Antiulcer Effects of Ethyl acetate Fraction of *Carpolobia lutea* Leaf

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**ABSTRACT**

*Carpolobia lutea* leaves (CLL) (Polygalaceae) were earlier screened and the antiulcer ethnomedicinal claim established. This article seeks to quantitatively isolate, elucidate the active compounds from most active CLL fraction. Fractionation was by semi-preparative HPLC; the active fraction was subjected to radical scavenging assays (RSA) and quantification of the total phenolic content (TPC) were also executed. Results: Ethyl acetate fraction (EAF) was observed to be the most pharmacologically active antiulcer fraction when screened using acute ulcer models induced in rodents. The EAF demonstrated significant (p < 0.05-0.001) antiulcer activity in various *in vivo* induced ulceration models by reducing the ulcer index and increasing the preventive ratio. The EAF demonstrated > 70% in TPC and < 20 % in RSA. Cinnamic and coumaric acids derivatives were isolated from EAF. Cinnamic acids have been implicated and patented as antiulcer agent. Isolated compounds could in part mediate the observed pharmacological activities which lend credence to its ethnobotanical uses.

**Keywords:** *Carpolobia lutea*; Polygalaceae; cinnamoyl-, coumaroyl- 1-deoxyglucoside; ulcerogenic.

**INTRODUCTION**

The burden of non–communicable diseases account for 69% of all global deaths by 2030 with 80% of these deaths in low-income and middle-income countries (Samb et al., 2010). Approximately 50% (over 3 billion) of the world populations are known to be infected with *Helicobacter pylori*, mainly in the developing countries. Among those, hundreds of millions of people develop peptic ulceration during their lifetime and still tens of millions might progress to gastric cancer (Barik, 2009). Besides, approximately 60% of the world’s population relies on plants for medication (Falbriant and Farnsworth, 2001) and plants have been the raw material for synthesis of many drugs and new therapeutic agents (Andre et al., 2006). Traditional remedies world-wide are used to treat various gastric disorders since thousands of years (Gurbuz and Yesilada, 2007). Plants with divers chemical compounds possessing anti-ulcerogenic property have been reviewed (Lewis and Hanson, 1991).
Polygalaceae is known to contain species with a variety of polyphenolic compounds such as xanthones, flavonoids and biphenyl derivatives, which exhibit significant biological activities (Cervellati et al., 2004). The plant *Carpolobia lutea* (CL) is called cattle stick (English), Ikpafum (Ibibio), Agba or Angalagala (Igbo) and Egbo Oshunshun (Yoruba) in Nigeria (Etukudo, 2003; Muanya, 2008). The leaf is used to cure rheumatism, fever, pains, insanity, dermal infection, venereal diseases, sterility and to promote child birth. In addition, it is used as vermifuge and stomach medicine (Burkil, 1984; Muanya and Odukoya, 2008; Irvine, 1961). The stem bark is dried and taken as snuff to cure migraine headache (Irvine 1961). The leaf is reported to have anti-inflammatory and anti-arthritis properties (Iwu and Ayanwu 1982) and to cure diabetes mellitus (Okon Etefia, Personal communication). Fever with diarrhea, headache, leprosy, snakebite, venereal disease and wounds are reported to be cured by the leaf extract (Lewis and Elvin-Lewis, 1977; Ajibesin et al., 2008).

The anti-diarrhoeal and anti-ulcerogenic potential of crude ethanolic extract of *Carpolobia lutea* leaf (CLL) have been established experimentally in rodents (Nwafor and Bassey, 2007). Preliminary evaluation of various fractions of the leaf extracts of CL revealed the ethyl acetate extract as the most gastro-protective (Nwidu and Nwafor, 2009). Although CLL is patronised traditionally for the management gastric ulcers and diabetes, the biological active molecules are yet to be elucidated. This work reports the single dose (770 mg/kg) protocol with cysteamin-induced duodenal ulcer and evaluates the antiulcer effects of different doses of ethyl acetate fraction (EAF) in rodent. HPLC fingerprint of the EAF revealed the presence of polyphenolics in high concentration.

Polyphenolics are secondary metabolites, ubiquitous in the plant kingdom and are widely present in the human diet. They have shown to exert beneficial influence on human health (Manach et al., 2004). Phenolic compounds are potent antioxidants, and epidemiological studies have suggested a direct correlation between their high intake with diet and reduced risk of coronary heart disease mortality by suppressing the oxidation of low-density lipoprotein [19]. Cinnamic acid and p-coumaric acid antiulcer effects have been demonstrated (Frey et al., 2001; Pereira et al., 2003; Funari et al., 2007; Siddaraju and Shylaja, 2007; Barros et al., 2008). Two new cinnamoyl 1-deoxyl glucosides, cinnamic acid, besides two new coumaroyl 1-deoxyl glucosides, were isolated by semi-preparative HPLC and the structures was established using NMR experiments Nwidu et al., 2011.

This study quantitatively isolate, elucidate the active compounds from the EAF by semi-preparative HPLC and subjecting the fraction to radical scavenging assays (RSA). The total phenolic content (TPC) was also quantified.

**MATERIALS AND METHODS**

**Chemicals**

The chemicals used were all of analytical grade: absolute ethanol, ethyl acetate, chloroform, acetic acid (Reidel-de Haen, Germany), methanol (Synth, Brazil), cimetidine, omeprazole, propanolol, cysteamin hydrochloride, acetyl salicylic acids, indomethacin were all purchased from Sigma Chemical Co (St. Louis, USA).

**Preparation of Plant Material**

*Carpolobia lutea* leaves were collected and supplied by Mr. Okon Etefia, the traditional herbalist, attached to the Pharmacognosy Department, University of Uyo. The plant was identified and authenticated by Dr. (Mrs.) Margaret Bassey of Department of Botany, University of Uyo, Akwa Ibom State, Nigeria. A voucher specimen (UUh 998) was deposited at the University Herbarium, University of Uyo, Akwa Ibom State, Nigeria. The CLL was air dried under shade for 4 days at 30˚C, powdered with pestle and mortar. The pulverized leaves were stored at room temperature.

**Extraction, Fractionation and HPLC Analysis of C. lutea**

Procedure of gradient solvent extraction is as described in previous article (Nwidu and Nwafor, 2009) and fractionation as described in Nwidu et al., 2011. The HPLC analysis and isolation have been described (Rodrigues et al., 2007; Latza et al., 1996; Hidradate et al., 2004). The structure elucidation of the isolated compounds is as reported in Nwidu et al., 2011.

**Determination of Total Phenolic Content (TPC)**

The TPC in CLL polar crude extract [crude ethanol extract (CETE) and crude ethyl acetate (CEAE)] and polar fraction [ethanol fraction (ETF) and ethyl acetate fraction (EAF)] were determined by *Folin-Ciocalteu’s* (FC) reagent using the methods of Zeng and Wang 2001 and Liu et al., 2002 with modifications. Gallic acid was used as standard. This test was performed by preparing each extract and fraction with a concentration of 100 µg/ml. To 1 ml solution of each extract (CEAE and CETE) and fraction (ETF and EAF) of *C. lutea* was added 1 ml deionised water and 0.5 ml of 1 N FC solution and thoroughly shaken in a test tube and allow standing for 1 minute. Later, 1 ml of 10 % Na2CO3 solution was added and vortex for 30 seconds. This was allowed to stand for 40 minutes. The same treatment was giving to gallic acid. The standard absorbance of both extract and fractions were read on a spectrophotometer at 760 nm. Blank samples without the test substances were prepared and the absorbance read also. For the analytical curve, 1 ml of aliquots of 20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 2200 µg/l aqueous gallic acid solution were used with 0.5 ml 1 N FC reagent. The linearity and reproducibility of the analysis was determined from six replicates. The results were expressed as mg of gallic acid equivalent (GAE)/l.

**Determination of Antioxidant Activity with 1, 1 Diphenyl, 2-Picryl Hidrazyl (DPPH)**

The antioxidant potential was evaluated by spectrophotometric assay according to the method of Germano et al., 2002 in the visible region, using a solution of 0.004% DPPH in methanol, which was added to the solution of test samples. Stock solutions of extracts and standards were prepared to obtain a final
concentration of 250 µg/ml (methanolic solution 20% Tween 80). Four standards samples were used: caffeic acid (CA), ferulic acid (FA) and gallic acid (GA). Each sample was prepared in a concentration of 5, 10, 20, 40, 80 and 160 µg/ml. Each sample (1 ml) was added to 2 ml of DPPH solution and after 30 minutes of reaction the absorbance of the solutions was measured at 517 nm. The reference solution (blank) consisted of DPPH in solution of 20% Tween 80. The spectrophotometer was calibrated with methanol. The sequestration of free radical activity of each sample was calculated by the inhibition of DPPH %Δ (decrease in absorbance) = (A₀ - Aₐ) x 100, (A₀ = absorbance of DPPH in methanol; Aₐ = absorbance of the sample after 30 min incubation). The values obtained were plotted on a graph of % change in absorbance versus concentration of extract, fraction and standards.

Animals
Adult albino mice and rats were used. All the animals were housed in standard cages under laboratory conditions in the University of Uyo, Pharmacology department. The animals used were fed with pellet feeds (Vital Feed and Flour Mill Limited, Edo State, Nigeria) and water ad libitum. All animals used have free access to tap water under standard conditions of 12 h dark–12 h light and temperature (21±1%). The protocols were approved by the University of Uyo Institutional Animal Care and Use Committee (UUAEC) which follows the guidelines of the internationally accepted principles for laboratory animal use and care as found in the European Community guidelines (EEC Directive of 1986; 86/609/EEC).

Determination of Median Lethal Dose (LD₅₀)
The median lethal dose LD₅₀ was determined by the method of Lorde, 1983 with modification. The Swiss albino mice used in this study were starved for 24 h with free access to water except for 2 h prior to experiment. Different doses (100, 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000 mg/kg i.p.) of the ETF, EAF and CEAE were administered to nine group of mice (n = 3 per dose) to establish the range of doses of the fraction and extract that would elicit toxic effects. The mice were observed for symptoms of toxicity and mortality intermittently for the next 6 hours if any and then again 24 hours post treatment for physical signs of toxicity. The LD₅₀ was estimated by the geometric mean of the highest dose that caused 0% mortality and the lowest dose that cause 100% lethality.

Pharmacological Assay
Single dose (770 mg/kg) of CLL fractions (n-hexane, chloroform, ethyl acetate and ethanol) were earlier evaluated using indomethacin-, ethanol-, reserpine in 0.5% acetic acid-, stress-, serotonin- and diethylthiocarbamate-induced ulcer models in rats. These assays were published in previous article Nwidu and Nwafor, 2007. In this work, we report the single dose fraction protocol with Cystaemin not published earlier in Nwidu and Nwafor, 2007. In addition, we report results of evaluation of three doses (192.5, 385.0 and 770.0 mg/kg) of EAF of CLL with indomethacin, ethanol and stress ulcer models. 20% of Tween 80 is used as vehicle for extract and fraction.

Effect of Fractions on Cystaemin-Induced Duodenal Ulceration in Rats
Male adult albino rats weighting between 113-123 g were used for this experiment. The rats were randomized and divided into 5 groups of 6 rats each. Food was withdrawn 24 h and water 2 h before the commencement of the experiment. Acute duodenal lesions were induced in rats using two doses cystaemin 300 mg/kg (p.o) in 1 ml of distilled water at 4 h intervals. Group 1 was given 300 mg/kg (p.o) as positive control. Groups 2 – 5 were pre-treated 0.5 h with 770 mg/kg CLL fractions of ethanol, ethyl acetate, chloroform and n-hexane, respectively before 300 mg/kg cystaemin. The CLL fractions were administered intragastrically via the aid of an orogastric cannula. 48 hours after the last dose of cystaemin, the animals were killed by cervical dislocation. The tissues were fixed with 10% formaldehyde in saline. The duodenum were removed and examined for lesion, the number and severity of gastric lesions and perforations were evaluated according to the procedure of Antonio et al., 2004. 

Effect of Ethyl Acetate Fraction on Indomethacin-Induced Gastric Ulceration in Rats
Pilot tests aimed at determining the effective dose of indomethacin needed to produce reliable acute gastric ulceration in rats were evaluated using varying doses of indomethacin: 0.03, 0.06 and 0.1 g/kg (b.wt.) on the rats. 0.1 g/kg of indomethacin per body weight of animal produced gastric ulceration in all rats in 5 h in the pilot study. Male adult albino rats weighting 140 – 145 g were used for this experiment. The rats were randomized and divided into 5 groups of 6 rats each. Food was withdrawn 24 h and water 2 h before the commencement of the experiment. Group 1 (positive control) was administered with 0.1 g/kg indomethacin, orally. Group 2 – 4 were pre-treated with 192.5, 385.0, 770.0 mg/kg EAF of CLL, respectively, 1 h prior to administration of 0.1 g/kg of indomethacin. Group 5 received cimetidine (1.0 x 10⁻³ g/kg, p.o) 1 h prior to administration of 0.1 g/kg of indomethacin. The drugs were administered intragastrically via the aid of an orogastric cannula 5 h later, the animals were killed by cervical dislocation. The stomach were removed and opened along the greater curvature. The tissues were fixed with 10% formaldehyde in saline. Macroscopic examination was carried out with the aid of a hand lens and scored for the presence of lesions using the method of Al-Said et al. 1986. Ulcer index (UI) of indomethacin alone, ulcer index and preventive ratio of each of the groups pre-treated with the EAF of CLL were calculated using the methods of Al-Said et al., 1986 and Nwidu and Nwafor, 2009.

Effect of Ethyl Acetate Fractions on Ethanol-Induced Gastric Ulceration in Rats
Male adult albino rats weighting between 215 – 225 g were used for this experiment. The rats were randomized and divided into 5 groups of 6 rats each. Food was withdrawn 24 h and water 2 h
before the commencement of the experiment. Ulcer lesion was established with 0.5 ml of 95% ethanol (p.o). Group 1 was given ethanol as positive control, groups 2-4 were pre-treated with 192.5, 385.0, 770.0 mg/kg EAF of CLL, respectively, while group 5 received omeprazole (3.0 x 10² g/kg, p.o) 1 h prior to administration of 0.5 ml of ethanol.

The three doses of EAF respectively were administered intragastrically via the aid of an orogastric cannula. The animals were killed 4 h later by cervical dislocation. The stomach were removed and opened along the greater curvature. The tissues were fixed with 10% formaldehyde in saline. Macroscopic examination was carried out with the aid of a hand lens and scored for the presence of lesions using the method of Barry et al., 1988. Ulcer index of ethanol alone, ulcer index and preventive ratio of each of the groups pre-treated with the EAF of CLL above and that of omeprazole (standard drug) were calculated using the method of Zaidi and Mukerji, 1958.

Effects of Ethyl Acetate Fractions on Water Immersion-Induced Gastric Ulceration in Rats

Male adult albino rats weighting 215–223 g were used for this experiment. The rats were randomized and divided into 5 groups of 6 rats each. Food was withdrawn 24 h and water 2 h before the commencement of the experiment. Group 1 (positive control) rats were placed individually in plastic cages measuring 5.0 x 5.0 x 30.0 cm. The animals were placed individually in each compartment of the cage and it was immersed vertically in water tank, water was added gradually to the level of the xiphoi. The temperature of the tank was maintained at 15 – 20 °C using ice pack to induce stress ulceration.

Group 1 was immersed in water without administration of the test samples. Group 2–4 were pre-treated with 192.5, 385.0 and 770.0 mg/kg of the EAF of CLL respectively, 1 h prior to immersion. While group 5 received cimetidine (1.0 x 10⁻³ g/kg, p.o) 1 h prior to immersion. The drugs were administered intragastrically via the aid of an orogastric cannula. 18 h later, the animals were killed by cervical dislocation. The stomach were removed and opened along the greater curvature. The tissues were fixed with 10% formaldehyde in saline. Macroscopic examination was carried out with the aid of a hand lens and scored for the presence of lesions using the methods of Takalgi and Okabe (1968). Ulcer index (UI) of rats immersed in water without drug alone, ulcer index and preventive ratio of each of the groups pretreated with the EAF of CLL were calculated using the method of Takalgi and Okabe (1968). The number and severity of gastric lesions were evaluated according to the following rating scale.

Statistical Analysis and Data Evaluation

Data obtained from this work were analysed statistically using Students’ T-test and by multiple comparisons of Mean ± S.E.M by one way and two way analysis of variance (ANOVA. One- or Two –way) followed by a post test (Turkey- Kramer multiple comparison test). A probability level of less than 5% was considered significant (P≤0.05).

RESULTS

Determination of Total Phenolics Content (TPC)

The quantification of TPC for the polar extract and fractions revealed that the percentage of TPC increased from 61.33, 78.67, and 90.78 to 136.22 µg/ml for ETF, EAF, CEAE and CETE of CLL respectively (Figure 1).

Determination of Antioxidant Activity with DPPH

The antioxidant activity of the C. lutea fractions and extract showed minimal radical scavenging activity when compared to standard (Figure 2).

Determination of Median Lethal Dose (LD₅₀)

The acute toxicity study LD₅₀ shows that median lethal dose is 3850.0, 3240.4 and 1414.2 mg/kg for the ETF, CETE and EAF, respectively. The signs of toxicity were observed from 1250 mg/kg, 3000 mg/kg and 3100 mg/kg for the EAF, ETF and CETE respectively. The mortality rate was observed from 1750, 3600 and 3200 mg/kg and rose to 2000, 4000 and 3500 mg/kg for the EAF, ETF and CETE respectively. The no observed-adverse-effect level (NOAEL) for the intraperitoneal dose was 1500 mg/kg and the lowest-observed-adverse-level (LOAEL) 3000 mg/kg. Signs of toxicity observed include restlessness, convulsion, salivation, defecation, urination, syncope, asthenia and death under 24 h doses.
Evaluation of Antiulcer Activity

The results of the antiulcer assays using these protocols are enumerated as follows:

Effect of CLL Fraction on Cysteamine-Induced Duodenal Perforation in Rats

The treatment with 770 mg/kg CLL fractions (ETF, EAF, CHF and n-HF) do not significantly reduced the ulcer index relative to control group (P ≥ 0.05). The preventive ulcer ratios are 32.64, 77.11, 55.79, 67.46 and 46.58 for the treated group with 770 mg/kg CLL fraction (ETF, EAF, CHF and n-HF) and famotidine, respectively as shown in Figure 3.

Effects of EAF of CLL on Indomethacin-Induced Ulceration in Rats

The result of gradient doses CLL EAF on indomethacin-induced ulceration in rat shows the median and the lowest dose to exert significant preventive ratio than the highest dose of the fraction. Pre-treatment with EAF (192.5-770 mg/kg) show the median and lowest dose statistically significant (p < 0.001) relative to control. The lowest dose followed by the median dose exhibit the highest antiulcer effect. The result is shown in Figure 4.

Effect of C. lutea Leaf Fractions on Stress-Induced (Water Immersion) Ulceration in Rats

The result shows that CLL EAF (192.5, 385.0, 770.0 mg/kg) and cimetidine (100 mg/kg) produce a dose dependent and significant reduction (P < 0.001) of the ulcer index as shown in Figure 6.

DISCUSSION

Dietary polyphenols has protective effects against various degenerative diseases (Scalbert et al., 2004; Manach and Scalbert, 2005) and possess antiulcer activity (Saito et al., 1988; Siddaraju and Shylaja, 2007b). Several phenolics compound have antiulcer
properties in rats (Sairam et al., 2002). Caffeic, ferulic, p-coumaric and cinnamic acids have demonstrated significant antiulcer activity (Barros et al., 2008). Phenolic acids play a major role in down regulating parietal cell H+K+-ATPase with cinnamic acid and p-coumaric exhibiting maximum inhibitory activity. They also inhibit ulcerogen- H. pylori- and exhibit anti-oxidative properties in vitro (Barros et al., 2008; Das et al., 1997). Oxidative stress is considered a common factor in the pathogenesis of ulcers in different experimental and clinical models (Das et al., 1997). The antioxidant activity of phenolics is associated to antiulcer activity because free radicals and ROS are the main causative factors for ulcer (Saito et al., 1988)[43]. Rajan et al., (2001) and Hung et al., (2005) demonstrated the strong antioxidant activity of phenolic acids and analogues. Besides, cinnamic acid is reported the best inhibitor of Helicobacter pylori (Siddaraju and Shylaja, 2007b). Therefore the isolation and characterisation of cinamoyl- and coumaroyl- 1-deoxyglucosides from the most antiulcer active fraction, EAF, could support the observed antiulcer properties. Free-radical-mediated cell injury and lipid peroxidation in various pathological phenomena have been reported (Halliwell, 1987). Numerous antioxidant methods and modifications have been proposed to evaluate antioxidant activity and to explain how antioxidants function. Of these, total antioxidant activity, reducing power, DPPH assay, metal chelating, active oxygen species such as H2O2, O2− and OH• quenching assays are most commonly used for the evaluation of antioxidant activities of extracts (Amarowicz et al., 2000; Chang et al., 2002).

In our study, we used DPPH test/method which is one of the oldest and most frequently used methods for total antioxidant potential/capacity of food extracts. DPPH is a stable free radical and is often used to evaluate the antioxidant activity of several natural compounds (Yokozawa et al., 1998). Antioxidants, on interaction with DPPH, transfer electron or hydrogen atom to DPPH, and thus neutralize its free-radical character. DPPH shows a strong absorption at 517 nm. It’s mechanism of action is based on the ability of an antioxidant to give hydrogen radical to synthetic long-lived nitrogen radical compound DPPH. A blue violet colour changes gradually to green and yellow (absorption maximum at 405 nm), and a decrease in absorbance at 517 nm is monitored. However, in our study, the RSA of the fraction was less than the extract. The RSA of both extract and fraction was less than 20%. This entails that the extract and fraction lack compounds which could donate either hydrogen or electron to DPPH. The RSA of fraction and extract was quite below that of caffeic acids, ferrulic acids and galic acids which are above 90%. The aromatic hydroxyl group plays a considerable antioxidative role by conferring stability to the radical form and participating in electron delocalization. Compounds with aromatic hydroxyl groups may be few in the extract and fraction as revealed by trans-cinnamic acids glucosides, the major isolated compounds from the EAF. The trans-cinnamic glucosides isolated (constitute 21.9% of isolated compounds) function as Michael’s electron acceptor and could not donate electron to reduce DPPH, hence the low RSA. Therefore, DPPH assay is not able to elucidate the mechanism of antioxidant activity of CLL as observed. The antioxidant activity of putative antioxidants have been attributed to various mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging (Oktay et al., 2003). The HPLC profiling of the extract and fraction reveal preconcentration of the polyphenols in EAF than the aqueous crude extract. Trans-cinnamic acids glucosides constitute 21.9% of these polyphenols in EAF. Present evaluation of the polar fractions and crude extract revealed the concentration of TPC to be 61.33, 78.67, 90.78 and 136.22 µg/ml for ETF, EAF, CEAE and CETE, respectively. The crude extract is richer in polyphenolic than the fraction. This result may in part be due to the presence in the crude extract many different phenolics components that were not isolated by EAF during the fractionation process. Different mechanisms have been proposed for the pathogenesis of cysteamin-induced gastrointestinal ulcer such as hypersecretion of gastric acid, reduced bicarbonate secretion in response to acid in duodenum and delayed gastric emptying (Szabo, 1987; Szabo, 1978; Poulsen et al., 1985). The results obtained shows that CLL EAF inhibited the formation of duodenal ulcer. This effect might be due to gastric acid inhibition or neutralization in the stomach or due to stimulation of the formation of mechanical adherent barrier (mucus) by EAF in the duodenum. Other possible mechanisms reported in literature are by stimulation of epidermal growth factor (EGF), endothelium-derived relaxing factor (nitric oxide) and non-protein sulphydryl derivatives, all are involved in maintaining the integrity of the mucosa (Salim, 1989; Donadel et al., 2005). It is also possible that the inhibition of gastric acid secretion and the mucus release in the duodenal mucosa may indirectly interfere with duodenal mucosa protection, thus maintaining its integrity. However, the exact mechanism of inhibition duodenal mucosa perforation is unknown but may not be unrelated to any of these reported mechanisms of maintaining gastric mucosal integrity. These results, taken together, suggest that the EAF may act by any of these mechanisms. However, other mechanisms may also be involved. Non-steroidal anti-inflammatory drugs such as indomethacin have the ability to cause gastroduodenal ulceration, and this effect is related to the ability of these agents to suppress prostaglandin synthesis (Wallace 2001). The gastrointestinal irritant property of indomethacin is the major impediment to their use as anti-inflammatory drugs (Chiba et al., 2008). Indomethacin inhibits prostaglandin production, increased acid production and decreased cytoprotective mucus formation leading to gastrointestinal ulcer (Wallace 2005; Hiruma-Lima 2009). The lower and middle dose of EAF were quite effective than the highest dose. This may be as a result of the higher dose of the extract exhibit pro-oxidant effects. That the EAF of CLL inhibited indomethacin - induced ulceration therefore suggests that it may partly mediate its effect through cyclo-oxygenase pathway. This finding indicates that active compounds present in EAF may enhance either gastric mucosal defensive factor and are effective than H2-receptor antagonists, cimetidine, the standard drug utilised in this assay.
Ethanol-induced ulcers predominate in the glandular part of the stomach, stimulate the formation of leukotriene C\(_4\) (LTC\(_4\)) and result in the damage of rat gastric mucosa (Dreiling \textit{et al.}, 1986; Cho \textit{et al.}, 1987). Ethanol-induced gastric lesions caused superficial damage to mucosal cells resulting in gastric mucosal change which may in part due to free radical formation (Oates and Hakkinen 1988), increase in lipid peroxidation and decrease of the levels of protein sulphhydryl compounds in gastric mucosa (Mui and Doteuchi 1986). Ethanol is metabolized in the body to cause increased production of O\(^2\)- within the tissues, and simultaneously increased free radical concentration. This free radical causes denaturation of DNA strands and protein; reduce gastric blood flow, haemorrhage, necrosis and solubilisation of mucus constituents in the stomach. These actions result in an increased flow of Na\(^+\) and K\(^+\), increased pepsin secretion, and a loss of H\(^+\) ions and histamine into the lumen (Szabo, 1987). It is also established that disturbances in gastric secretion, damage to gastric mucosa, alterations in permeability, gastric mucosa depletion and free radical production are observed after administration of ethanol (Salim 1990). Ethanol is one of the ulcerogenic agents that induce intense damage in gastric mucosa by promoting disturbances of mucosal microcirculation, ischemia and appearance of free radicals, endothelin release, degranulation of mast cell and inhibition of prostaglandins and decrease of gastric mucus production (Abdel-Salam \textit{et al.}, 2001). All the doses of EAF utilised in the experiment were very effective than the 30 mg/kg omeprazole. At a critical dose of 770 mg/kg, the same dose that gave 100% inhibition of intestinal transit (Nwidu \textit{et al.}, 2001), the ulcers induced by ethanol were protected 100%. The low RSA may preclude DPPH assay as a possible mechanism of elucidating its antioxidant effects.

Stress plays an important role in the etiopathology of gastroduodenal ulceration (Favier \textit{et al.}, 1990). Stress – induced gastric stress is characterized by increase in gastric acid and peptic secretion; decrease in gastric blood flow; suppression of endogenous generation of prostaglandins; inhibition of mucus growth and cell proliferation; and alteration of gastric motility (Konturek \textit{et al.}, 1986a; Konturek \textit{et al.}, 1986b; Soll 1983). Gastric stress-induced ulcer is probably mediated by the release of histamine. It not only increases gastric secretion, often called the aggressive factor, but also causes disturbances of the gastric mucosal microcirculation and abnormal motility, and reduces mucus production, known as the defensive factor. Moreover, stress-induced ulcer in animal models may be partially or entirely prevented by vagotomy, since increased vagal activity has been suggested as the main factor in stress-induced ulceration (Singh and Majumdar 1999). The vagus nerve stimulates stomach acid secretion via interaction of its chemical mediator (acetylcholine) with the muscarinic receptor. The activation of the muscarinic receptor gives rise to sequential events that result in increased gastric acid secretion (Clapham 1995). According to some authors, those receptors are located in the parietal cells and histamine secretary cells. Therefore, the increase in acid secretion is a consequence of acetylcholine activity on the histamine cell and parietal cell activity (Schubert 2000). It is important to consider that vagal activity is up regulated in situations of stress. Some antiulcer drugs, such as cimetidine, ranitidine and famotidine, are used in ulcer treatment by blocking H\(_2\) histaminic receptors. Moreover, it is important to state that 770 mg/kg dose of EAF reduced ulcer index comparable to the standard drug, cimetidine (100 mg/kg). At that same dose of the fraction in another study, the intestinal propulsive movements (IPM) in rat was inhibited by 100% through \(\alpha_2\)- adrenergic mechanism (Nwidu \textit{et al.}, 2011). Thus the possible mechanism of antiulcer activity may not be unrelated with the inhibition muscarinic receptors in the parietal cells.

\textit{C. lutea} fractions interrupt diethyldithiocarbamate (DTC) induces antral lesions in earlier report by 68.79% (Nwidu and Nwafor 2009). DTC is reported to initiates its effects by mobilization of superoxide and hydroxyl radicals (oxygen derived free radicals) (Oka \textit{et al.}, 1990). Superoxide radical and hydrogen peroxide play pathogenic role in this ulcer model Salim (1989). The low RSA observed in our study could not be explained in light of present findings. Gastric cyto-protection may be mediated by at least two different mechanisms. The first one is by stimulation of release of prostaglandins (PG) and the second one by Michael’s acceptor interaction with sulphhydryl-containing compounds of the mucosa. The latter, mechanism of cyto-protection might be mediated by CLL EAF, at least in part, by the reaction between electrophilic moiety on the trans-cinnamic acid and sulphhydryl-containing compounds of the gastric mucosa (Donadel \textit{et al.}, 2005). In this mechanism of cyto-protection, antioxidant activity might be mediated by the reaction between the electrophilic acceptor, trans-cinnamic acid, (which constitute 21.9% of isolated compounds) from the EAF with sulphhydryl-containing compounds of the gastric mucosa (Maria \textit{et al.}, 2000)[79]. Trans-cinnamic acid (Michael’s acceptor) and electron donation by sulphhydryl moiety present in proteins or oxidants containing compounds has form a new basis of cytoprotection (Souza-Brito \textit{et al.}, 1998; Hiruma-Lima \textit{et al.}, 1999; Hiruma-Lima \textit{et al.}, 2001; Melo \textit{et al.}, 2003; Yesilada \textit{et al.}, 2004; Hamauzu \textit{et al.}, 2008).

These results, taken together, suggest that the EAF active compounds mechanism of RSA is yet to be elucidated; but may be
mediated by trans-cinnamic acid (Michael’s acceptor) interaction with oxidants. This assertion is predicated on the significantly large amount of trans-cinnamic acids in the isolated compounds. The antioxidative activity of trans cinnamic acid like any caffeic acid analogue, depends on several other factors such as: unsaturated 2,3-double bond of the side chain (which maximizes the stabilization of the phenolic radical), the electron-donating and withdrawing substituent on the catechol ring, the number of hydroxyl groups or catechol moieties, the involvement of other H-donating groups (—NH, —SH), the chemical stability, and the hydrophobicity or partition coefficient (log P) of the compounds (Rice-Evans et al., 1986). However, other mechanisms may also be involved. The antiulcer assays with isolated compounds need further evaluation to elaborate possible mechanism of antiulcer effects.

**CONCLUSION**

The demonstration of inhibition of acute antiulcer models couple with the characterisation and isolation phenolics with reported antiulcer potential gave credence to *C. lutea* folkloric patronage as stomach medicine. However, the explicit antiulcer mechanism of the *C. lutea* leaf fractions is non-specific in nature, evident by the various ulcerogenic agents it inhibited. Though two cinnamoyl 1-deoxyglucopyranosides and two new p-coumaroyl 1-deoxyglucopyranosides, besides cinnamic acid were isolated and characterised, further bioactivity guided evaluation of these new molecules may lead to a novel antiulcer drug discovery.

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