**

**Collection Identification and Preparation of the Plant Material**

The plant material was collected at “Yankarfi” village, Sabo Gari Local Government Area of Kaduna State, in May, 2015. The plant was taxonomically authenticated at the Herbarium unit, Department of Biological Sciences, Ahmadu Bello University Zaria, Nigeria with Voucher specimen number 2848. The stem-bark was dusted, cleaned and all foreign matter removed, it was then air-dried and comminuted to powder form, stored in an air-tight container for subsequent use.

**Preparation of Extracts from Commiphora africana Stem-bark**

The solvents used during the course of this study were of analytical grade. Powdered sample (1kg) was extracted with n-hexane, ethyl acetate and methanol (JHD, Lobal Chem, India) gradient wise in a soxhlet apparatus at 50ºC. The plant material was exhaustively extracted with hexane (2 L) until the solvent became clear and the same procedure was applied consecutively to ethyl acetate and methanol. The gradient extracts obtained with each solvent were concentrated on a rotary evaporator and finally dried to a constant weight after which it was stored in an air-tight container for subsequent use (Kokate, 2003) with modification. The extracts were subsequently coded as CAHE (*C. africana* Hexane Extract), CAEE (*C. africana* Ethyl Acetate Extract) and CAME (*C. africana* Methanol Extract). The percentage yield was calculated using the formula:

\[
\text{Percentage Yield of extracts} = \left(\frac{\text{Weight of total extract}}{\text{Weight of powdered material}}\right) \times 100
\]

**Phytochemical Analysis**

Preliminary phytochemical screening of hexane, ethyl acetate and methanol extracts was carried out for the detection of steroids/triterpenes, flavonoids, saponins, tannins, cardiac glycosides, alkaloids and
anthraquinones using standard chemical tests (Harborne, 1992; Evans, 2002 and Sofowora, 1993).

**Thin Layer Chromatographic Profile**

Thin layer chromatographic analysis was performed on pre coated silica gel plates with silica gel 60 F<sub>254</sub> (Merck, Germany) using the one way ascending technique. Hexane: ethyl acetate (3:2) and n-butanol: acetic acid: water (6:1:1) were used as the mobile phase. Developed plates were visualized using general detecting reagent (anisaldehyde/H<sub>2</sub>SO<sub>4</sub>) and specific detecting reagents (Gennaro, 2000).

**Experimental Animals**

Male albino rats weighing 150–180 g were obtained from the animal house of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. The animals were maintained under standard conditions (12 hours light /12 hours dark cycle, temperature of 37 ± 2°C, 35–60% humidity). The rats were fed with standard (grower) mash (Vital feed, Jos, Nigeria) and water ad-libitum. Animals were procured two weeks before the experiments to acclimatize with the laboratory environment. Ethical rules guiding the use of animals for experimentation were strictly adhered to (DHHS, 1985).

**Acute Toxicity Study**

The acute toxicity of the *C. africana* stem-bark extract was determined by method of Lorke (1983). The study was carried out in two phase. The first phase consist of nine rats divided into three groups of three rats each and were treated with the n-hexane extract at doses of 1600, 2900 and 5000 mg/kg body weight orally. The oral median lethal dose (LD<sub>50</sub>) was calculated as the geometric mean of the minimum toxic dose and maximum tolerated dose using the second phase. This procedure was repeated for ethyl acetate, and methanol extracts.

**Anti-Ulcer Activity**

Ethanol-induced ulcer, were evaluated in albino rats as described by modified method of Nwafor *et al.*, (2000). The rats were fasted for 48 hours to produce significant effect of the drug. Fifty five adult albino rats were weighed, marked and randomly assorted into 11 groups (1-11) of five rats each. The separated groups were pretreated with 10 ml/kg distilled water as negative control, standard drug (Omeprazole 20 mg/kg) as positive control, *C. africana* stem-bark n-hexane, ethyl acetate and methanol extracts (250, 500, 1000 mg/kg) orally using an orogastric cannula. After 45 minutes, gastric lesion was induced in all the groups with absolute ethanol at a dose of 1 ml, administered by orogastric intubation. After an interval of one hour the rats were sacrifice by cervical dislocation and their stomach were carefully removed. Each stomach was cut open through the greater curvature with a scissor and rinsed, stretched lightly and spread on a filter paper for proper viewing and assessment of ulcers.

**Measurement of Ulcer Index**

The stomachs were examined for ulcer macroscopically. The extent of the mucosal damage were measured by using a calibrated meter rule (in millimeters) and the ulcer indices measurement was done from left to right of each tissue. The average mucosal damage was determined and the ulcer index (U.I) was calculated (Okasha *et al.*, 2008). The effectiveness of the extract and drugs
was calculated using the following formula (Kayode and Kayode, 2009).

\[
\% \text{ ulcer inhibition} = \frac{\text{U.I.(Ulcer control)} - \text{U.I.(Treated)}}{\text{U.I.(Ulcer control)}} \times 100
\]

**Statistical Analysis**

The results were presented in tables and expressed as mean ± standard errors of the mean (SEM) for all values. The data was statistically analyzed using one-way ANOVA followed by Dunnett’s post hoc. Results were considered to be significant at (P<0.05).

**Results and Discussion**

**Preparation of the Extract**

Percentage yield of the stem-bark of C. africana is given in the Table 1. The percentage yield from the gradient wise extraction of C. africana stem bark showed methanol to be the highest which was followed by n-hexane and finally ethyl acetate. These could be attributed to the ability of highly polar solvents to attract more of the phytochemical constituents present in a plant material.

**Phytochemical Screening**

The results of preliminary phytochemical screening of the C. africana stem-bark extracts had revealed the presence of some secondary metabolites namely alkaloids, tannins, flavonoids, cardiac glycosides, saponins, steroids/triterpenes and anthraquinones (Table 2). These secondary plant metabolites are known to posses various pharmacological effects and may be responsible for various action of C. africana. This result is in agreement with the findings of (Ezekiel et al., 2010).

**Thin Layer Chromatographic Profile**

Thin layer chromatographic analysis of n-hexane, ethyl acetate and methanol extracts from C. africana stem bark in different solvent systems in different ratios gave various degrees of separations. The n-hexane (A) and ethyl acetate (B) extracts developed in hexane: ethyl acetate (6:4) and visualized with p-Anisaldehyde revealed twelve (12) clear and distinct spots for both extracts, while methanol (C) extract revealed eleven (11) clear spots in butanol: acetic acid: water (6:1:1) visualized with p-Anisaldehyde and with their Rf values (Plate 1). The chromatogram of hexane extract was positive to Liebermann-Buchard reagent which revealed the presence of steroids/triterpenes (Plate 2) and it was negative to both ferric chloride and aluminum chloride reagent. The chromatogram of ethyl acetate extract was positive to Liebermann-Buchard, ferric chloride and aluminum chloride reagent (which was observed under UV light at 254nm after spraying the plate) which revealed the presence of steroids/triterpenes, phenolic compounds and flavonoids respectively (Plate 3). Methanol extract was positive to Liebermann-Buchard, ferric chloride and aluminum chloride reagent which revealed the presence of steroids/triterpenes, phenolic compounds and flavonoids respectively on TLC (Plate 4). The yellow fluorescence of aluminum chloride reagent (which was observed under UV light at 254nm after spraying the plate) was faint which would be as a result of the degree of concentration of the compound in the methanol extract. The successful separation of bio-molecules by chromatographic technique depends upon suitable solvent system which needs an ideal range of partition coefficient (k) for each target compounds (Ito, 2005). The solvent system, hexane: ethyl acetate (3:2) gave a
better separation for n-hexane and ethyl acetate extracts in this study. For methanol extract, butanol: acetic acid: water (6:1:1) was a good solvent combination for TLC of *C. africana* stem-bark. Furthermore, chromatograms from the extracts confirmed the presence of steroids/triterpenes, phenolic compounds and flavonoids on TLC. The presence of these compounds supports the traditional use of the plant in treatment of ulcer. Thin layer chromatographic analysis is a simple and cheap method for detection of plant active constituents due to its good selectivity and sensitivity of detection providing convincing results (Patra et al., 2012).

**Table 1** Mass and Percentage Yield for the Crude Extracts of *C. africana*

<table>
<thead>
<tr>
<th>S/No.</th>
<th>Extract</th>
<th>Mass (g)</th>
<th>Percentage Yield (w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>CAHE</td>
<td>24.12</td>
<td>2.41</td>
</tr>
<tr>
<td>2.</td>
<td>CAEE</td>
<td>18.54</td>
<td>1.85</td>
</tr>
<tr>
<td>3.</td>
<td>CAME</td>
<td>112.98</td>
<td>11.30</td>
</tr>
</tbody>
</table>

CAHE = *C. africana* hexane extract, CAEE = *C. africana* ethyl acetate extract, CAME = *C. africana* methanol extract

**Table 2** Preliminary Phytochemical Screenings of Extracts of *C. africana* Stem-bark

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>CAHE</th>
<th>CAEE</th>
<th>CAME</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saponins</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Steroids/Triterpenes</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Key: Present (+), Absent, (-)

**Table 3** Effect of *C. africana* Stem-bark N-hexane, Ethyl Acetate and Methanol Extract on Ethanol Induced Gastric Ulcer

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose</th>
<th>MUI (mm) ± SEM</th>
<th>UI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>1ml</td>
<td>12.80 ± 0.97</td>
<td>68.75</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>20 mg/kg</td>
<td>4.00 ± 0.71*</td>
<td></td>
</tr>
<tr>
<td>CAHE</td>
<td>250 mg/kg</td>
<td>11.60 ± 0.40*</td>
<td>9.38</td>
</tr>
<tr>
<td>CAHE</td>
<td>500 mg/kg</td>
<td>7.20 ± 0.37*</td>
<td>43.75</td>
</tr>
<tr>
<td>CAHE</td>
<td>1000 mg/kg</td>
<td>5.40 ± 0.40*</td>
<td>57.81</td>
</tr>
<tr>
<td>CAEE</td>
<td>250 mg/kg</td>
<td>5.20 ± 0.37*</td>
<td>59.38</td>
</tr>
<tr>
<td>CAEE</td>
<td>500 mg/kg</td>
<td>4.00 ± 0.32*</td>
<td>68.75</td>
</tr>
<tr>
<td>CAEE</td>
<td>1000 mg/kg</td>
<td>1.80 ± 0.58*</td>
<td>85.94</td>
</tr>
<tr>
<td>CAME</td>
<td>250 mg/kg</td>
<td>4.40 ± 0.51*</td>
<td>65.63</td>
</tr>
<tr>
<td>CAME</td>
<td>500 mg/kg</td>
<td>3.40 ± 0.24*</td>
<td>73.44</td>
</tr>
<tr>
<td>CAME</td>
<td>1000 mg/kg</td>
<td>1.20 ± 0.20*</td>
<td>90.63</td>
</tr>
</tbody>
</table>

Key: MUI-mean ulcer index, SEM-standard error mean, UI- ulcer inhibition, (-) - negative, (+) - positive. *: values are statistically significant (p<0.05) with negative control.
Fig. 1 Mean Ulcer Indices of Various Groups

Fig. 2 Percentage Ulcer Inhibition of Various Groups
Plate 1 Chromatogram of Hexane (A), Ethyl Acetate (B) and Methanol (C) Extracts Sprayed with *P*-Anisaldehyde/H$_2$SO$_4$ with Rf Values

Plate 2 Chromatogram of Hexane Extract in Hexane: Ethyl Acetate (3:2) Spray with (LB) Lieberman-Buchard Reagent and its Rf Values
Plate.3 Chromatogram of Ethyl Acetate (EA) Extract in Different Spraying Reagent (AlCl3-Aluminium Chloride (A), FeCl2- Ferric Chloride (B) and LB-Lieberman-Buchard (C) with Rf values

Plate.4 Chromatogram of Methanol Extract in Different Spraying Reagent (LB-Lieberman-Buchard (A) and FeCl2- Ferric Chloride (B) with Rf Values

Acute Toxicity

The Acute toxicity ($LD_{50}$) of the extracts (hexane, ethyl acetate and methanol) of the stem bark of *C. africana* was carried out orally in rats. The $LD_{50}$ was found to be greater than 5000 mg/kg when administered orally in rats. These studies showed that *C.*
"Commiphora africana" stem bark is practically non-toxic when administered using the oral route. Based on the toxicity classification by Loomis and Hayes (1996), substances with LD$_{50}$ values of 5000 to 15,000 mg/kg body weight is practically non-toxic.

**Antiulcer Evaluation**

"Commiphora africana" stem-bark extracts produced significant dose-dependent antiulcer activity at all the doses tested. The least percentage ulcer inhibition was observed in n-hexane extract while methanol extract recorded the highest percentage ulcer inhibition (Table 3). Hexane extract of "C. africana" at dose 250 mg/kg did not show significant (p>0.05) reduction in the mean ulcer index (11.60 ± 0.40) after pretreatment. For ethyl acetate and methanol extracts all the doses tested showed significant (p<0.05) reduction in the mean ulcer index. Ethanol triggered severe gastric ulcer with mean ulcer index (12.80 ± 0.97 mm) and pretreatment with "C. africana" stem-bark of n-hexane, ethyl acetate and methanol extracts at (250, 500 and 1000 mg/kg) showed a dose dependent decrease in mean ulcer index. This result is in agreement with the finding of Al-Harbi and coworkers who report "Commiphora molmol" (oleo-gum resin) pretreatment at doses of 250, 500 and 1000 mg/kg provided dose-dependent protection against the ulcerogenic effects of different necrotizing agents used (Al-Harbi et al., 1997).

Both Methanol and ethyl acetate extracts at 1000 mg/kg showed better gastro-protective effect over omeprazole (20 mg/kg). The increase in the antiulcer activity of ethyl acetate and methanol extracts could be attributed to the presence of flavonoid, steroids/triterpenes and phenolic compound in the extracts. Mahran et al., (1991) have reported that plant drugs containing saponins, terpenoids or amino acid have anti-ulcer activity. Flavonoids have anti-inflammatory activity and protect the gastric mucosa against a variety of ulcerogenic agents in different mammalian species (Harborne and Williams, 2000). Tannins are known to ‘tan’ the outermost layer of the mucosa and to render it less permeable and more resistant to chemical and mechanical injury or irritation (Asuzu and Onu, 1990). Several plants containing high amounts of saponins have been shown to possess anti-ulcer activity in several experimental ulcer models (Izzo et al., 2000), this may have accounted for the highest activity observed in methanol extract compared to other extracts (Table 2). Therefore "C. africana" stem-bark extracts possesses strong gastro-cytoprotective properties against ethanol-induced gastric ulcer. Various phytochemicals like flavanoids, tannins, saponins, terpinoids showed their anti-ulcer activity due to their cytoprotection, antisecretory and antioxidant property (Sen et al., 2009). Some phytochemical compounds such as flavonoid groups may prevent or suppress ulcerogenic process. This is in agreement with previous reports which shown that "Cassia singueana" leaf has flavonoid compound which exhibit a gastro protective effect against ethanol – induced stomach ulcers (Ode and Asuzu, 2011). Ethanol is widely used to induced ulcers (Kayode and Kayode, 2009). This are done by suppressing the protective action of the mucus secreted by mucus membrane the increased synthesis of mucus can be explained as the probable cytoprotection mechanism in this case (Cho and Ogle, 1992).

In conclusion, Stem-bark extracts of "C. africana" exhibited a significant anti-ulcer activity in experimental rats. The results support the traditional claim of the plant in treatment of ulcer.
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