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Research Article
Anti-*Helicobacter pylori* Activity and Gastroprotective Effect of *Emilia coccinae* (Asteraceae) Against Ethanol-induced Gastric Mucosal Hemorrhage in Rats

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Abstract

Background: This study was performed to evaluate the antibacterial activity and gastroprotective effect of methanol and ethyl acetate extracts of *Emilia coccinae* whole plant against ethanol-induced gastric mucosal injury in rats. **Methodology:** The antibacterial properties were examined using broth micro dilution method against 10 clinical strains of *Helicobacter pylori*. For the gastroprotective assessment, rats were divided into five groups, respectively pre-treated orally with vehicle (ulcer control groups), omeprazole 20 mg kg^{-1} (reference group), 250, 500 and 1000 mg kg^{-1} of *E. coccinae* (experimental groups) 1 h before oral administration of absolute ethanol to generate gastric mucosal damage. After an additional hour, the rats were sacrificed and the ulcer areas of the gastric walls were determined. **Results:** The best antibacterial activity was recorded with *E. coccinae* methanol extract with MIC value of 64 µg mL^{-1} against 6 of the 10 (60%) tested strains. This results revealed that treatment with *E. coccinae* prior to absolute alcohol has significantly protect gastric mucosa as ascertained grossly by significant reduction of ulcer area, increases in gastric mucus production and decrease the acidity of gastric content compared to ulcer control group. **Conclusion:** *Emilia coccinae* was able to decrease the acidity of gastric content and increase gastric mucus production, there by justifying its use as an anti-ulcerogenic agent.

Key words: Gastroduodenal disorders, *Helicobacter pylori*, Asteraceae, anti-ulcerogenic

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Gastric ulcer a major gastrointestinal disorder, results from an imbalance between offensive (gastric acid secretion) and defensive (gastric mucosal integrity) factors[1]. It is estimated that at some time in life, nearly 20% of all people suffer from peptic ulcers caused by factors such as stress, smoking, alcohol, nutritional deficiencies and infections[2]. According to Peckenpaugh and Poleman[3] some other factors, such as bad dietary habits, excessive intake of non steroidal anti-inflammatory agents, hereditary predisposition and *Helicobacter pylori* infection, which is reported to account for more than 70% of cases are responsible for the development of peptic ulcer diseases[4].

There are several models that are used to evaluate antiulcer medicines. Absolute ethanol method of inducing gastric lesions is a convenient way of screening plant extracts for anti-ulcer potency and cytoprotection in macroscopically and microscopically visible lesions. Administration of absolute ethanol into the gastric lumen induced gross lesions in the glandular part of the stomach[5]. It was also apparent that ethanol caused gastric damage, which was confirmed by significant increase in the number of hemorrhage and gastric erosions[6]. Disturbances in gastric secretion, damage to gastric mucosa, alterations in permeability, gastric mucus depletion and free-radical production are reported to be the pathogenic effects of ethanol[7]. Ethanol induced gastric lesion formation may be also due to stasis in gastric blood flow, which contributes to the development of the hemorrhagic and necrotic aspect of tissue injury[8].

Several orthodox pharmaceutical drugs, such as anticholinergic drugs, histamine H2-receptor antagonists, antacids and more recently proton-pump inhibitors have been employed in the management of peptic ulcers. In the case of *H. pylori* infection, antibiotics are also used. However, the innumerable adverse effects caused by these allopathic medicines[9] and the emergence of drugs resistance *H. pylori* strains evidence the need for safer and more effective antigastric ulcer agents. Moreover, due to the high cost of orthodox medical care, rural dwellers and low-income earners depend mainly on traditional medicine for health care[10].

In recent years, there has been growing interest in alternative therapies especially from plant sources due to their perceived lower side effects, ease of accessibility and affordability[11]. Plants are some of the most attractive sources of new drugs and some have been shown to have promise for the treatment of gastroduodenal ulcer with minimum side effects[12,13]. *Emilia coccinea* (SIMS) G. (Asteraccae) is an annual herb commonly found throughout the plain of the Central Africa and in dry areas up to 2000 m altitude in the Eastern Africa. This species belongs to the genus *Emilia* represented by about 100 species, with 50 of them found in Africa[14]. In traditional medicine, this plant is used for the treatment of fever, convulsions and epilepsy in children[15]. The sap is also applied to ulcers, body rashes and abscesses. The dry leaves are used for the treatment of wounds, sores and sinusitis ulcer, ringworm[16], but also to treat jaundice, abdominal pains and gastritis. In some tribes in the Western part of Cameroon, the infusion of the dry leaves of this plant is used as a potent sedative and restorative. Previous phytochemical studies on *E. coccinea* have reported the presence of alkaloids, tannin, saponin, steroid, terpenoid, flavonoid and cardiac glycoside[17,18]. Various pharmacological activities of this plant, including antibacterial, antioxidant and anti-inflammatory activities have been documented[19]. Using an agar diffusion assay, Ndip *et al.*[20] have observed that the methanol extract of *E. coccinae* had a weakly antimicrobial activity against clinical isolates of *H. pylori.* The evaluation of the susceptibility of plant-extract against microorganism using microdilution method is more sensitive and recommended than disk diffusion method[21]. So, in this study, we have evaluated the antimicrobial activity of the tested plant-extract against ten clinical isolates of *H. pylori* using micro dilution method in addition to its anti-ulcerogenic effect against ethanol-induced gastric mucosal injury in experimental rats.

MATERIALS AND METHODS

Bacterial strains: Ten strains of *H. pylori* were isolated from gastric biopsies of patients with gastric related morbidities undergoing endoscopy at Laquintinie Hospital in Douala-Cameroon. The study was approved by local ethical committee of Laquintinie Hospital (Approval No. 425/AR/MINSANTE/HLD/SCM/CR) and the specimens were only collected from patients who had given consent. The isolates were identi ed by Gram staining and enzymatic activity (catalase, oxidase, urease)[22]. Pure cultures were suspended in Brian Heart Infusion (BHI) broth supplemented with 5% horse serum and 20% glycerol and stored at -80°C until used.

Experimental animals: Wister Albino healthy adult male rats (150-200 g) obtained from the Animal House, Faculty of Medicine and Biomedical Sciences, University of Yaounde I, Cameroon were used for this study. The animals were fed with standard pellet diet and tap water. These animals were used for the ethanol drug-induced gastric ulcer assessment and for acute toxicity. The study was approved by the Cameroon

National Ethical Committee for Animal Experimentation (Ref No. FW-IRB 00001954). Throughout, the experiments, all animals received human care according to the criteria outlined in the internationally accepted principle guidelines of the European Union on Animal Care (CEE Council 86/609).

Plant material: The whole plant of *Emilia coccinea* (Asteraceae) was collected in Baham, West region of Cameroon in May, 2015. Identification of the plant was done at the National Herbarium Yaoundé-Cameroon (voucher specimen No. 59675 HNC). The plant materials were then air dried at room temperature and ground into a fine powder.

Drugs: Omeprazole (Cipla Ltd., India), a proton pump inhibitor obtained from the local pharmacy was used as reference drug. The drug was dissolved in distilled water and administered orally to the rats at the dose of 20 mg kg^{-1} b. wt. (5 mL kg^{-1}) according to Abdulla *et al.*[23]. Culture media (Columbia agar, brain heart infusion, lacked horse blood, horse serum, vitox supplement) and the CampyGen gas pack were all purchased from Oxoid, Basingstoke, England. Doxycycline (Doxycycline 200 mg, Combitic Global Caplet, India), erythromycin (Erythromycine stearate 500 mg, cipla, India), Amoxicillin (Amoxicillin trihydrate 500 mg, maxheal pharmaceutical, India), ciprofloxacin (ZOFLOX, ciprofloxacine 750 mg, Odypharm), clarithromycin (Clarithromycin 500 mg, aurechem Laboratories, India) and metronidazole (Metronidazole 500 mg, strides Arcolab, India) used as reference antibiotics were purchased from a local pharmacy. The P-Iodonitrotetrazolium chloride (INT, Sigma-Aldrich) was used as a microbial growth indicator[24]. Ethanol and phenolphthalein were purchased from Sigma chemical company (Saint Louis, MO, USA). All other chemicals used were of analytical grade.

Preparation of the plant-extract: Air-dried and powdered plant material was weighed (300 g) and soaked in 1 L of methanol (MeOH) or Ethyl Acetate (EA) for 72 h at room temperature. The filtrate obtained through Whatman filter paper No. 1 was concentrated under reduced pressure in a vacuum to obtain the crude extract. All crude extracts were kept at 4°C until further use.

Anti-*Helicobacter pylori* assays: Plant extracts were further used for the determination of MICs by INT broth micro dilution method[24] using 96 well plates. Two-fold dilutions of the extract were prepared in the test wells in BHI broth supplemented with 5% horse serum (BHI-serum). The final extract concentrations ranged from 2-1024 μg mL^{-1}. One hundred microliters of inoculums prepared from a 48 h colonies on supplemented Columbia agar (Columbia agar+5% (v/v) lacked horse blood and 1% (v/v) vitox) at McFarlands turbidity standard 3 was added to 100 μL^{-1} of the extract-containing culture medium. Control wells were prepared with culture medium and bacterial suspension and broth only, respectively. Doxycycline, erythromycin, amoxicillin, ciprofloxacin, clarithromycin and metronidazole at concentrations ranging from 0.0625-512 μg mL^{-1} were used as positive controls. The plates were covered with a sterile plate sealer, then the contents of the wells were mixed with a shaker and incubated for 3 days at 37°C under micro aerophillic conditions. After incubation, 40 μL^{-1} of 0.2 mg mL^{-1} INT was added per well and incubated at 37°C for 30 min. Living bacteria reduced the yellow dye to pink. The sample concentration that prevented the color change of the medium and exhibited complete inhibition of microbial growth is known as the MIC. All the experiments were carried out in triplicate.

Acute toxicity bioassay: The acute toxicity study was used to determine a safe dose for *Emilia coccinae* extract. The bioassay was conducted according to the Organization for Economic Cooperation and Development Guideline protocol year 2001[25]. Eighteen adult male and female Wistar Albino healthy rats (9 males and 9 females) weighed between 150-180 g were used. The animals were given standard rat pellets and tap water *ad libitum* and assigned equally each into 3 groups labelled as vehicle (0.5% tween 80), 2 and 5 g kg^{-1} of *Emilia coccinae* whole plant extract preparation, respectively. The animals were fasted overnight (water but not food) prior to dosing. Food was withheld for further 3-4 h after dosing. The animals were observed after the administration of plant-extract for 48 h for clinical, physical signs of toxicity (restlessness, dullness, agitation) and mortality, if any was observed over a period of 2 weeks. The acute toxicity LD_{50} was calculated as the geometric mean of the dose that resulted in 100% lethality and that which caused no lethality at all. On day 15, body weight variation was determined and all the animals were sacrificed under an overdose of ether anaesthesia. The abdominal cavity of each animal was opened and organs namely, the heart, liver, lungs, brain, spleen and kidneys were removed, cleaned and weighed and the Relative Organ Weight (ROW) of each animal was then calculated as follows:

$$\text{ROW} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of rat on day of sacrifice (g)}} \times 100$$

Gastroprotective bioassay: For the gastric ulcer assessment, the rats were divided randomly into 5 groups of 6 rats each. They were fasted for 48 h before the experiment[23], but were allowed free access to drinking water up till 2 h before the experiment. Gastric ulcer was induced by orogastric intubation of absolute ethanol (5 mL kg^{-1}) according to the method described by Mahmood *et al.*[26]. Ulcer control group were orally administered vehicle (0.5% tween 80). The reference group received oral dose of 20 mg kg^{-1} omeprazole in distilled water as positive control. Experimental groups were orally administered *Emilia coccinea* methanol extract in 5% tween 80 at doses of 250, 500 and 1000 mg kg^{-1}. One hour after this pre-treatment all groups of rats were administered with absolute ethanol (5 mL kg^{-1}) in order to induce gastric ulcers[23]. The rats were euthanized 60 min later[27] under an overdose of ether anaesthesia and their stomachs were immediately excised.

Measurement of volume and pH of gastric juice: At the end of the experimental period, the rats were sacrificed and the stomach removed. The gastric content was collected and centrifuged for 10 min at 3000 rpm and the supernatant was separated. The volume and pH of centrifuged gastric juice were measured with a graduated cylinder and digital pH meter respectively. The volume was expressed in mL[28].

Determination of total acidity: An aliquot of 1 mL gastric juice diluted with 1 mL of distilled water was taken into a 50 mL conical flask and 2-3 drops of phenolphthalein as indicator was added to it and titrated with 0.01 N NaOH until a permanent pink colour was observed. The volume of 0.01 N NaOH consumed was noted. Total acidity was calculated by using the following formula:

$$\text{Acidity} = [\text{Vol. of NaOH} \times \text{Normality} \times 100 \text{ meq } L^{-1}]/0.1$$

Measurement of mucus production: Gastric mucus production was measured in the rats that were subjected to absolute ethanol-induced gastric lesions. The gastric mucosa of each rat was obtained by gentle scraping the mucosa with a glass slide and the collected mucus were weighed by using a precision electronic balance[27,29].

Gross gastric lesions evaluation: Gastric mucosa of each rat was thus examined for damage. A 2x hand lens was used to locate and score the lesions according to the method described by Ohara *et al.*[30]. The length and width of the ulcer (mm) were measured with a ruler under magnifying glass and the ulcerated area of each ulcer band was calculated. The severity of mucosal damage was graded as follows[31].

Scoring ulcer grading: The 0 = no lesion, 0.5 = hemorrhage, 1 = ulceration area≤0.5 mm^2, 2 = ulceration area between 0.5-2.5 mm^2, 3 = ulceration area between 2.5-5 mm^2, 4 = ulceration area between 5-10 mm^2 and 5 = ulceration area>10 mm^2. The results were expressed as total Ulcer Area (UA), Ulcer Index (UI), percentage of ulcerated area (UA%) and inhibition percentage (I%). Ulcer Index (UI) was determined using the following formula[32]:

$$UI = UN+US+UP\times 10$$

where, UN is average number of ulcers per animal, US is average of severity score and UP is percentage of animals with ulcer.

The sum of the areas of all lesions for each stomach was determined as the total Ulcer Area (UA). The percentage of ulcerated area (UA%) was determined using the following formula[33]:

$$UA(\%) = \frac{\text{Total ulcer area (UA)}}{\text{Total gastric area}} \times 100$$

stomach taken as a circle with area = $\pi d^2/4$.

The percentage protection or inhibition percentage (I%) was calculated according to the recommendation of Wasman *et al.*[29]:

$$I(\%) = \frac{\text{UI control-UI treated group}}{\text{UI control}} \times 100$$

Statistical analysis: All values were expressed as Mean±SEM. Statistical analyses were carried out using the software GraphPad Prism 5.01. The significance of the differences observed between the doses was achieved by analysis of variances (ANOVA) of the multiple tests of comparison of Tukey-Kramer. Differences between concentrations were considered statistically significant when $p<0.05$.

RESULTS

Anti-*Helicobacter* study: The *E. coccinae extracts* showed different anti-*Helicobacter* activity each with MIC values ranging from 64-1024 µg mL^{-1} (Table 1). The best activity was recorded with *E. coccinae* methanol extract with MIC values ranging from 64-256 µg mL^{-1} against 9/10 (90%) of the tested strains. The lowest MIC value (64 µg mL^{-1}) was recorded with

Table 1: MIC of ethyl acetate and methanol crude extracts of *E. coccinae* and antibiotics (μg mL^{-1})

	Microorganisms									
Plant-extract/antibiotics	*H. pylori* α_1	*H. pylori* α_2	*H. pylori* α_3	*H. pylori* α_4	*H. pylori* α_5	*H. pylori* α_6	*H. pylori* α_7	*H. pylori* α_8	*H. pylori* α_9	*H. pylori* α_{10}
Emilia coccinae (MeOH)	64	256	512	64	256	64	128	64	64	64
Emilia coccinae (EA)	1024	1024	1024	1024	256	1024	512	1024	512	512
Ciprofloxacin	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125
Doxycycline	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125
Erythromycin	128	128	128	128	128	128	128	128	128	128
Amoxicillin	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512
Clarithromycin	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125
Metronidazole	256	256	256	256	256	256	256	256	256	256

All the values given in the table are means of three determinations, EA: Ethyl acetate, MeOH: Methanol

Table 2: Relative organ weight

Treatments	Liver	Kidneys	Lungs	Spleen	Heart	Brain
Normal group	2.12±0.13	0.24±0.05	0.62±0.11	0.21±0.05	0.26±0.05	0.54±0.10
Emilia coccinae (2 g kg^{-1})	2.06±0.39	0.26±0.03	0.64±0.10	0.17±0.06	0.25±0.03	0.60±0.05
Emilia coccinae (5 g kg^{-1})	2.10±0.36	0.25±0.26	0.78±0.17	0.17±0.02	0.23±0.03	0.50±0.05

All values are expressed as Mean±Standard Error (n = 6), means with different superscripts are significantly different from the normal group, the mean difference is significant at the p<0.05 level

Table 3: Effect of *E. coccinae* on acidity of gastric content and mucus production

Treatments	Gastric juice (mL)	Gastric (pH)	Gastric acidity (meq L^{-1})	Mucus production
Negative control group	3.64±0.68	5.97±0.33	5.16±0.74	1.44±0.12
Emilia coccinae (250 mg kg^{-1})	2.50±0.75	7.65±0.16*	2.82±0.24*	1.68±0.17
Emilia coccinae (500 mg kg^{-1})	3.12±0.30	7.96±0.08*	2.70±0.16*	2.21±0.35*
Emilia coccinae (1000 mg kg^{-1})	2.60±0.21	7.83±0.08*	2.90±0.27*	2.67±0.86*
Omeprazole (20 mg kg^{-1})	2.60±0.51	8.02±0.11*	1.15±0.12**	1.72±0.18

All values are expressed as Mean±Standard Error (n = 6), means with different superscripts are significantly different from the negative control group, the mean difference is significant at the *p<0.05 and **p<0.01 level

the methanol extract from *E. coccinae* against 60% of the tested strains (*H. pylori* α_1, *H. pylori* α_4, *H. pylori* α_6, *H. pylori* α_8, *H. pylori* α_9 and *H. pylori* α_{10}) (Table 1).

Acute toxicity study: Methanol extract of *E. coccinae* was used for the acute oral toxicological study for appreciating the usefulness and safety of the chosen plant and in understanding its effects in relation to the ulcer disease. For this purpose, animals were treated with methanol extract of *E. coccinae* at a dose of 2 and 5 g kg^{-1} and were kept under observation for 14 days. Animals were found to be safe without mortality even up to 5000 mg kg^{-1} b.wt., it reflects that LD_{50} value of *E. coccinae* extract is >5000 mg kg^{-1} b.wt., There were no abnormal signs, behavioural changes, body and organs weight changes (Table 2) or macroscopic finding at any time during the observation period. Therefore, there are no acute toxicological changes for the herbal drug *E. coccinae* up to 5000 mg kg^{-1} b.wt., of the animal. The fact that no toxic effect occurred in animals that were administered acutely *E. coccinae* up to 5000 mg kg^{-1} b.wt., suggest that the margin of safety of the extract is high at dosages used clinically and justify the choice of dose up to 1000 mg kg^{-1} for the anti-ulcerogenic assay.

Acidity of gastric content and mucus production: The acidity of gastric content in experimental animals pre-treated with *E. coccinae* decreased significantly, while its pH significantly increased compared to that of the ulcer control group ($p<0.05$). The mucus production of gastric mucosa also increased significantly ($p<0.05$) in animals pre-treated with *E. coccinae* compared to the ulcer control group (Table 3).

Gross appearance of the gastric mucosa in rats: In this study, pronounced destructive changes associated with haemorrhage in the stomach were observed in the ethanol-induced control group. Pre-treatment of rats with a single oral dose of *E. coccinae* partly reduced ulcer area and promote healing of gastric lesions induced by acute intake of ethanol (Fig. 1).

Effect of *E. coccinae* extract on gastric lesions induced by ethanol in rats: This results indicate that *E. coccinae* extract showed potent gastroprotective effect against ethanol induced gastric damage on rats. Table 4 shows that, the gastric lesions were significantly reduced by the administration of *E. coccinae* extract at 250, 500 and 1000 mg kg^{-1} doses compared with the ethanol-treated

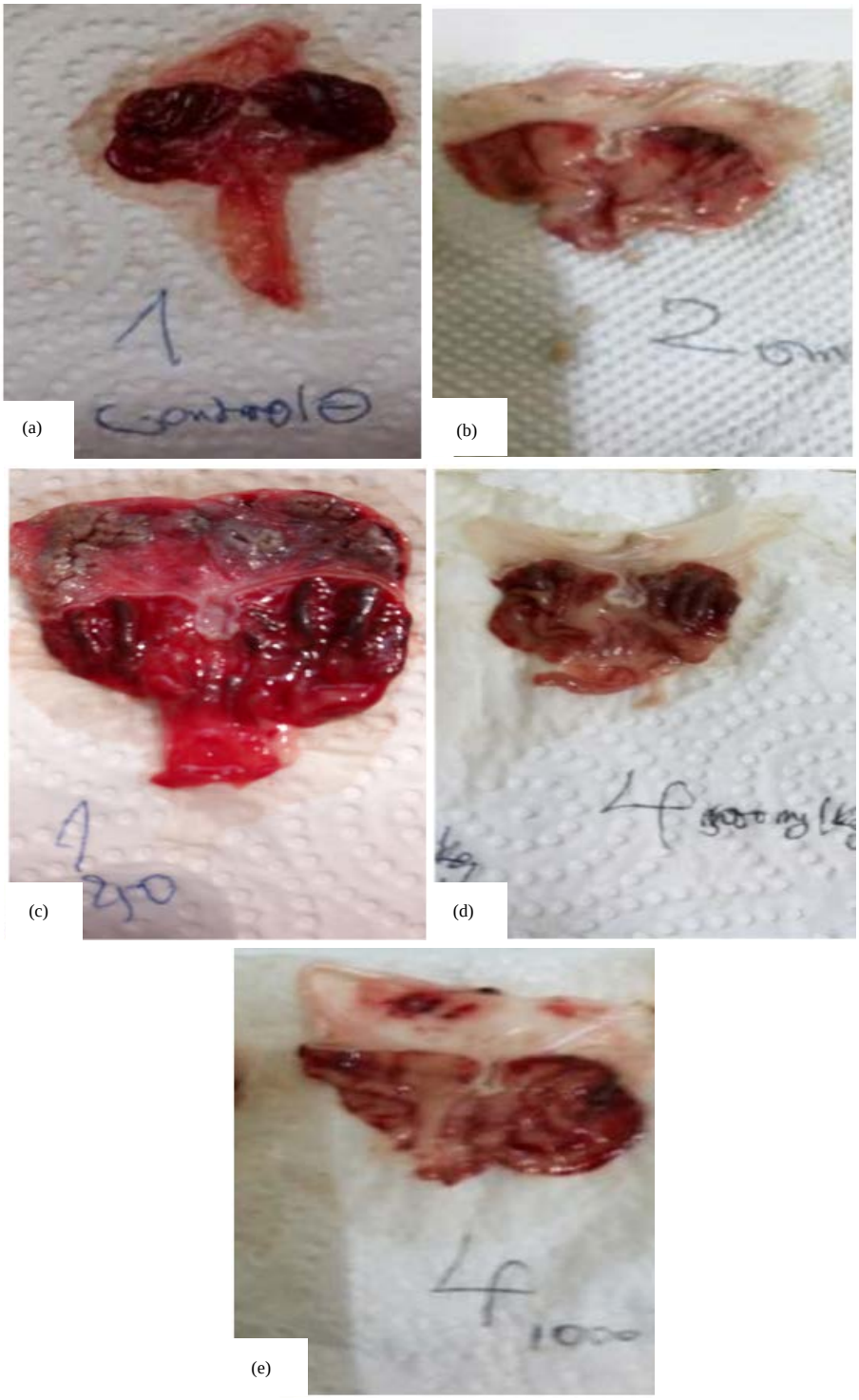

Fig. 1(a-e): Gross appearance of the gastric mucosa in rats, (a) Rats pre-treated with 5 mL kg^{-1} 5% tween 20 (ulcer control). Severe injuries are seen in the gastric mucosa. Absolute ethanol produced extensive visible hemorrhagic necrosis of gastric mucosa, (b) Rats pre-treated with of omeprazole (20 mg kg^{-1}). Injuries to the gastric mucosa are milder compared to the injuries seen in the ulcer control rats, (c) Rat pre-treated with *E. coccinae* (250 mg kg^{-1}). Mild injuries are seen in the gastric mucosa. The extract reduces the formation of gastric lesions induced by absolute ethanol, (d) Rat pre-treated with *E. coccinae* (500 mg kg^{-1}). Mild injuries are seen in the gastric mucosa and (e) Rats pre-treated with *E. coccinae* (1000 mg kg^{-1}). Very mild injuries to the gastric mucosa are seen

Table 4: Effect of *E. coccinae* on ulcer area and inhibition percentage in rats

Treatments	UA (mm^2)	UI	UA (%)	I (%)
Negative control group	388.1±40.96	7.86±0.66	57.20±5.05	0.00
Emilia coccinae (250 mg kg^{-1})	235.9±37.72*	5.00±0.98*	12.76±0.84*	36.38
Emilia coccinae (500 mg kg^{-1})	126.3±10.47**	4.94±0.21*	9.96±0.59*	37.15
Emilia coccinae (1000 mg kg^{-1})	63.05±9.13***	4.10±0.35*	9.22±0.76**	47.83
Omeprazole (20 mg kg^{-1})	50.15±3.53***	3.56±0.42**	4.70±0.97**	54.70

All values are expressed as Mean±Standard Error (n = 6), means with different superscripts are significantly different from the negative control group, the mean difference is significant at the *p<0.05, **p<0.01 and ***p<0.001 level

control group. All tested doses of *E. coccinae* extract also showed a greater gastroprotective effect in comparison to omeprazole, which was used as a reference drug. The ulcer indexes in rats receiving *E. coccinae* extract at doses of 250, 500 and 1000 mg kg^{-1} were 5.00±0.66, 4.94±0.21 and 4.10±0.35, respectively. The *E. coccinea* treatment also significantly ($p<0.05$) diminished the percentage of ulcerated area of ethanol-induced ulcerations relatively to the negative control group. The mean percentage of ulcerated area decreased from 57.20±5.05% (negative control group) to 9.22±0.76 (*E. coccinae* at 1000 mg kg^{-1} b.wt.). Gastric lesions in rat's stomachs were dose-dependent and significantly ($p<0.05$) inhibited from 36.38-47.83% by treatment with *E. coccinae* at doses varying from 250-1000 mg kg^{-1} b.wt., while omeprazole (20 mg kg^{-1} b.wt.) exhibited inhibition of 54.70% (Table 4).

DISCUSSION

In this study, the *in vitro* antimicrobial activity of the ethyl acetate and methanol extract of *E. coccinae* were investigated against 10 clinical strains of *Helicobacter pylori.* The tested plant-extracts displayed different antibacterial activities from each other. In fact, the best anti-*Helicobacter* activity was obtained with the methanol extract of the plant, indicating that the antimicrobial compounds of this plant are more soluble in the methanol than in ethyl acetate. The activity observed with *E. coccinae* methanol extract (MIC of 64 µg mL^{-1}) against 60% of the tested *H. pylori* strains can be considered important since it is documented that the antibacterial activity of a plant products is significant when MIC values were below 100 µg mL^{-1}, moderate when 100>MIC<625 µg mL^{-1} and weak[34] when MIC>625 µg mL^{-1}.

It has been demonstrated that the methanol extract from the whole plant of *E. coccinae* exhibits a weak antimicrobial activity at 200 mg mL^{-1} against clinical isolates of *H. pylori* with inhibition zone diameters that varied from16-22 mm. Although, this results showed higher activity of this extract relative to that reported previously by Ndip *et al.*[20], they however corroborate their reports. This variation may be due to the method of bioassay employed, the age of the plant and the region of the plant collection.

Previous phytochemical studies on *E. coccinea* have reported the presence of alkaloids, tannin, saponin, steroid, terpenoid, flavonoid and cardiac glycoside[17,18]. The active principles of the extract of this plant should be assignable to these different secondary metabolites. Anti-*Helicobacter* activity of the methanol extract of *E. coccinae* was better than that of metronidazole, erythromycin and amoxicillin antibiotics used in the treatment of *H. pylori* infected individuals.

Disturbances in gastric secretion, damage to gastric mucosa, alterations in permeability, gastricmucus depletion and free-radical production are reported to be the pathogenic effects of ethanol[35]. The products of the 5-Lipooxigenase pathway may also play a key role in the development of ulcer induced by irritant agents, such as ethanol[36]. The present study on the cytoprotective assessment demonstrated that pre-treatment of rats with methanol extract of *E. coccinea* significantly protect the rat gastric mucosa against hemorrhagic lesion induced by absolute ethanol, which may be indication of the gastroprotective activity of this plant-extract. The protective action of *E. coccinae* against ethanol-induced gastric mucosa damage was demonstrated by the reduction of the gastric ulcer area, increase production of gastric mucous and decrease acidity of gastric content in treated animals compared to negative control animals.

The mucus of the gastric wall is thought to play an important role as a defensive factor against gastrointestinal damage[29]. The *E. coccinae* prevented ethanol induced-gastric damage with mucous production increase which is a strong defense factor of gastric mucosa.

Contrary to gastric mucosal content, gastric acidity is an important indicative factor for gastro-intestinal damage. Experiments show that the volume of gastric acid produced determines the permeability of the gastric mucosal wall which triggers and accelerates the development of ulcers[37]. Ethanol production of necrotic lesions in the gastric mucosa is also due to its direct toxic effect reducing the secretion of bicarbonate, then increasing gastric acidity[38]. Neutralization of the acid content in the stomach may well be reasoned enough for speeding up the healing of gastric ulcers[39]. In this study, it was realized that pre-treatment of rats with methanol extract of

E. coccinea significantly decrease the acidity of gastric content. Inhibition of acid production by *E. coccinea* may well maintain the permeability of the gastric mucosal membrane and help reduce the formation of gastric ulcers.

Lowering of gastric acidity and increase the gastric mucosal content by *E. coccinae* methanol extract act as its potential defensive factor against the gastric damage and ulceration induced by ethanol. Similar findings exist in the literatures, where plant extracts have been shown to prevent gastric mucosal ulceration in rats[27,29].

Omeprazole a reference drug used is a proton pump inhibitor which has been widely used as an acid inhibitor agent for the treatment of disorders related to gastric acid secretion[39]. It inhibits acid secretion by acting on the hydrogen-potassium exchanger (H±, K±-ATPase) for the apical plasma membrane of the gastric mucosa[40]. The activity of the methanol extract of *E. coccinea* against ethanol induced gastric damage herein was comparable to that of omeprazole, a standard drug in the purified form whereas the extracts are mixture of substances.

The *E. coccinea* has been shown to contain flavonoids[17,41]. Previous studies have shown that flavonoids may be related to the antiulcer activity[42] and play a major role in the mechanism of gastroprotection[43]. It could be conceivable that the anti-ulcer activity of this plant could be linked to the flavonoids since flavonoids are reported to protect the mucosa by preventing the formation of lesions by various necrotic agents[44]. It is well known that many flavonoids display anti-secretory and cytoprotective properties in different experimental models of gastric ulcer[45]. Flavonoids possess antioxidant properties in addition to strengthening the mucosal defense system through stimulation of gastric mucus secretion[46] and flavonoids can scavenge for the reactive oxygen species (super-oxide anions) and free radicals produced by ethanol. These reactive intermediates are potentially implicated in ulcerogenicity[47].

CONCLUSION

The present study demonstrate that the methanolic extract of *E. coccinea* possesses an important anti-*Helicobacter* property and protects the rat's gastric mucosa against hemorrhagic lesions produced by absolute ethanol and could be a potential source for novel antiulcer drug discovery and development.

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