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Full Length Research Paper

Gastroprotective effects of leaf extracts of *Carpolobia lutea* (polygalaceae) G. Don. in rats

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The preliminary screening of the gasroprotective effects of *Carpolobia lutea* leaf extracts was investigated through bioactivity guided gradient extraction. Experimentally induced gastric ulceration was affected using ulcerogens such as indomethacin, ethanol, reserpine in 0.5% acetic acid, stress, serotonin and diethylthiocarbamate in rats. The median lethal dose (LD 50) of the ethanol extract was also investigated intraperitoneally in mice. Preliminary phytochemical screening of the ethanol extract was conducted. The acute toxicity shows the median lethal dose to be 3850.0 mg/kg. The phytochemical screening of C*. *lutea* revealed that alkaloids, saponins, tannins, anthraquinone, cardiac glycosides, flavoniods were presents. The ethanol extract gave a preventive ratios (PRs) of 3.08, 90.09, 22.17, 70.00, 43.44 and 51.58; the ethyl acetate extract gave 57.50, 100.00, 83.33, 63.61, 84.80, and 68.79; the chloroform extract gave 4.85, 45.05, -13.80, 46.37, 35.88 and 70.29; n-hexane extract gave 38.02, 34.83, 55.50, 100.00, 68.49 and 31.30 PRs respectively for the indomethacin, ethanol, reserpine in 0.5% acetic acid, stress, serotonin and diethylthiocarbamate induced ulceration in rats. The PRs of cimetidine are 90.26, 66.67, 91.82, and 49.97 respectively for indomethacin, reserpine in 0.5% acetic acid, stress and serotonin induced ulceration in rats. The ethyl acetate extract (770 mg/kg) consistently and effectively reduced the ulcer index significantly ($p<0.01 - 0.001$) than the ethanol, chloroform and n-hexane extracts of *C. lutea* in all the experimentally induced ulcer models studied. *C. lutea* could be exploited in the treatment of peptic ulcer in man justifying its ethnomedical use as stomach medicine.

Key words: *Carpolobia lutea*, gradient bioactivity guided extraction, gastroprotectives, gastric ulcers

INTRODUCTION

Carpolobia lutea G.Don (family: polygalaceae) occurred as a dense overgrowth or an evergreen shrub or small tree, up to 5 m high. It grows in rainforest and Guinea savannah of Sierra Leon to Cameroon from April to September. *C. lutea* is called cattle stick (English), Ikpafum (Ibibio), Agba or Angalagala (Igbo) and Egbo Oshunshun (Yoruba) in Nigeria (Inyang, 2003; Chukwuma, 2008). It is reputed in Southern Nigeria as “ogun aleko” that is invigorating tonic or aphrodisiac. The aphrodisiac and androgenic action of the root is recorded in Ivory Coast where it is used to manage impotence (Burkill, 1985). The bark decoction is used to cure rheumatism, while the powdered bark is used as snuff, produces tearing and sneezing (Irvine, 1961). The root decoction of *C. lutea* is used locally to manage fever, general pain and insanity. The powdered bark is taken as snuff for headache and to prevent sleep due to fatigue thus sustaining vitality. Also root decoction of *C. lutea* is given in enema form to women to combat sterility and to promote childbirth, and as a vermifuge and taenifuge (Burkill, 1985). The roots are also used in Nigeria for dermal infections and venereal diseases. An infusion of the leaves and twigs is reported to be stomach medicine (Irvine, 1961).

Natural products like plants present promise of cure as they have been the raw materials for the synthesis of drugs and as an important source of new therapeutic agents (Andreo et al., 2006). Diverse chemical compounds have been isolated from medicinal plants with antiulcer activity (Lewis and Hanson, 1991). Several factors known to increase the incidences of peptic ulcer diseases (PUD) are smoking, nutritional deficiencies, alcohol consumption and frequent ingestion of non-steroidal anti-inflammatory drugs (NSAIDs) (Peskar and Maricic, 1998; Belaiche et al., 2002). The proportion of ulcer unrelated to *Helicobac-*

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ter pylori or NSAIDs has increased tremendously and may likely have implication on the management of PUD (Chan and Leung, 2002). Therefore the demand of the most reliable, available, accessible, cost effective and proven stomach medicine will be on the increase. Following an initial investigation of the pharmacological activity of the crude ethanolic extract of the leaf as a potent antiulcerogenic and antidiarrhoeal properties (Nwafor and Bassey, 1997), a bioactivity guided extraction was conducted. This is a preliminary screening of the gastroprotective effects of the leaf *C. lutea* using a single dose of 770 mg/kg of the ethanol, ethyl acetate, chloroform and n-hexane fractions using indomethacine, ethanol, and acetic acid in reserpin, stress, serotonin and diethythiocarbamate induced ulceration in rat.

MATERIALS AND METHODS

Collection of plant materials

C. lutea leaves was collected from the wild in Itak Ikot Akap-Ikono Local Government Area, about 100 km from Uyo, the capital of Akwa Ibom State, Nigeria. The plant was identified by Dr (Mrs.) Margaret Bassey, a botanist in the Department of Botany, University of Uyo, Nigeria. A voucher specimen was deposited in Department of Pharmacognosy, Faculty of Pharmacy, and University of Uyo, Nigeria.

Extractions

The fresh leaves were sun dried for over 72 h before it was powdered with mortar and pestle. 750 g of the dry powder was weighed with weighing balance and subjected to solvent-guided or gradient extraction by maceration (Trease and Evanse, 1983). The leaf was first defatted in n-hexane for 72 h, filtered to obtain the n-hexane extract. The residue was sun dried and the process repeated for chloroform, ethyl acetate and ethanol solvent (Reidel-de Haen, Germany). The filtrate from each solvent was evaporated to dryness in a water bath at 45°C. The yield was 3.66, 3.03, 3.63, and 15.07% for n-hexane, chloroform, ethyl acetate and ethanol respectively. The various extract was stored in desiccators containing silica gel until used.

Phytochemical test

The phytochemical analysis (Trease and Evans, 1983) on the ethanol extracts revealed the following compounds: flavonoids, tannins, saponins, alkaloid and cardiac glycosides.

Animals

Wistar rats of male sex, weighing between 91 – 252 g and albino mice weighing between 14 – 23 g purchased from University of Jos, Animal house, Plateau State, Nigeria were used in this study. The animals were housed in cross ventilated room in cages at 22 ± 2.5°C) with 12 h dark/12 h light cycles and were feed with standard growers mash feeds (Vital Veterinary Service, Uyo, Nigeria) and water *ad libitum*. Animals were acclimatized for one week and fasted overnight, with free access to water, prior to experiments. This study was approved by the Ethical committee of the Faculty of Pharmacy, University of Uyo, Nigeria.

Drugs reagent and solvents

The drugs used in the study are obtained from Sigma, Aldrich (St. Louis, USA), solvents from Reidel-de Haen (Germany) and the chemicals were of analytical grade.

Acute toxicity studies (LD_{50})

The determination of median lethal dose LD_{50} was by the method of Lorke (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. Wide doses (100, 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, mg/kg i.p.) of the ethanol extract were given to nine group of mice (n = 3) to establish the range of doses of the extract that would elicit toxic effects. The mice were observed for 24 h post treatment for sign of excitement, dullness, nervousness, alertness, ataxia or even death. The LD_{50} was estimated by the geometric mean of the dose that caused 100% mortality and the dose that cause no lethality at all.

Pharmacological assays

The ethnopharmacological information of *C. lutea* leaf about the employed posology is inexact. This necessitate the utilization of the fivefold lower dose obtain from acute toxicity (770 mg/kg) as the only dose in all the experiments to determine the common preliminary profile of antiulcer effects of *C. lutea* leaf (Andreo et al., 2006). The antiulcer assays were executed using these protocols: indomethacin, ethanol-induced ulcer, reserpine in 0.5% acetic acid, stress induced ulcer, serotonin induced ulcer and diethylthiocarbamate ulcer models.

Effect of extract 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 137 – 148 g were used for this experiment. The rats were randomized and divided into 6 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 – 5 were pretreated with 770 mg/kg *C. lutea* leaf extracts of ethanol, ethyl acetate, chloroform and n-hexane, respectively, 1 h prior to administration of 0.1 g/kg of indomethacin. While group 6 received cimetidine (1.0×10^{-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 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 pretreated with these extracts of *C. lutea* were calculated using the method of Al-Said et al. (1986).

Effect of extracts on ethanol–induced gastric ulceration in rats

Male adult albino rats weighting between 147 – 216 g were used for this experiment. The rats were randomized and divided into 6

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 - 5 were pretreated with 770 mg/kg *C. lutea* leaf extracts of ethanol, ethyl acetate, chloroform and n-hexane, respectively, while group 6 received propranolol (4.0 x 10^{-2} g/kg, p.o) 1 h prior to administration of 0.5 ml of ethanol. The extract was administered intragastrically via the aid of an orogastric cannula. 4 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 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 pretreated with these extracts of *C. lutea* were calculated using the method of Zaidi and Mukerji (1958) with modification.

Effect of extract on reserpin 25 mg/kg in 10 ml/kg of 0.5% acetic acid–induced gastric ulceration in rats

Male adult albino rats weighting 130 – 206 g were used for this experiment. The rats were randomized and divided into 6 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 0.25 g/kg reserpin in 10 ml of 0.5% acetic acid, orally. Groups 2 – 5 were pretreated with 770 mg/kg *C. lutea* leaf extracts of ethanol, ethyl acetate, chloroform and n-hexane, respectively, 1 h prior to administration of 0.25 g/kg reserpin in 10 ml of 0.5% acetic acid. While group 6 received cimetidine (1.0 x 10^{-3} g/kg, p.o) 1 h prior to administration of 0.25 g/kg reserpin in 10 ml/kg 0.5% acetic acid. The drugs were administered intragastrically via the aid of an orogastric cannula. 4 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 a hand less and scored for the presence of lesions using the method of Lau and Ogbe (1981). The umber and severity of gastric lesions were evaluated according to the following rating scale: 0, no lesion; 1, mucosal oedema and petechiae; 2, 1 - 5 small lesions (1 – 2 mm); 3, more than 5 small lesions or 1 intermediate lesion (3 – 4 mm); 4, 2 or more intermediate lesions or 1 gross lesion (greater than 4 mm); 5, perforated ulcers. Ulcer index (UI) of 0.25 g/kg reserpin in 10 ml of 0.5% acetic acid alone, ulcer index and preventive ratio of each of the groups pretreated with these extracts of *C. lutea* were calculated using the procedure of Lau and Ogbe (1981).

Effects of extract on water immersion and immobilization-induced gastric ulceration in rats

Male adult albino rats weighting 175 – 226 g were used for this experiment. The rats were randomized and divided into 6 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 xiphoid. 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 – 5 were pretreated with 770 mg/kg *C. lutea* leaf extracts of ethanol, ethyl acetate, chloroform and n-hexane, respectively, 1 h prior to immersion and immobilization. While group 6 received cimetidine (1.0 x 10^{-3} g/kg, p.o) 1 h prior to immersion and immobilization. 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 a hand lens and scored for the presence of lesions using the methods of Takalgi and Okabe (1968).

The number and severity of gastric lesions were evaluated according to the following rating scale; 0: no lesion; 1: mucosal oedema and petechiae; 2: 1 - 5 small lesions (1 - 2 mm); 3: more than 5 small lesions or 1 intermediate lesion (3 - 4 mm); 4: 2 or more intermediate lesions or 1 gross lesion (greater than 4 mm); 5: perforated ulcers. Ulcer index (UI) of rats immobilized and immersed in water without drug alone, ulcer index and preventive ratio of each of the groups pretreated with these extracts of *C. lutea* were calculated using the method of Takalgi and Okabe (1968).

Effect of extracts on serotonin–induced gastric ulceration in rats

Male adult albino rats weighting between 110 – 131 g were used for this experiment. The rats were randomized and divided into 6 groups of 6 rats each. Food was withdrawn 24 h and water 2 h before the commencement of the experiment. Glandular lesions were established with a single 0.5 ml 50 mg/kg subcutaneous injection of serotonin creatinine sulphate (5-HT, Sigma). Group 1 was given only 50 mg/kg serotonin in 0.5 ml physiological saline as positive control, groups 2 – 5 were pretreated with 770 mg/kg *C. lutea* leaf extracts of ethanol, ethyl acetate, chloroform and n-hexane, respectively, while group 6 received cimetidine (1.0 x 10^{-3}g/kg, p.o) 1 h prior to administration of 0.5 ml of serotonin. The extract was administered intragastrically induced via the aid of an orogastric cannula. 6 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 a hand lens and scored for the presence of lesions using the method of Okabe et al. (1976). Ulcer index of serotonin alone, ulcer index and preventive ratio of each of the groups pretreated with these extracts of *C. lutea* were calculated using the method of Okabe et al. (1976).

Effect of extracts on diethyldithiocarbamate-induced ulceration in rats

Male adult albino rats weighting between 91 – 126 g were used for this experiment. The rats were randomized and divided into 6 groups of 5 rats each. Food was withdrawn 24 h and water 2 h before the commencement of the experiment. Acute glandular lesions were induced with subcutaneous administration of 1 ml of diethyldithiocarbamate 800 mg/kg (Sigma Co.) in saline followed by 1 ml oral dose of 0.1 N HCl. Group 1 undergo a midline laparatomy to ligate the pylorus before given subcutaneously, 800 mg/kg of diethyldithiocarbamate in 1 ml of saline followed by 1 ml oral dose of 0.1 N HCl as positive control, groups 2 – 5 were pretreated 0.5 h before surgery to ligate the pylorus with 770 mg/kg *C. lutea* leaf extracts of ethanol, ethyl acetate, chloroform and n-hexane, respectively, 800mg/kg diethyldithiocarbamate + 0.1N HCL. The extract was administered intragastrically via the aid of an orogastric cannula. 5 h after the last dose of diethyldithiocarbamate + 1 ml oral dose of 0.1N HCl, 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 a hand lens and scored for the presence of lesions using the method of Oka et al. (1990). Ulcer index of diethylthiocarbamate alone; ulcer index and preventive ratio of each of the groups pretreated with these extracts of *C. lutea* were calculated using the method of Oka et al. (1990).

Statistically analysis

Values for the results were expressed as a mean ± SEM. The statistical significance of each test group in relation to the control was calculated using one way analysis of variance followed by Turkey-Krammer multiple comparisons tests. A probability of less than 5% was considered significant.

RESULTS

The preliminary phytochemical screening of *C. lutea* revealed that alkaloid, saponins, tannins, anthraquinone, cardiac glycosides and flavoniods were present. The acute toxicity studies LD50 shows that median lethal dose is 3850.0 mg/kg. Signs of toxicity observed includes restlessness, convulsion, salivation, defecation, urination, and death under 24 h at doses above >3000 mg/kg.

In the indomethacin-induced ulcer model, it was observed that treatment with 770 mg/kg *C. lutea* leaf extract and each of ethanol, ethyl acetate, chloroform and n-hexane and cimetidine 100 mg/kg significantly reduced ulcer index in comparison with the negative control group ($p<0.01$ - 0.001). The preventive ratio of ulcers are 3.08, 57.5, 4.85, 38.02, and 90.26 for the treated groups with 770 mg/kg of *C. lutea* leaves extracts and each of ethanol, ethyl acetate, chloroform and n-hexane extracts and positive control (cimetidine), respectively. The obtain results suggest that the ethyl acetate leaf extract of *C. Lutea* present a significant antiulcer effects in this ulcer model.

For the ethanol model, the treatment with 770 mg/kg *C. lutea* leaf extract of ethanol, ethyl acetate, chloroform and n-hexane and propanolol (40 mg/kg) reduced significantly the ulcer index in comparison with control group ($P <0.01$ - 0.001). The preventive ulcer ratio are 90.09, 100.00, 45.05, 34.83 and 39.94 for the treated group with 770 mg/kg *C. lutea* leaf extract of ethanol, ethyl acetate, chloroform and n-hexane and positive control (propanolol), respectively. Ethanol and ethyl acetate extracts of *C. lutea* showed significant gastric protection when compared to the control.

For the 25 mg/kg reserpin in 10 ml/kg 0.5% acetic acid induced ulcer model, it was observed that treatment with 770 mg/kg *C. lutea* leaf extract of ethanol, ethyl acetate, chloroform and n-hexane and cimetidine (100 mg/kg) reduced significantly the ulcer index in comparison with control group ($p<0.001$ - 0.05). In this model, the preventive ulcer ratio are 22.17, 83.33, -13.8, 55.50 and 66.67 for the treated group with 770 mg/kg *C. lutea* leaf extract of ethanol, ethyl acetate, chloroform and n-hexane and positive control (cimetidine), respectively. Pretreatment with ethyl acetate, chloroform and n-hexane extracts were more significant in gastroprotection in this model.

For the water immersion and immobilization stress induced ulcer model, it was observed that treatment with 770 mg/kg *C. lutea* leaf extract of ethanol, ethyl acetate, chloroform and n-hexane and cimetidine (100 mg/kg) reduced significantly the ulcer index in comparison with control group ($p< 0.001$ - 0.05). In this model, the preventive ulcer ratio are 70.00, 63.61, 46.37, 100.00 and 91.82 for the treated group with 770 mg/kg *C. lutea* leaf extract of ethanol, ethyl acetate, chloroform and n-hexane and positive control (cimetidine), respectively. Ethanol, ethyl acetate and n-hexane extract of *C. lutea* effectively reduced ulcers induced in this model.

In the serotonin-induced ulcer model, it was observed that pretreatment with 770 mg/kg *C. lutea* leaf extract of ethanol, ethyl acetate, chloroform and n-hexane and cimetidine 100 mg/kg significantly reduced ulcer index in comparison with the negative control group ($p<0.01$ - 0.001). The preventive ratio of ulcers are 43.44, 84.80, 35.88, 68.49 and 49.97 for the treated groups with 770 mg/kg of *C. lutea* leaf extract of ethanol, ethyl acetate, chloroform and n-hexane extract and positive control (cimetidine) respectively. Our data also show that pretreatment with ethyl acetate and n-hexane leaf extracts of *C. lutea* significantly protected (84.8 and 68.5% respectively) gastric mucosa against serotonin induced ulcers in rats.

Regarding the diethyldithiocarbamate induced ulcer model, the treatment with 770 mg/kg *C. lutea* leaf extract of ethanol, ethyl acetate, chloroform and n-hexane reduced significantly the ulcer index in comparison with control group ($P < 0.01$-0.001). In this model, the preventive ulcer ratio are 51.58, 68.79, 70.29, and 31.30 for the treated group with 770 mg/kg *C. lutea* leaf extract each of ethanol, ethyl acetate, chloroform and n-hexane respectively. The ethanol, ethyl acetate and chloroform leaf extract of *C. lutea* significantly exhibit antioxidant activity. These results are summarized in Table 1 - 6.

DISCUSSION

Currently, effective multi-drug products exist for peptic ulcer diseases (PUD) management. However they are very expensive and presents with multiple side effects that limit their usage. Attention is now focused on antiulcer agents that are less expensive, less toxic and very effective. Medicinal plants are among the most attractive source of new drugs with promising results in PUD management (Borelli and Izzo, 2000). *C. lutea* is a medicinal plant briefly reported as a stomach remedy in folklore medicine (Irvine, 1961). Little is known in literature except the antiulcerogenic and antidiarrhoeal effects of crude ethanolic extract recently reported (Nwafor and Bassey, 2007).

The effects of four extracts from *C. lutea* leaf: ethanol, ethyl acetate, chloroforms and n-hexane, on gastric ulcers induced by different damaging agents (indomethacin, ethanol, 25 mg/kg of reserpin in 0.5% acetic acid, stress, serotonin and diethyldithiocarbamate) was investigated in rats. The mechanisms by which these extracts produced these effects seem unclear.

Table 1. Effects of various leaves extracts of *C. lutea* on indomethacin induced ulceration in rats.

Treatment	(mg/kg)	Ulcer index	Preventive ratio
Control	-	18.83 ± 7.20	-
Ethanol	770	18.25 ± 7.16	3.08
Ethyl acetate	770	8.00 ± 4.23**	57.50
Chloroform	770	17.92 ± 3.64	4.85
N-hexane	770	11.67 ± 3.88	38.02
Cimetidine	100	1.83 ± 0.22***	90.26

Significance relative to control: **P< 0.01; ***p<0.001; values represent mean ± S.E.M (n = 6).

Table 2. Effects of various leaves extracts of *C. lutea* on ethanol induced ulceration in rats.

Treatment	(mg/kg)	Ulcer index	Preventive ratio
Control	-	3.33 ± 0.54	-
Ethanol	770	0.33 ± 0.37***	90.09
Ethyl acetate	770	0.00 ± 0.0***	100.00
Chloroform	770	1.83 ± 1.00 ***	45.05
N-hexane	770	2.17 ± 0.18 **	34.83
Propanol	40	2.00 ± 0.57 ***	39.54

Significance relative to control: **P< 0.01; ***p<0.001; values represent mean ± S.E.M (n = 6).

Table 3. Effects of various leaves extracts of *C. lutea* on 25 mg/kg reserpin in 10 ml/kg 0.5% acetic acid induced ulceration in rats.

Treatment	(mg/kg)	Ulcer index	Preventive ratio
Control	-	6.0 ± 1.44	-
Ethanol	770	4.67 ± 2.80	22.17
Ethyl acetate	770	1.0 ± 0.49 ***	83.33
Chloroform	770	6.83 ± 2.18	-13.8
N-hexane	770	2.67 ± 1.12*	55.50
Cimetidine	100	2.0 ± 1.10**	66.67

Significant relative to control: *p<0.05; **p<0.01; ***P<0.001; values represent mean ± S.E.M (n = 6).

Table 4. Effects of various leaves extracts of *C. lutea* on water immersion and immobilization induced stress ulcer in rats.

Treatment	(mg/kg)	Ulcer index	Preventive ratio
Control	-	18.83 ± 6.12	-
Ethanol	770	5.50 ± 2.41 ***	70.00
Ethyl acetate	770	6.67 ± 3.79***	63.61
Chloroform	770	9.83 ± 7.33*	46.37
N-hexane	770	0.00 ***	100.00
Cimetidine	100	1.50 ± 1.64***	91.82

Significance relative to control: *p < 0.05;**p < 0.01; ***P < 0.001; values represent mean ± S.E.M (n = 6).

Table 5. Effects of various leaves extracts of *C. lutea* on serotonin induced ulceration in rats.

Treatment	(mg/kg)	Ulcer index	Preventive ratio
Control	-	15.33 ± 0.73	-
Ethanol	770	8.67 ± 3.23***	43.44
Ethyl acetate	770	2.33 ± 1.29 ***	84.80
Chloroform	770	9.83 ± 2.36 **	35.88
N-hexane	770	4.83 ± 1.43 ***	68.49
Cimetidine	100	7.67 ± 3.04 ***	49.97

Significance relative to control: **p<0.01;***P<0.001; values represent mean ± S.E.M (n = 6).

Table 6. Effects of various leaves extracts of *C. lutea* on diethyldithiocarbamate induced ulcer in rats.

Treatment	(mg/kg)	Ulcer index	Preventive ratio
Control	-	10.67 ± 2.36	-
Ethanol	770	5.17 ± 1.80 **	51.58
Ethyl acetate	770	3.33 ± 1.08 ***	68.79
Chloroform	770	3.17 ± 0.87 ***	70.29
N-hexane	770	7.33 ± 3.71	31.30

Significance relative to control: **p<0.01;***P<0.001; values represent mean ± S.E.M (n = 6).

Oral pretreatment of *C. lutea* leaf extracts to the indomethacin group reduced the intensity of gastric damage induced by indomethacin. Indomethacin is an established ulcerogen especially in an empty stomach (Bhargava et al., 1973). The incidence of indomethacin-induced ulceration is mostly on the glandular (mucosal) part of the stomach (Evbuonwa and Bolarinwa, 1990). The mechanism underlying the ulcerogenicity of indomethacin is the inhibition of prostaglandin synthesis (Vane, 1971), thus prostaglandin is cytoprotective to gastric mucosa. Prostaglandin maintains gastric microcirculation (Vane, 1971; Ferreira and Vane, 1974) and cause gastric secretion of bicarbonate (Garner et al., 1979) and mucus (Menguy and Desbaillets, 1967), in contrast to the blockers of the prostaglandin synthesis such as indomethacin which inhibit the non-parietal secretions (Kollberg et al., 1981). Significant inhibition of gastric ulcer was only observed with the ethyl acetate leaf extract of *C. lutea* in this ulcer induced model.

The incidence of ethanol-induced ulcers which is predominant in the glandular part of the stomach has been reported to stimulate the formation of leukotriene (LTC_4) resulting in the damage of rat gastric mucosa (Drelying et al., 1986; Peskar et al., 1986; Cho et al., 1987). Indeed, in the rat gastric mucosa, some of the effects elicited by oxygenous LTC_4 resemble those produced by ethanol (Guth et al., 1984; Szabo et al., 1985; Whittle et al., 1985). Since vascular changes appears to be the most pronounced features of ethanol-induced lesions, maintenance of the mucosal vasculature and normal blood flow may be the major mechanism of cytoprotection (Matsuda et al., 1999).

It has been proposed that mucosal protection induced by non-prostannoid compounds may be mediated through the mobilization of endogenous prostaglandins (Cho et al., 1983; Konturek et al., 1987). It is possible that one of the mechanisms of anti-ulcerogenic effects of the extracts may be their ability to mobilize prostaglandins in gastric mucosa. Suppression of alcohol-induced ulceration indicated that the extract suppress lipoxygenase path-way, this may in part be one of its mechanisms of action. Pretreatment with ethanol and ethyl acetate leaf extract produced a significant protective effects but no significant difference with the positive control group, propanol and the negative control. The obtained results suggest that the ethanol and ethyl acetate extracts may induce their antiulcer effects through the same mechanism in this model.

Reserpin in acetic acid induced gastric ulceration by the depression of the adrenergic activity with an increase of cholinergic tone. It is acid dependent in its mediation and hyper motility seems to be more important than hyper secretion for the induction of gastric mucosal lesion (Kagoshima and Suguro, 1982). Stimulation of the cholinergic afferent fibers exerts gastroprotective effects mediated by the calcitonin gene related peptides (CGRP), nitric oxide (NO) and various other peptides. Activation of the adrenergic system results in gastric acid production and pathogenesis of gastric mucosal lesionsmediated through the presynaptic α-adrenoceptor. Stimulation of these receptors mediates the antisecretory and the gastric mucosal protective effects. The ethyl ace-

tate leaf extract was tested and showed a significant inhibition in the ulcerative index. Stress induced gastric mucosal damage is attributed to breakdown of gastric defense mechanisms under both experimental and clinical condition (Haglund, 1990). The structural elements (mucus and epithelial cell barrier) and the physiological component (mucine production, bicarbonate secretion and mucosal micro-circulation) are preventive mechanism evoked in acute gastric acid damage development (Goldman and Rosof, 1968; Holzer, 2000).

Mucosal ischemia is the main mechanism of stress induced gastric mucosal damage and not gastric acid hypersecretion which may only play a permissive role (Haglund, 1990). Locally secreted prostaglandins (PG's), sensory neuropepeptides and nitric oxide contribute to regulation of gastric blood flow and maintenance of mucosal integrity (Pawlik et al., 2001; Gustaw et al., 1994; Whittle et al., 1990). In the gastric ulcer induced by the hypothermic-restraint stress all the extracts except the chloroform extracts tested showed significant inhibition in the preventive ratio. These potent extracts may mediate their effect by depressing vagal overactivity (Grijalva and Novin, 1990).

Serotonin is known in rat to cause vasoconstriction, thus reducing gastric mucosal blood flow (GMBF) resulting in acute mucosal injury (Le Pard and Stephens, 1994). Like in stress restraint model, all the extract except the chloroform extract mediated the same significant antiulcer effects. They may probably be stimulating increased GMBF as the mechanism of preventing ulcerogenesis (Okabe et al., 1976).

Diethyldithiocarbamate induces antral lesions by mobilization of superoxide and hydroxyl radicals (oxygen derived free radicals). It was used to asses the role of the antioxidant activity of the compound in the extracts in the prevention of gastric damage (Oka et al., 1990). Superoxide radical and hydrogen peroxide play pathogenic role in this ulcer model (Salim, 1989). Chloroform and ethyl acetate leaf extracts were very effective than other extracts, and significantly prevented antral lesion.

The results from each experimental model of induced gastric ulcer shows greatest gastro-protective action of the ethyl acetate extract than other extracts of *C. lutea*. The phytochemical analysis of the extracts reveal the presence of tannins, saponins and flavonoids, substances known to affect the integrity of mucous membranes (Oliver, 1960). Flavonoids and tannins from the plant are extracted by the oxygenated ethyl acetate.

Tannins with its protein precipitating and vasocontrictory effects could be advantageous in preventing ulcer development (Aguwa and Nwako, 1988). Tannin being an astringent, may have precipitated microproteins on the site of ulcer thereby forming an impervious protective pellicle over the lining to prevent absorption of toxic substances and resist the attack of proteolytic enzymes (John and Onabanjo, 1990; Nwafor et al., 1996). Flavonoids have been reported to offer some protection in ulcer development by increasing capillary resistance. Flavonoids improve microcirculation which renders the cells less injurious to precipitating factors (Hashizume et al., 1978).

Cytoprotection (Robert et al., 1979) in rats as evidenced by the ability of prostaglandins to protect the mucosa of the stomach against the erosive effects of absolute ethanol, 0.6 NHCl, 0.2 N NaOH or boiling water administered intragastrically has been discussed. This pharmacological induced property is independent of inhibition of gastric secretion. Mechanisms that have been proposed include stimulation of mucus and bicarbonate secretion, stimulation of sodium pump, and strengthening of the gastric mucosal barrier (Guth, 1982; Chiu et al., 1984).

This bioactivity guided screening of these extracts showed that they exhibit cytoprotective action that affords protection of the stomach against potentially noxious stimuli. The gastroprotective potential of the extracts may in part be due to its ability to mobilize prostaglandins, inhibition of lipoxygenase pathway, antihistaminic, anticholinergic, antiserotonergic and anti-oxidant effects; their chemical constituents which includes tannins, saponins and flavonoids, and their cytoprotective mechanism on gastric mucosa. The ethyl acetate fraction of *C. lutea* was very consistent and effective than other extracts in all experimental ulcer models studied and may be useful in the treatment of peptic ulcer in man. Further investigation is ongoing in our laboratory with the ethyl acetate fraction to elucidate dose dependent gastro-protective effects and isolation and characterization of the active ingredient.

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