



Preliminary phytochemicals, *non-enzymatic in-vitro* antioxidant, microbicides, anti-ulcerogenic and toxicological properties of *Emilia coccinea*

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Abstract: *Emilia coccinea* is a shrub herb that belongs to the family Compositae (Asteraceae). This study evaluates the preliminary phytochemicals, *in-vitro* antioxidant, anti-ulcerogenic, microbicides, and toxicological properties of *E. coccinea* ethylacetate extract. Fresh leaf of *E. coccinea* was gotten, rinsed, shade dried, powdered, and processed into ethyl acetate extract. Qualitative and quantitative phytochemicals, 1, 1 Diphenyl -2-Picrylhydrazine (DPPH) antioxidant property and the antimicrobial effect carried out via standard method. Adults Wistar rats weighing 180-220 were acclimatized and allotted into groups of five (n=5) (twenty-five rats) such as ulcer control, 10 mg/kg cimetidine, and graded doses (100, 200, and 400 mg/kg) of ethyl acetate extract were evaluated. A toxicological study was evaluated. The preliminary phytochemicals present include; phenol (107.46 mg), saponins (10.42 mg), alkaloids (184.30 mg), cardiac glycosides (83.82 mg), and anthraquinone (124.94 mg). The *in-vitro* antioxidant assay exhibited scavenging property against DPPH radicals when compared with ascorbate. The microbicide effect of *E. coccinea* ethyl acetate extract at graded concentration showed inhibitory action against *Escherichia coli*, *Helicobacter pylori*'s, and *Salmonella typhi*. Ulcerative defect in the mucosal lining of the rats' stomach was pre-treated with ethyl acetate crude extract, which showed a significant decrease in ulcer count and ulcer index with a considerable increase in ulcer percentage inhibition compared to ulcer control and 10 mg/kg cimetidine. At <5000 mg/kg of the acute study showed no dead or adverse effect. Haemato-pathological and histopathological analysis of the extract displayed no significant difference with the normal architecture of the organs compared with the control. In conclusion, further studies will be required for compound isolation.

Keywords: Preliminary Phytochemicals, non-enzymatic *in-vitro* antioxidant, Anti-ulcerogenic, Microbicides, toxicological, *Emilia coccinea*



INTRODUCTION

Plants are enriched with several culinary and medicinal applications identified as medicinal plants (Wainright, 2001). Many plant type's supports are a viable entity to propagate scientific and clinical approval. Plant constituents like the leaves, flowers, fruits, seeds, stem bark, and roots are useful in several therapeutic forms (Kokate et al, 2006). Characteristically, they synergized in most cases as polyherbal agents or more active without combination. Natural herbs can be utilized as either nutraceuticals or spices. More so, the efficacy present in inherent herbs displayed its potency as an antimicrobial with the basis of conventional remedies (Ozumba, 2003). *Emilia coccinea* is from the family Compositae (Asteraceae). At times, it is known as a shrubs and woody herbs as a climber (Olorode, 1984). In Nigeria, the leaf is consumed when cooked as either salad or spinach and, a fresh juice from the leaf aid in the treatment of eyesore (Sofowora, 1982). The leaf can be eaten raw or mixed with fluid obtained from lime and guinea corn to manage sore throat (Ayitey-Smith, 1989; Sofowora, 1993). The roots portion of the plant is applicable for the treatment of diarrhea. *E. coccinea* stimulated some bioactivities includes; antimicrobial, anti-diarrhea, and anti-fungi (Ogbebor and Adekunle, 2005; Ndip et al, 2007). The usual phytochemicals found in them are; alkaloids and cardiac glycoside (Edeoga et al, 2005). The chief bioactive ingredients are tannins, flavonoids, alkaloids, and phenolic (Hill, 1952). It has been vaguely utilized as a conventional method in the treatment of diseases. Sap derived from the leaf help in treating vertigo (Oliver, 1960). It is also active in managing lice, cough, ringworm, ulcers, seizure, measles, and gonorrhea (Edeoga et al, 2005; Odugbemi, 2006). The leaf sap is helpful in the treatment of epilepsy (Kerharo and Bouquet, 1950). Peptic ulcer ailments, commonly known as stomach ulcers, are associated with severe injury in the mucosal lining of the stomach, which is the chief small intestinal portions infrequent to the lower esophagus. It is more observable than other forms of ulcer, perhaps as a result of mucosal thickening to shield the stomach wall against digestive juice, which could be intrusive to stomach lining tissues. Stomach ulcers can easily be treated when not in a severe case or when appropriate drugs are applicable. The segment inevitably can be initiated contingent on the main discrete factor (Milosavljevic et al, 2011). Decrease in the mucosal lining of the stomach developed into ulcer features activated by prolonging nonsteroidal anti-inflammatory drugs abuse (ibuprofen and aspirin) or extreme discharge of the stomach acid instigated by stress, smoking, and drinking or *Helicobacter pylori* or genetic origin or Zollinger-Ellison disorder, exposing the stomach into a redundant synthesis of stomach acid (Najm, 2011; Steinberg, 2002). Aged people are more disposed to the forms of ulcer initiated by NSAIDs. Diagnosis is typically ascertained by re-occurring signs and symptoms either with barium swallows or endoscopy. *H. pylori* can also be diagnosed via blood test, signs of microbes invasion detected from a stool test, urea test, biopsy, and breathe Test of the samples (Najm, 2011). Specific objectives of this study investigated the bioactive ingredients, *in-vitro* antioxidant property, microbicides, and antiulcer effect of *E. coccinea*.

METHODS

Plant collection: Fresh leaves of *Emilia coccinea* were gotten in June around the University of Benin botanical garden, Benin City, Edo State, Nigeria. The plant was identified and authenticated by Dr. O. Timothy, a plant taxonomist in the herbarium unit of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria. The voucher number of the deposited specimen was obtained as GW: E198



The extraction procedure: The fresh leaf of *E. coccinea* was rinsed in clean water, shade dried, and powdered with an electric blending machine. 1.25 kg of the pulverized sample was achieved and macerated in ethylacetate solvent for 72 hr. The filtrate was concentrated in a rotary evaporator, and a crude dark greenish semi-solid matter weighed 42.9 g was obtained. The percentage yield of the sample was calculated via the formula below. The percentage yield of the extract is calculated as follows:

$$\text{Percentage (\%)} \text{ yield of the extract} = \frac{\text{weight of extract}}{\text{Weight of pulverized leaves}} \times 100$$

The extract was refrigerated at a suitable temperature for further use.

Preliminary qualitative and quantitative Phytochemical screening: Qualitative and quantitative phytochemical chemical tests were carried out on ethyl acetate extract in a standard method described by Sofowora (1993), Harborne (1973) to display the color changes of the various qualitative phytochemicals present and at what quantity of tannins, phlobatannins, saponin, flavonoids, steroids, Test for terpenoids (Salkowski test) and cardiac glycosides were measured (Keller-Killani test).

DPPH free radical-scavenging assay: Antioxidant capacity of ethyl acetate extract was evaluated using free radical-scavenging effect on 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical. Ten (10) μl of an aliquot in the extract was thoroughly mixed in 90 μl of distilled water and 3.9 ml of 25 mM DPPH in methanol solution. The mixture was thoroughly eclectic and kept in the dark for 30 min. Absorbance measurement was recorded at 515 nm. The blank used was methanol without DPPH. Obtained optical values were expressed as percentage inhibition calculated according to the following;

$$\% \text{ Inhibition of DPPH}' = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

Antimicrobial assay: Preparations of the solution of the crude extract were reconstituted in distilled water to attain 2000, 1000, 500, 250, and 125 mg/ml concentrations gradient. This was done by dissolving 0.5, 0.25, 1.0, and 2.0 g of ethyl acetate crude extract in 1ml of distilled water. The reconstituted extract was stored at 4°C in sample bottles for use.

Collection fluid specimen: Fluid specimens were obtained from the University of Benin Teaching Hospital and St. Philomena Hospital, Benin City. The blood fluid specimens were collected into sterile sample bottles and conveyed instantaneously to the laboratory at 37°C temperature.

Isolation and Identification of bacteria isolates: A blood fluid specimen was streaked on prepared Nutrient agar in a petri dish to isolate non-fussy microbes. Plates were all incubated at a temperature of 37°C for 24-48 hours. After incubation, isolated bacterial species (*Mycoplasma pneumonia*, *Helicobacter pylori*, *Klebsiella pneumonia*, *Escherichia coli*, *Streptococcus pneumoniae*, and *Salmonella Typhi*) were identified correctly using Gram staining and biochemical tests following the standard technique described by Ellof (2008).

Standardization of tested organisms: A loopful of standard culture organisms was inoculated in 5 ml of prepared sterile nutrient broth and incubated for 24 hours. Overnight



cultures of 0.2 ml of the microorganisms were inoculated into 20 ml of sterile nutrient broth and incubated for 3-5 hours. Turbidity of culture was compared to that of 0.5 Mac-Farland in a standardized culture of 10^6 CFU/ml.

Susceptibility testing: Method described by Emeruwa (2009) was used to determine the antibacterial effect of ethyl acetate crude extract. Homogeneous culture at 0.5 ml was banquet into two distinct sterile plates for proper growth. And 18.0-20 ml Nutrient agar at 45°C was introduced to the plate and adequately rocked even for relevant bacteria and agar mixture. Content of the plates was left to thicken, and the wells were approximately 6 mm in diameter bored in the agar medium surfaces via sterile cork borer, and the bottom holes were sealed with molten agar. The reconstituted at 0.2 ml of ethyl acetate crude extract of the tested concentrations were released into the holes. An aqueous solution of chloramphenicol and streptomycin were at an equal concentration (positive control). The plates were left for 30 minutes to pre-diffusion of ethyl acetate extract rate then incubated at 37°C for 24 hours, and zones of inhibition were measured at mm using translucent meter rule. Mean of the triplicate results were taken.

Determination of minimum inhibitory concentrations (MIC): Bacterial strains were cultured overnight at 37°C on Nutrient broth and adjusted to 10^6 cfu/ml density. This aids in inoculating 96-well microtitre plates comprising of serial 5-fold dilutions of the extract (50-6.25% v/v) under aseptic situations. Aqueous extract of *E. coccinea* was dissolved in water. Plates were incubated in aerobic conditions at 37°C and observed after 24 hours. This serves as an indication of bacterial growth; $40\ \mu\text{l}$ of $0.2\ \text{mg}\ \text{ml}^{-1}$ p-iodonitrotetrazolium solution was added to the well and incubated for 30 min at 37°C . The colorless tetrazolium salt was reduced to a red-colored product by the biological action of the organisms. The treatment was completed in triplicate, and widespread subdual of the growth in an explicit concentration of the extract was specified by a clearer solution obligatory to affirmed vigorous (Ellof, 2008). Chloramphenicol was employed as reference controls with sample-free solutions and distilled water as the controls.

Determination of minimum bactericidal concentrations (MBC): MBC values were determined via eradicating a loopful of bacterial deferral from MIC tubes without any growth, sub-cultured in the Nutrient agar plates. The plates were at 37°C incubated for 24 hours. Afterward, the concentrate with no observable increase was recorded in MBC.

Experimental animals Thirty-two adult Wistar rats weighing 180-220 were obtained and kept in the animal compartment of Animal and Environmental Science, University of Benin, Benin City Nigeria, incapacious and well-ventilated cages with suitable temperature, food, drinking water, and relative humidity for 14 days of acclimatization. Approval for the use and proper handling of animals was obtained in Life Sciences ethical committee and certified with the ethical number LS019213.

Experimental design: Animals with relative body weight (180 to 220 g) were allotted into five groups (n=5). Group A serves as normal control, group B received 10 ml/kg cimetidine, group C serves as ulcer control treated with 10 ml/kg DmsO₄, and Group D to F was treated with graded doses (100, 200, and 400 mg/kg) of ethylacetate *E. coccinea* extract. The treatment persisted for 14 days. Albino rats fasted for 24hrs preceding the induction of ulcer. An hour after treatment, Wistar rats were administered with 1 mL/kg oral administration of 75% ethanol excluding normal control, administered with 0.5 mL of 1% Tween 80. All the animals were anesthetized in chloroform, and the stomachs were isolated and analyzed.



Acute toxicity study: Twelve mice were indiscriminately apportioned into groups of three (n=3). Graded doses (10, 100, and 1000 mg/kg) of the extract were administered alongside the control group in phase first of the study. Four to Twenty four hours later, without any death, recorded, phase 2 of the procedure took place involving three mice exposed to 1600, 2900, and 5000 mg/kg *E. coccinea* ethylacetate extract. Animals were observed for four hours through twenty-four hours to 14 days with the method described by Lorke (1983).

Sub-chronic toxicity study: Two four albino rats of male sex weighing 195 to 250 g, randomly selected into groups of four (n=5). There were all administered with graded doses of *E. coccinea* extract for 28. Ethylacetate extract at 100, 200, and 400 mg/kg and the control administered with 0.5 ml/kg distilled water. The animals weight was taken at an interval of 7 days for 28 days. The whole group of rats was mildly anesthetized and sacrificed; a Blood sample was collected via abdominal aortal.

Hematological analysis: Blood samples were obtained via 5 ml syringes into an ethylene diamine tetraacetic acid (EDTA) bottle for full blood count (FBC) analysis. Blood indices (red blood cell count, white blood cell count, and hematocrit) were procured for full blood count analysis by Sysmex (R) XT- Series Automated Haematology Analyser.

Histological study: The stomach was harvested from the various groups during the experiment, and the following histological procedures were performed. The harvested organ was fixed in bouins fluid to arrest metabolic activity in the tissues and avoid autolysis and protein precipitation, thus preventing the enzymatic digestion of dead tissues. The fixed tissues were passed through several changes of alcohol, 70% alcohol for 24 hours and 90% alcohol for 12hours, and absolute alcohol. This was done to remove water from the fixed tissues and allow complete infiltration of tissue by paraffin. The tissues were passed through Xylene for 3 hours to avert shrinkage and tissue brittleness in paraffin. Blocks of paraffin were melted and placed in beakers in the oven at a temperature of 60°C. After melting, the tissues were placed in paraffin wax. Forceps were used to arrange the tissues in the desired plane, after which the wax was collected to cool for one and half hours in a water bath. Blocks of wax were attached to the microtome's block holder, which was trimmed it using a Plano concave knife with the microtome gauge set at 5um. Upon exposure of the whole tissue surface, sections were placed one at a time on a slide and flooded with egg albumin. Sections were exposed to absolute alcohol, 90 and 70% alcohol for 2 minutes, respectively, and distilled water. Slides were then stained with hematoxylin for 15 minutes at room temperature, and the excess stains were in absolute alcohol. Differentiation was done using 1% acid alcohol and counter-staining with eosin for 3 minutes. Stained sections were cleared with Xylene. Canada balsam was carefully placed on the stained section on the slide, and a cover was carefully placed over the tissue to prevent air bubbles from been trapped in the slide. Slides were viewed under an Olympus Microscope (light microscope) (Nikon Eclipse E400). All alterations from the normal structure were registered. Photomicrographs were obtained at different magnifications to show the differences in tissues for the rats from each experimental group and stage.

Statistical analysis: Statistically, the investigation was done using the general descriptive statistics and a one-way ANOVA at the $p < 0.05$ significant level. Significant differences were defined at $p < 0.05$. Computer software, statistical package for social scientists (SPSS), and Microsoft Excel were used for statistical analysis.

RESULTS

Table 1 presents the various qualitative and quantitative phytochemicals such as Phenols, Saponins, Alkaloids, Cardiac glycoside, and anthraquinone present at what level of concentrations.

Table 1: Qualitative and quantitative phytochemical screening of *Emilia coccinea*

Phytochemicals	Qualitative	Mean±SEM Quantitative mg/100g
Phenols	+++	107.46±15.13
Saponins	+	10.42±1.93
Alkaloids	+++	184.30±19.40
Cardiac glycoside	++	83.82±5.52
Anthraquinone	+++	124.94±22.06

Figure 1 shows the competitive scavenging effect of ethyl acetate extract of *E. coccinea*. The extract showed a wide range of inhibitory effects against the DPPH scavenging effects compared with ascorbic acid, whose scavenging actions showed a competitive effect, as shown in the Figure 1.

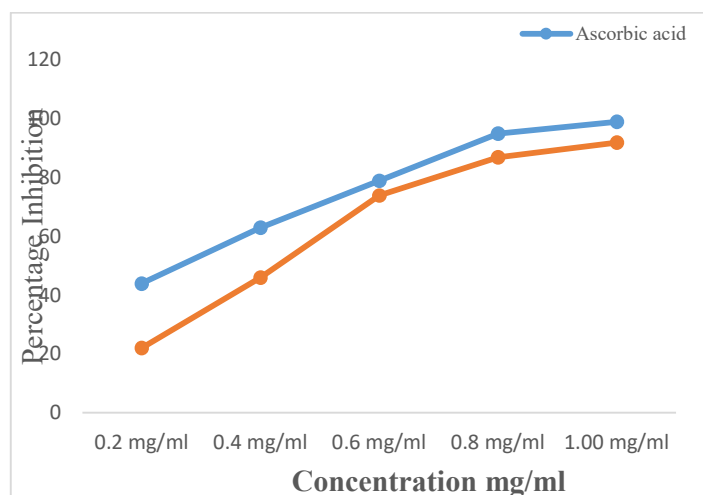


Figure 1: Effects of *Emilia coccinea* on DPPH radical scavenging property.

Table 2 shows the zone of inhibition of *Salmonella spp* when compared across treated groups. It was observed that the treated group highly inhibit *Salmonella spp* when compared across the control groups except for reference control with no significant difference from the treated groups.

Table 2: Zone of inhibition of *Salmonella* isolates on *Emilia coccinea* ethyl acetate extract

Treatment	Dosage µg/mg/ml	Mean±SEM <i>Salmonella spp</i>	X ² value compared to control 1	X ² value compared to control 2	X ² value compared to control 3
Ciprofloxacin	30	22.00±0.29	-	-	-
Septtrin	30	0.00±0.00	(X ² =19.17, **P<0.01)	-	-
Tarivid	10	28.00±0.31	(X ² =0.72, P>0.05)	(X ² =25.14,	-



				**P<0.01)	
<i>E. coli</i>	100	0.00±0.00	(X ² =19.17, **P<0.01)	(X ² =0.00, P>0.05)	(X ² =25.14, **P<0.01)
<i>E. coli</i>	200	0.00±0.00	(X ² =19.17, **P<0.01)	(X ² =0.00, P>0.05)	(X ² =25.14, **P<0.01)
<i>E. coli</i>	500	0.00±0.00	(X ² =19.17, **P<0.01)	(X ² =0.00, P>0.05)	(X ² =25.14, **P<0.01)

P>0.05- Not Significant, *P<0.05 – Significant, **P<0.01- Highly Significant

Table 3 showed the effect of the reference controls for the zone of inhibition of *Escherichia coli* compared with treated groups. It was observed that test groups showed an inhibitory effect against *Escherichia coli* when compared with Tarivid across the groups except ciprofloxacin and Septrin, which displayed no significant inhibitory effect compared with the treated groups.

Table 3: Zone of inhibition of *E.coli* isolates on *Emilia coccinea* ethyl acetate extract

Treatment	Dosage µg/mg/ml	Mean±SEM <i>E. coli</i>	X ² value compared to Ciprofloxacin	X ² value compared to Septrin	X ² value compared to Tarivid
Ciprofloxacin	30	0.00±0.00	-	-	-
Septrin	30	0.00±0.00	(X ² =0.00, P>0.05)	-	-
Tarivid	10	15.00±1.17	(X ² =12.25, **P<0.01)	(X ² =12.25, **P<0.01)	-
<i>E. coli</i>	100	0.00±0.00	(X ² =0.00, P>0.05)	(X ² =0.00, P>0.05)	X ² =12.25, *P<0.05)
<i>E. coli</i>	200	0.00±0.00	(X ² =0.00, P>0.05)	(X ² =0.00, P>0.05)	X ² =12.25, *P<0.05)
<i>E. coli</i>	500	3.00±0.25	(X ² =1.00, P>0.05)	(X ² =0.00, P>0.05)	(X ² =8.00, *P<0.05)

Note: P>0.05- Not Significant, *P<0.05 – Significant, **P<0.01- Highly Significant

Table 4 showed the standard control in the zone of inhibition of *Helicobacter pylori* when compared across test groups. It was observed that the test groups significantly inhibit *Helicobacter pylori* across the grade concentration 100, 200, and 500 mg/ml of ethyl acetate extract when compared with Ciprofloxacin and Tarivid except for Septrin that is not significant in the treated groups.

Table 4: Zone of inhibition of *Helicobacter* isolates on *Emilia coccinea* ethyl acetate extract

Treatment	Dosage µg/mg/ml	Mean±SEM <i>H. pylori</i>	X ² value compared to Ciprofloxacin	X ² value compared to Septrin	X ² value compared to Tarivid
Ciprofloxacin	30	13.00±0.62	-	-	-
Septrin	30	0.00±0.00	(X ² =10.29, **P<0.01)	-	-
Tarivid	10	11.00±0.77	(X ² =0.17, P>0.05)	(X ² =8.33, **P<0.01)	-
<i>E. coli</i>	100	0.00±0.00	(X ² =10.29, **P<0.01)	(X ² =0.00, P>0.05)	(X ² =8.33, **P<0.01)
<i>E. coli</i>	200	4.00±0.51	(X ² =4.77, *P<0.05)	(X ² =1.80, P>0.05)	(X ² =3.27, P>0.05)
<i>E. coli</i>	500	8.00±0.59	(X ² =1.19, P>0.05)	(X ² =5.44, *P<0.05)	(X ² =0.48, P>0.05)

P>0.05- Not Significant, *P<0.05 – Significant, **P<0.01- Highly Significant



Results from Table 5 exhibited the ulcer index of the ulcer control and 10 mg/kg cimetidine when compared with test groups. It was observed that ethyl acetate extract at 400 mg/kg showed a significant reduction in ulcer index when contrasted with a standard drug has no significant difference with Cimetidine, unlike 100 and 200 mg/kg, which displayed no significant difference.

Table 5: Effect of *Emilia coccinea* on Ulcer Indexes in rats

Treatment	Dosage mg/kg	Mean±SEM Ulcer index	X ² value compared to DMSO ₄	X ² value compared to Cimetidine
DMSO ₄	10 %	56.00±3.11	-	-
Cimetidine	100	44.00±2.69	(X ² =1.44, P>0.05)	-
<i>E. coccinea</i>	100	36.00±2.85	(X ² =4.35, *P>0.05)	(X ² =0.80, P>0.05)
<i>E. coccinea</i>	200	10.00±1.10	(X ² =32.06, **P>0.05)	(X ² =21.41, **P>0.01)
<i>E. coccinea</i>	400	10.00±1.09	(X ² =32.06, **P<0.01)	(X ² =21.41, **P>0.01)

Note: P>0.05- Not Significant, *P<0.05 – Significant, **P<0.01- Highly Significant.

Table 6 exhibited the Mean ulcer index in the ulcer control and 10 mg/kg cimetidine used in comparing graded doses of the test groups. It was observed that across the treated groups, there was no significant difference.

Table 6: Effect of *Emilia coccinea* aqueous extract on Mean Ulcer Index in rats

Treatment	Dosage mg/kg	Mean±SEM ulcer index	X ² value compared to DMSO ₄	X ² value compared to Cimetidine
DMSO ₄	10 %	14.00±1.11	-	-
Cimetidine	100	11.00±1.04	(X ² =3.60, P>0.05)	-
<i>E. coccinea</i>	100	9.00±0.58	(X ² =1.09, P>0.05)	(X ² =0.20, P>0.05)
<i>E. coccinea</i>	200	10.00±0.94	(X ² =0.67, P>0.05)	(X ² =0.05, P>0.05)
<i>E. coccinea</i>	400	10.00±1.00	(X ² =0.67, P>0.05)	(X ² =0.05, P>0.05)

Table 8 exhibited ulcer control and 10 mg/kg cimetidine percentage inhibition when compare across the test groups. It was recorded that 100 and 200 mg/kg of ethyl acetate showed a promising effect when contrasted with 10 mg/kg cimetidine had no significant difference. In comparison, the 400 mg/kg treated group was highly significant when compared across the groups.

Table 8: Effect of *Emilia coccinea* aqueous extract on percentage inhibition in rats

Treatment	Dosage mg/kg	Percentage inhibition	X ² value compared to DMSO ₄	X ² value compared to Cimetidine
DMSO ₄	10 %	0.0	-	-
Cimetidine	100	76.0	(X ² =73.05, **P<0.01)	-
<i>E. coccinea</i>	100	62.5	(X ² =60.06, **P<0.01)	(X ² =1.22, P>0.05)
<i>E. coccinea</i>	200	16.0	(X ² =13.24, **P<0.01)	(X ² =39.13, **P>0.01)
<i>E. coccinea</i>	400	00.0	(X ² =0.00, P>0.05)	(X ² =73.05, **P<0.01)

Note: P>0.05- Not Significant, *P<0.05 – Significant, **P<0.01- Highly Significant

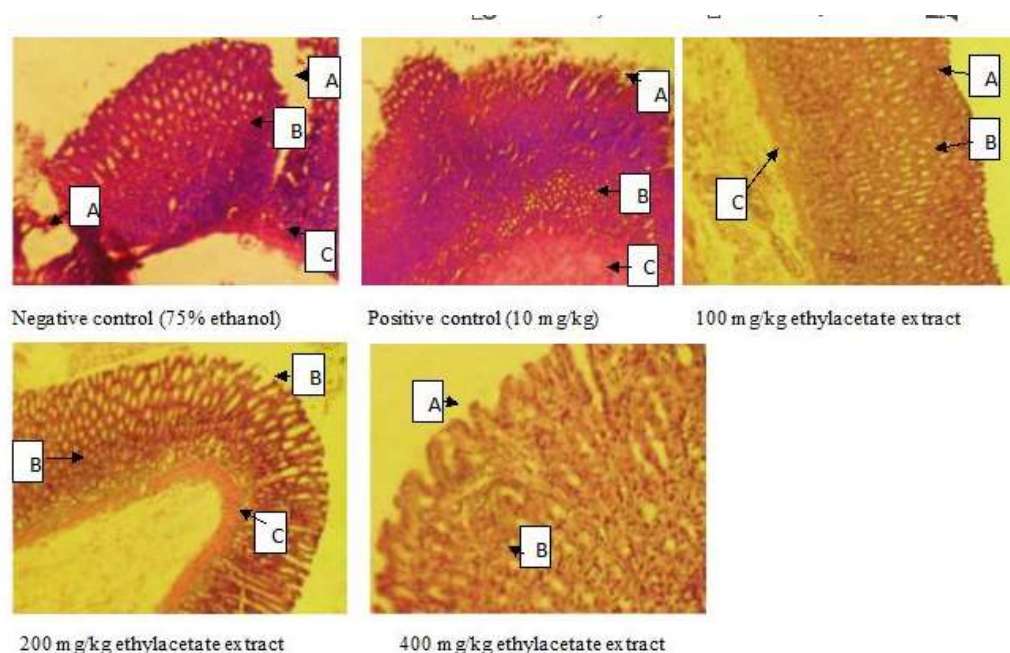


Plate 1: Effects of *Emilia coccinea* ethyl acetate extract on mucosa lining of the stomach
 Negative control (75% ethanol): The rat stomach treated with Ethanol only showing A. patchy funnel-shaped mucosal erosion, B. glands, and C. muscularis mucosa. Positive control (10 mg/kg): Rat stomach treated with Ethanol plus Cimetidine showing A. relatively normal mucosal lining B. glands and C. muscularis mucosa. 100 mg/kg aqueous extract: Rat stomach treated with 100mg/kg ethyl acetate extract plus Ethanol showing A. normal mucosa lining B. glands and C. muscularis mucosa. 200 mg/kg aqueous extract Rat stomach treated with 200mg/kg ethyl acetate extract plus Ethanol showing A. normal mucosal lining B. glands and muscularis mucosa. 400 mg/kg aqueous extract: Rat stomach treated with 400mg/kg ethyl acetate extract plus Ethanol showing A. normal mucosa lining, B. glands, and C. muscularis mucosa.

The study observed that the LD50 of the ethylacetate extract after 4 to 24 hours all through 14 days noted no dead on the extract-treated groups, signifying that it showed no acute toxicological defect.

Table 9: Effect of acute toxicity of ethyl acetate extract of *Emilia coccinea* in mice

Treatment	Dose (mg/kg)	No of mortality	Mortality ratio	No of Mortality and adverse effect
<i>E. coccinea</i>	10	0/3	0	Absent mortality
<i>E. coccinea</i>	100	0/3	0	Absent mortality
<i>E. coccinea</i>	1000	0/3	0	Absent mortality
<i>E. coccinea</i>	1500	0/1	0	Absent mortality
<i>E. coccinea</i>	2900	0/1	0	Absent mortality
<i>E. coccinea</i>	5000	0/1	0	Absent mortality
Control	DMSO ₄ +water	0/3	0	Absent mortality

DW= distilled water

Table 10 shows the significance level across graded doses of the ethylacetate extract compared with the control exhibited no significant difference.



Table 10: Effect of *Emilia coccinea* aqueous extract on the haematological parameters in rats

Parameters	Control (10% DmsO4)	100 mg/kg aqueous	200 mg/kg Aqueous	400 mg/kg aqueous
WBC x103/ul	8.65±1.18	8.25±1.88	7.30±0.73	6.15±1.97
LY x103/ul	3.15±0.72	3.10±0.62	3.30±0.70	2.73±0.63
MO x103/ul	1.13±0.27	0.88±0.26	0.65±0.10	0.48±0.15
GR x103/ul	4.35±1.44	4.25±1.20	3.33±0.68	2.70±1.36
LY%	39.33±10.8	38.48±4.52	45.43±7.83	46.73±4.69
MO%	10.83±2.49	10.95±2.63	8.68±0.60	8.23±1.20
GR%	47.33±10.29	50.58±3.69	45.65±8.24	45.05±4.46
RBC x106/ul	7.02±0.41	6.94±0.11	7.11±0.33	6.39±0.29
Hgb (g/dl)	17.58±1.12	17.15±0.38	18.05±0.77	15.83±0.94
HCT%	45.50±1.92	43.63±1.15	43.60±1.41	39.08±1.85
MCV (fl)	64.98±1.54	62.45±1.71	61.38±0.89	61.10±0.67
MCH (pg)	24.98±0.21	24.50±0.35	25.38±0.15	24.65±0.35
MCHC (g/dl)	38.48±1.04	39.30±0.86	41.33±0.45	40.40±0.54
RDW%	48.40±5.09	49.20±6.42	42.70±1.27	40.90±1.51
PLT x103/ul	785.50±56.99	606.00±126.18	775.00±44.04	375.00±176.19
PCT (%)	0.54±0.03	0.39±0.07	129.61±129.13	0.24±0.11
MPV (fl)	6.88±0.19	6.60±0.30	6.35±0.22	6.75±0.18
PDW (%)	10.70±0.61	10.48±0.74	9.25±0.27	9.00±0.66

p>0.05- Not Significant

DISCUSSION

Plant phytochemicals serve as a promising therapeutic ingredient with several medicinal uses known as "active or potent ingredient" in therapeutic agents. They are liable to numerous factors implicated in herbal plant preparation. Preliminary qualitative and quantitative phytochemicals in ethyl acetate extract of *E. coccinea* exhibited the presence of Phenols, Saponins, Alkaloids, Cardiac glycoside, and anthraquinone at a significant concentration with the least had (10.42 mg/100g of saponins) and most abundant at (184.30 mg/100g of alkaloids) responsible for diverse forms of ulcer healing and antimicrobial efficacy. This exhibited a similar work of Karau et al. (2005) on the antibacterial activity of alkaloids from *Sida acuta*. Antioxidants of a plant are active components capable of inhibiting oxidation stress via response to free radicals (Sies, 1997). Nature endorse most biological cells with suitable protective mechanisms in combating numerous reproachful properties linked with free radicals such as glutathione reductase, glutathione peroxidase, superoxide dismutase (SOD), disulfide affinity, thiols, and thioredoxin. A-tocopherol is an indispensable nutrient with efficient chain-breaking antioxidant properties, reinforced in preventing free radical proliferative responses linked with human cell membranes. Ascorbic acid is an active component capable of eliciting protective measures in their mode of action (Cadenas, 1997). Plant with natural occurring remedies, commonly acclaimed to be non-toxic. Hence, researchers proposed a suitable toxicological screening, which affirmed safety in natural medicine (Oyewole et al, 2007; O'Hara et al, 1998). Antioxidant investigation of ethyl acetate extract of *E. coccinea* showed a scavenging effect with a defensive measure against DPPH free radical at (92%) compared with ascorbic acid. as shown in Figure 1, a significant increase was observed from



percentage inhibition of ethyl acetate extract with scavenging property on free radicals. This agreed with the study of Cadenas (Cadenas, 1997). Antimicrobial properties of ethylacetate extract of *E. coccinea* at various concentrations elicited inhibitory effect against gram-positive microbes. Ciprofloxacin, Septrin, and Tarivid from the zone of inhibition against *Salmonella spp* were used to compare the activities of the graded concentration in the test groups. The extract showed a highly inhibitory effect in *Salmonella spp* compared with Ciprofloxacin and Tarivid, except for Septri, which exhibits no significant differences compared with the test groups (Idu et al, 2007). Also, Ciprofloxacin, Septrin, and Tarivid showed a distinct zone of inhibition against *Escherichia coli* compared with test groups, showed increased inhibition effect against *Escherichia coli* compared with Tarivid excluding Ciprofloxacin and Septrin with no significant difference in the treated groups (Ogbebor and Adekunle, 2005). Ciprofloxacin, Septrin, and Tarivid at highly significant differences in a zone of inhibition against *Helicobacter pylori* when compared across the treated groups by impeding *Helicobacter pylori* at a graded concentration of ethyl acetate extract when compared with Ciprofloxacin and Tarivid excluding Septrin that exhibited no significant difference when compared with the treated groups. This concurred with the report of Idu et al. (2007). Ulceration of stomach tissue in gastrointestinal lining triggered by NSAIDs like indomethacin, ibuprofen with inhibitory effect on cyclooxygenase I (COX-I) enzyme by metabolic activity to quash inflammatory disease (Agrawal and Dajani, 1993), with receptiveness over severe adverse effects in drugs ensued in the finding of new antiulcer mediator resultant from plants basis with no minute toxicity and side effects (Curtis and Griffin, 1991). This study on ethyl acetate extract of *E. coccinea* displayed numerous bioactive constituents, including saponin, tannins, phenolic, flavonoids, and glycosides present to be liable as an antiulcer healing effect. A result from ulcer control exhibited a significant increase in ulcer index compared with test groups, eliciting a significant reduction in ulcer index. Ethylacetate extract at 100 mg/kg exhibited a significant decrease compared with 10 mg/kg cimetidine. At 400 mg/kg of ethyl acetate of *E. coccinea* extract elicited a significant reduction in ulcer index compared with ulcer control showed a potent effect of the test groups explicitly at higher doses. This study agreed with the report derived from Cho and Ogle (1992) on the pharmacological variances and correspondences between ethanol-induced mucosal damage and stress. The mean ulcer index revealed a significant reduction in the graded doses of ethyl acetate extract compared with ulcer control. Untreated control (DMSO₀) showed a significant reduction in percentage inhibition compared with test groups, indicating a significant increase with dose dependant. It was also detected that 100 and 200 mg/kg of ethyl acetate extract revealed no significant difference with the 10 mg/kg cimetidine group with insignificant percentage inhibition of ulcer. At increase dose, a significant increase was recorded across the test groups when compared with ulcers (Cho and Ogle, 1992). Histopathological report from the rat's stomach treated with 75% ethanol alone showed patchy shaped of ulceration in the mucosal lining, leading to leisure when contrasted with 10 mg/kg cimetidine and test groups showed typical architecture structure of the stomach having a well-structured mucosa lining of the stomach inhibiting digestive juice from intrusive stomach lining (Schneeweiss et al, 2006). The ulcer control exhibited severe and pervasive erosion, having a slight stomach lesion with definite hemorrhage signifying the presence of ulcer as revealed in Plate 1. 10 mg/kg cimetidine showed a fairly protective effect against gastrointestinal mucosa lining to exposed perceptible mucosa that is quantitatively normal with columnar epithelium as revealed in Plate 1. The *in-vivo* results from the antiulcer study showed the effect of the extract precisely at 200 and 400 mg/kg; compared with 10 mg/kg, cimetidine had a healthy mucosa wall with less or no sporadic ulceration in the mucosa of the stomach. Hence, the exploit of ethyl acetate extract indicated a decrease in acid release regardless of the secretory source of stimuli. This showed the potency of action in treating



peptic ulcer disease with gastroesophageal reflux having short or prolonged uses. This is comparable to the report of Schneeweiss et al. (2006) work. Ethanol is a chemical mediator that intrudes gastric mucosal lining, thereby instigating an extreme micro-vascular disparity with sturdy vasocontractile effect via the arteriolar reduction in the distension of mucosal capillaries (Cho and Ogle, 1992). The potential pathogenesis of stomach mucosal damage is implicated in the rebirth of ROS responsible for interaction with crucial eccentric in lipid peroxides development with successive adverse anti-oxidative enzyme feat in vital cells. This falls in line with Konturek et al. (2000) report. *Emilia coccinea* crude ethyl acetate extracts introverted ulcerogenic tendencies with Ethanol induced ulcer test groups with 100, 200, and 400 mg/kg exhibited an active reminiscent of antioxidant potential. This study had compelling evidence on the acute toxicity of ethyl acetate extract with defensive mechanisms having less or no side effects. Acute toxicity study exposed the plant with a comparative absence of toxicity and observed LD₅₀ of ethyl acetate extract after 14 days of lethality and pathological adverse effect investigation (Adedapo et al, 2004). The sub-chronic effect of the hematological indexes supports the resolve of lethality revealed with novel agents found in blood cells associated with maximum plant material different from normal blood effect. More so, it explains blood-associated defects disposed to t chemical constituent. Such investigation has been remarkably receptive, particular, and reliable with the rests to articulate ethical study, ailment diagnosis, prophylaxis, and curative properties (Okonkwo et al, 2004; Yakubu et al, 2007). The physiological standards of these indexes can be influenced by the absorption of certain toxic substances from plant materials (Adedapo et al, 2004). *At graded doses, Emilia coccinea ethyl acetate extract* displayed no significant variation in leucocytes, erythrocytes, and thrombocytes after 28 days of oral administration. A slight significant increase in MCH (mean corpuscular hemoglobin) was recorded in Table 10. These reports align with the previous study of Omodamiro and Nwankwo (2013), whose reports elicited a significant increase in MCH MCV, PCV, red blood cells, and total hemoglobin.

CONCLUSION

In conclusion, ethyl acetate extract of *E. coccinea* showed a productive effect in stomach mucosa linings against ulceration. Also, ethyl acetate extract of *E. coccinea* displayed a microbicides effect against gram-positive and Gram-negative microbes with dosage dependant. The biosafety of this study exhibited no adverse effect or mortality in the understudied doses.



Emilia coccinea

DECLARATION OF CONFLICT OF INTEREST

No conflict of interest to declare.

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