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Original Article

Phytochemistry, *In-vitro* antioxidant, Microbicidal, Anti-ulcerogenic and Biosafety Potential of *Emilia coccinea* Aqueous Extract in Animal models.

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ABSTRACT

Emilia coccinea enhances the treatment of several disease conditions include; vertigo management, ringworm, cough, gonorrhoea, ulcers, lice, measles, seizure, eye drop. This study investigates the phytochemistry, *In-vitro* antioxidant, anti-ulcerogenic, microbicidal and biosafety effect of aqueous extracts of *E. coccinea* using animal model. *E. coccinea* leaf was freshly obtained, shade dried, pulverized and prepared into aqueous extract. Standard procedure were used for the evaluation of the phytochemicals, *in-vitro* antioxidant and antimicrobial activities. Twenty five (25) Wistar rats were acclimitized and randomly selected into five groups (n=5) such as untreated group, 10 mg/kg cimetidine and graded doses (100, 200 and 400 mg/kg b.w.) of aqueous extract of *E. coccinea* to evaluate antiulcer and twenty Wistar rats were used for the biosafety effects. Results from the qualitative and quantitative phytochemical screening showed the present of phenol (47.19 mg), saponins (84.64 mg), alkaloids (75.17 mg), cardiac glycosides (63.12 mg) and anthraquinone (48.79 mg). The antioxidant property showed a competitive scavenging effect against 1,1 diphenyl-2-picrylhydraxyl radical when compared with ascorbic acids. Microbial activities of the aqueous extract of *E. coccinea* at various concentration elicited inhibitory effect against *Salmonella typhii, Escherichia coli, Helicobacter pyloris*. The ulceration in rat stomach lining induced with 70% ethanol and pretreated with prophylactic measure of aqueous crude extract showed significant reduction in ulcer count, ulcer index with increased percentage inhibition of ulcer when compared with untreated and reference control. The haematological and histopathological study shows no significant difference in the aqueous crude extract with absent toxicity when comparisons with the control

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1. Introduction

Plants with several medicinal and culinary benefits are documented as herbal plants [1]. Numerous plant species serves as useful entity in scientific and clinical trials. Plant materials include; roots, stem, bark, leaves, seeds, fruits and flowers are being utilized into various therapeutic forms [2]. Typically, they are either synergized as polyherbal drug or use as a single material. Most natural herbs are exploited as nuticeuticals or spices. Conversely, report have shown its efficiency in intrinsic herbs as potent anti-microbial, serves as the basis for conventional remedy [3]. *Emilia coccinea* belongs to the family Compositae (Asteraceae). It is primarily a shrub and woody herbs, acting also as a climber and trees herbs [4]. It is known as "tassel flower" with erect bushy herbs of approximately 120 cm high. In Nigeria, the leaves are usually consumed when prepared into salad and spinach of fresh juice derived from it in the management of eyes sore [5]. The leaves are usually ingested raw and it is mostly mixed with lime juice and guinea corn in the treatment of sore throat [6, 7]. Also, it

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aid in the treatment of wounds. The roots part is used as anti-diarrhoea. *E. coccinea* elicited several bioactivities such as; anti-fungi, antimicrobial and anti-diarrhoea potential [8, 9]. The major phytochemicals present include; cardiac glycoside and alkaloids [10]. The main bioactive constituents present are tannins, phenolic, flavonoids and alkaloids [11]. It has been sketchily used conventionally in managing severe diseases. The sap obtained from the leaf aid in the management of vertigo [12]. It is also effective in treating ringworm, ulcers, lice, cough, gonorrhoea, seizure and measles in children [10, 13]. The leaf sap aid in the treatment of epilepsy [14].

Small intestine and stomach lining leisure are usually sores with severe pain called stomach ulcers. It is more perceptible than peptic ulcer, due to the result procure from the layer of mucosal thickening protecting the stomach against gastric juice, interfering with the stomach lining tissues. Stomach ulcers can be merely treated, but severe in the absence of appropriate action. Its section unavoidably can be triggered depending mainly on distinct factors [15]. Decrease in the mucosal lining of the stomach progresses into ulcer characteristically instigated either by or lengthen use of nonsteroidal anti-inflammatory drugs (ibuprofen and aspirin) or *Helicobacter pylori* bacterium or excessive acidity secretion of the stomach caused by lifestyle (drinking, stress and smoking) or genetic makeup or Zollinger-Ellison syndrome, subjecting the body into excessive production of stomach acid [16, 17]. Elderly are prone to ulcer caused by NSAIDs. Diagnosis is usually ascertain by occurring symptoms either by endoscopy or barium swallows. *H. pylori* is detected via blood test, signs of bacteria infestation are gotten from the stool test, breath test, urea test and biopsy of subject samples [16]. Since drug resistance and chemo-preventive substances are no longer effective against pathogenic infectious progressing to the possible screening of frequent herbal medicine. The objectives of this study evaluates the chemical constituents, anti-oxidant property, anti-microbial and anti-ulcer effect of *E. coccinea*

1. Materials and Methods

2.1. Plant collection

Fresh leaf of *Emilia coccinea* was obtained in the months of June in University of Benin botanical garden, Benin City, Edo State, Nigeria. It 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. Voucher number of the deposited specimen was obtained as GW: E198

1.2. Extraction procedure

The fresh leaf of *E. coccinea* was washed in clean water, shade dried for a period of 2 weeks and pulverized with the aid of electric blending machine. 1.25 kg of the pulverized sample was obtained and macerated using distilled water for 72 hr. The filtrate obtained via Whatman filter paper was concentrated using rotary evaporator and a crude dark greenish semi-solid substance weighing 52.6 g was gotten. And percentage yield was with the formula below.

The percentage yield of the extract is calculated as follows: Percentage (%) yield of the extract =

$$\frac{\text{weight of extract}}{\text{Weight of pulverized leaves}} \text{X } 100$$

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

2.3. Phytochemical screening

qualitative and quantitative phytochemical chemical tests were carried out on aqueous extract using standard method as described by Sofowara [7], Harborne [18] to show the present and quantity of tannins, phlobatannins, saponin, flavonoids, steroids, Test for terpenoids (Salkowski test) and cardiac glycosides (Keller-Killani test).

2.4. DPPH free radical-scavenging assay

Antioxidant capacity of the aqueous extract was evaluated using free radical-scavenging effect on 1, 1-diphenly-2-picrylhydrazyl (DPPH) radical. Ten (10) µl of 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 mixed and kept in dark for a period of 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 follow;

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

2.5. Antimicrobial assay

Preparations of solution for 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 the aqueous crude extract in 1ml of distilled water. The reconstituted extract was stored at 4^{o}C in sample bottles for use.

2.6.1. Collection Fluid specimen

Fluid specimens were gotten from 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 37ºC temperature.

2.6.2. Isolation and Identification of bacteria isolates

Blood fluid specimen was streaked on already prepared Nutrient agar in a petri dish for isolation of non-fussy microbes. Plates were all incubated at temperature of 37°C for 24-48 hours. Subsequent to incubation, isolated bacterial species (*Escherichia coli, Streptococcus pneumoniae, Mycoplasma pneumoni, Helicobacter pyloris, Klebsiella pneumonia, Salmonella typhi*) were properly identified using Gram staining and biochemical tests following standard technique described by Ellof [19].

2.6.3. Standardisation 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 culture of 0.2 ml of the organisms 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 standardize culture of 10^6 cfu/ml.

2.6.4. Susceptibility Testing

Method described by Emeruwa [20] was used for the determination of antibacterial effect of the aqueous crude extract. Standardized culture at 0.5 ml was banquet into 2 distinct sterile plates for a proper growth. Then 18.0-20 ml Nutrient agar at 45°C was introduced to the plate and properly rocked even for suitable bacteria and agar mixture. Content of the plates were left to congeal 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 the aqueous crude extract of the tested concentrations were released into the holes and aqueous solution of chloramphenicol and streptomycin at same concentration serves as positive control. The plates were left for 30 minutes for pre-diffusion of aqueous extract occurrence then incubated at 37°C for 24 hours and zones of inhibition were measured at mm using transparent metre rule. Mean of the triplicate results were taken.

2.6.5. Determination of Minimum Inhibitory Concentrations (MIC)

Bacterial strains were cultured overnight at 37°C on Nutrient broth and adjusted to a density of 10^6 cfu/ml. This aid in the inoculate of 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 condition at 37°C and observed after 24 hours. This serves as indication to bacterial growth, 40 µl of 0.2 mg ml^{-1} p-iodonitrotetrazolium solution was added to the well and incubated for 30 min at 37°C. The colourless tretrazolium salt was reduced to a red-coloured product by biological action of the organisms. The treatment was completed in triplicate and widespread subdual of growth at a explicit concentration of the extract was specified by a clearer solution obligatory to be avowed vigorous [19]. Chloramphenicol was employed as reference controls with sample free solutions and distilled water as the controls.

2.6.6. Determination of Minimum Bacteriocidal Concentrations (MBC)

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

2.7. Experimental Animals

Adult Wistar rats were utilized for this study. They were kept in a capacious and a well ventilated cages with appropriate temperature, food, drinking water and relative humidity for consecutive 14 days of acclimatization. Animal were house in the animal compartment of Animal and Environmental Science, University of Benin. Approval for the use of animals by Life Sciences ethical committee was certified and ethical number (LS019213) was obtained.

2.8. Experimental Design

Animals with relative body weight from 180 g to 220 g were randomly selected into five groups (n=5). Group A serves as normal control, group B received 10 ml/kg cimetidine, group C serves as untreated control treated with 10 ml/kg Dmso4 and Group D to F were treated with aqueous extract of *E. coccinea* at graded doses of 100, 200 and 400 mg/kg). The treatment persisted for 14 days. Albino rats were fasted for 24hrs preceding the induction of ulcer. An hour subsequent to 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 anaesthetized in chloroform, and the stomachs were isolated and analysis.

2.9. Acute Toxicity Study

Twelve mice were indiscriminately apportion into groups of three (n=3). Graded doses (10, 100 and 1000 mg/kg) of the extract was administered alongside the control group in phase first of the study. Four to Twenty four hours later without no death recorded, phase 2 of the procedure took place involving three mice exposed to

1600, 2900 and 5000 mg/kg *E. coccinea* aqueous extract. Animals were observed for four hours all through twenty four hours to 14 days with the method described by Lorke [21].

2.10. Sub-chronic Toxicity Study

Twenty albino rats of male sex weighing 195 to 250 g, arbitrarily selected into four groups (n=5). There were all administered with graded doses of *E. coccinea* extract orally for 28. Graded doses of aqueous extract at (100, 200 and 400 mg/kg) with the control, was administered using 0.5 ml/kg of distilled water. The animals weight was taken at interval of 7 days for a period of 28 days. The whole groups of rats were mildly anaesthetized and sacrificed, Blood sample was collected using abdominal aortal.

2.10.1. Haematological Analysis

Blood samples were gotten via 5 ml syringes into ethylene diamine tetra acetic acid (EDTA) bottle for full blood count (FBC) analysis. Blood indices (red blood cell count, white blood cell count and haematocrit) were procured for full blood count analysis by Sysmex (R) XT- Series Automated Haematology Analyser

2.10.2. 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, avoid autolysis and protein precipitation thus preventing 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 through 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 with a temperature of 60^0C. 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 1 and half hours in a water bath. Blocks of wax were attached to the block holder of the microtome which 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 then in distilled water. Slides were then stained with haematoxylin for 15 minutes at room temperature and the excess stains 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 magnification to show the differences in tissues for the rats from each experimental group and stage.

2.10.3. Statistical Analysis

Statistically, the analysis were 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.

1. Results and discussion

Table 1 exhibited the present of various qualitative and quantitative phytochemical properties such as Phenols, Saponins, Alkaloids, Cardiac glycoside and anthraqunone with their respective level of concentrations in the plant.

Figure 1 showed the scavenging activity of aqueous extract of *E. coccinea*. It was obvious from the graph that aqueous extract exhibited scavenging effect against free radicals (DPPH), when compared with standard (ascorbic acid) whose level of scavenging activities was of no significant as shown in the graph below.

Table 2 exhibited the zone of inhibition of *Salmonella spp* compared with the 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 3 showed the various reference control for zone of inhibition of *Escherichia coli* compared with treated groups. It was observed that the treated groups showed significant inhibitory effect against *Escherichia coli* when compared with Tarivid across the groups except ciprofloxacin and Septrin that displayed no significant inhibitory effect when compared with the treated groups.

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

Table 5 exhibited the ulcer index of the negative and

reference control when compared with the treated groups. It was observed that the aqueous extract at 400 mg/kg showed significant reduction in ulcer index when contrasted with standard drug has no significant different with Cimetidine, unlike 100 and 200 mg/kg that displayed no significant difference.

Table 6 exhibited the Mean ulcer index in the negative and positive control group used in comparing across the treatment group. It was observed that across the treated groups there was no significant different.The table below exhibited that; the negative and positive control group percentage inhibition used to compare across the treated groups. It was observed that 100 and 200 mg/kg aqueous group when contrasted with the standard drug has no significant different with the positive control while the remaining treated groups were significant and highly significant when compared across the groups.

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

Phytochemicals	**Quantitative**	**Mean±Standard error of mean (SEM) Quantitative mg/100g**
Phenols	+	47.19±3.18
Saponins	++	84.64±4.31
Alkaloids	++	75.17±4.46
Cardiac glycoside	++	63.12±3.93
Anthraqunone	+	48.79±2.89

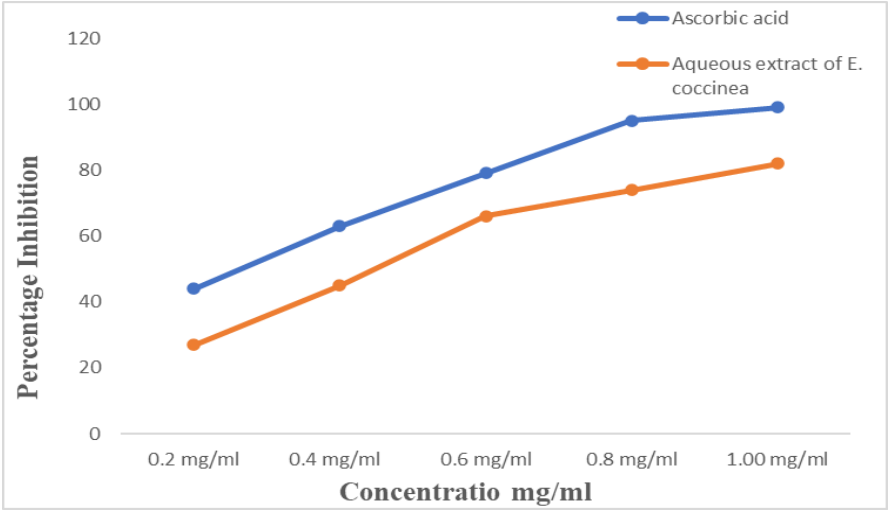

Figure 1: Scavenging property of aqueous extract of *Emilia coccinea* on DPPH radical.

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

Treatment	Dosage µg/mg/ml	Mean±SEM *Salmonella spp*	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	-	-	-
Septrin	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)	-
E. coccinea	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)
E. coccinea	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)
E. coccinea	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: Zone of inhibition of *E-coli* isolates on *Emilia coccinea* aqueous extract

Treatment	Dosage µg/mg/ml	Mean±SEM *E. coli*	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)	-
E. coccinea	100	0.00±0.00	(X^2=0.00, P>0.05)	(X^2=0.00, P>0.05)	X^2=12.25, **P<0.01)
E. coccinea	200	5.00±0.17	(X^2=2.67, P>0.05)	(X^2=2.67, P>0.05)	X^2=5.00, *P<0.05)
E. coccinea	500	10.00±0.92	((X^2=7.36, P>0.05)	((X^2=7.36, P>0.05)	(X^2=1.00, P>0.05)

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

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

Treatment	Dosage µg/mg/ml	Mean±SEM *H. pyloris*	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)	-
E. coccinea	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)
E. coccinea	200	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)
E. coccinea	500	3.00±0.16	(X^2=6.25, **P<0.01)	(X^2=1.00, P>0.05)	(X^2=4.57, *P<0.05)

*P>0.05- Not Significant, *P<0.05 – Significant, **P<0.01- Highly Significant*
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Table 5: Effect *Emilia coccinea* aqueous extract on the 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)	-
E. coccinea	100	45.00±2.85	(X^2=1.20, P>0.05)	(X^2=0.011, P>0.05)
E. coccinea	200	43.00±3.02	(X^2=1.71, P>0.05)	(X^2=0.011, P>0.05)
E. coccinea	400	29.00±2.29	(X^2=8.58, **P<0.01)	(X^2=3.08, P>0.05)

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

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)	-
E. coccinea	100	11.30±0.97	(X^2=3.62, P>0.05)	(X^2=0.00, P>0.05)
E. coccinea	200	10.80±1.00	(X^2=3.60, P>0.05)	(X^2=0.67, P>0.05)
E. coccinea	400	7.25±0.73	(X^2=2.33, P>0.05)	(X^2=0.89, P>0.05)

Table 7**:** 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)	-
E. coccinea	100	78.6	(X^2=76.05, **P<0.01)	(X^2=0.06, P>0.05)
E. coccinea	200	75.0	(X^2=72.05, **P<0.01)	(X^2=0.07, P>0.05)
E. coccinea	400	50.0	(X^2=47.08, **P<0.01)	(X^2=5.37, *P<0.05)

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

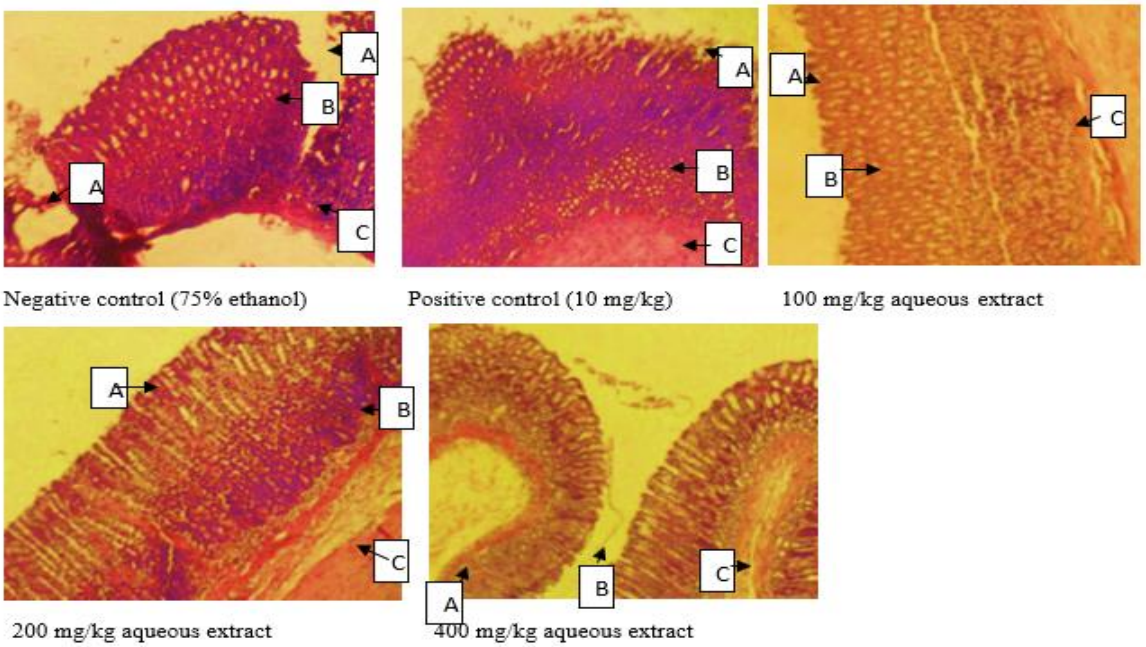

Plate 1: Effect of *Emilia coccinea* aqueous extract on the mucosa linning of the stomach

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, fairly normal mucosal lining, B, glands and C, muscularis mucosa. **100 mg/kg aqueous extract:** Rat stomach treated with 100mg/kg aqueous extract plus Ethanol showing A, normal mucosa, B, glands and C, muscularis propria. **200 mg/kg aqueous extract:** Rat stomach treated with 200mg/kg aqueous extract plus Ethanol showing A, normal mucosal lining, B, glands and muscularis propria. **100 mg/kg aqueous extract:** Rat stomach treated with 400mg/kg aqueous extract plus Ethanol showing A, normal mucosa, B, glands and C, muscularis mucosa.

From the study it was observed that the LD_{50} of the aqueous extract after 4 to 24 hours all through 14 days noted no dead on the extract treated groups, signified that it showed no acute toxicological defect.
Table 10 showed the level of significant across graded doses of the aqueous extract when compared with the control exhibited no significant different.

Table 9**:** Effect of acute toxicity of ELNA aqueous extract in mice

Treatment	**Dose (mg/kg)**	**No of mortality**	**Mortality ratio**	**No of Mortality and adverse effect**
E. coccinea	10	0/3	0	Absent mortality
E. coccinea	100	0/3	0	Absent mortality
E. coccinea	1000	0/3	0	Absent mortality
E. coccinea	1500	0/1	0	Absent mortality
E. coccinea	2900	0/1	0	Absent mortality
E. coccinea	5000	0/1	0	Absent mortality
Control	$DMSO_4$+water	0/3	0	Absent mortality

DW= distilled water

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	7.50±0.57	9.00±2.02	7.95±1.13
LY x103/ul	3.15±0.72	3.10±0.70	2.05±0.32	3.13±0.82
MO x103/ul	1.13±0.27	1.05±0.27	0.88±0.11	0.83±0.14
GR x103/ul	4.35±1.44	3.33±0.18	6.08±2.03	4.05±0.93
LY%	39.33±10.8	40.35±6.19	25.35±5.22	39.45±8.69
MO%	10.83±2.49	14.25±3.46	11.18±2.78	9.83±1.73
GR%	47.33±10.29	45.40±5.10	63.48±6.51	50.73±7.88
RBC x106/ul	7.02±0.41	7.19±0.49	6.58±0.40	7.00±0.30
Hgb (g/dl)	17.58±1.12	17.75±1.28	16.28±1.09	16.85±0.60
HCT%	45.50±1.92	44.30±2.03	40.63±3.02	42.80±1.14
MCV (fl)	64.98±1.54	61.88±1.75	61.65±1.58	61.35±2.09
MCH (pg)	24.98±0.21	24.60±0.27	24.70±0.23	24.13±0.36
MCHC (g/dl)	38.48±1.04	39.85±1.12	40.10±0.82	39.38±0.84
RDW%	48.40±5.09	48.23±5.16	45.28±4.25	48.23±6.34
PLT x103/ul	785.50±56.99	660.00±79.98	482.00±127.11	711.50±101.45
PCT (%)	0.54±0.03	0.45±0.06	0.33±0.09	0.44±0.05
MPV (fl)	6.88±0.19	6.83±0.09	6.90±0.11	6.30±0.23
PDW (%)	10.70±0.61	9.83±0.20	9.93±0.26	8.98±0.38

p>0.05- Not Significant

Chemicals with promising health effect with medicinal benefits are usually called “active or potent ingredients” or “active principles” acting as therapeutic agent in which their presence is contingent to several factors involving plant species either during the time and period of harvest, soil type and method of the herb preparation. Phytochemical screening of the aqueous extract of *E. coccinea* showed the present of Phenols, Saponins, Alkaloids, Cardiac glycoside and anthraqunone with their respective level of concentrations with the least (47.19 mg/100g of phenol) and most abundant (84.64 mg/100g of saponins) responsible for their wound healing and

antimicrobial activities. This showed similar report of Karau et al. [22] with the work on antibacterial activity of alkaloids from *Sida acuta*.

Antioxidants are active ingredient that inhibit oxidation via responce to free radicals or its action [23]. Most cells are promoted by nature using appropriate defensive mechanisms against several oxidative properties triggering the release of free radicals such as superoxide dismutase (SOD), glutathione reductase, glutathione peroxidase, thioredoxin, disulphide affinity and thiols. The a-tocopherol serves as an essential nutrient prone to efficient chain-breaking antioxidant, interfering with the proliferation of free radical reaction linked with cell membranes in human body. Ascorbic acid is a known for its protective action [24]. Scientists propose thar Plant with natural occurring medicines, prevalently commended to be non-toxic, suitable toxicological screening affirmed safety in natural medicine [25, 26]. Anti-oxidant evaluation of the aqueous extract of *E. coccinea* showed a scavenging and protective measure against DPPH radical at (83%) when compared with ascorbate. It was obvious from Figure 1 with a significant increase in the level of percentage inhibition of the aqueous extract with scavenging effect on free radicals. This concurred with the report derived from Cadenas [24]. The antimicrobial properties of the aqueous extract of E. coccinea at graded concentration exhibited an inhibitory effect against the tested organisms. The reference drugs showed significant decrease in the zone of inhibition against *Salmonella spp* and other tested organisms when compared with the treatment control hat showed similar effect across the graded concentration [27, 28]. Also, the presence of Ciprofloxacin, Septrin and Tarivid showed an increased in the significant level with a wider zone of inhibition against *Helicobacter pyloris* compared with the tested groups that had similar effect except for 500 mg/kg that displayed no significant differenc. This is in line with the study of Idu et al. [27].

Ulceration of gastric intestinal lining of the stomach triggered by non-steroidal anti-inflammatory drugs (NSAID) such as, indomethacin, ibuprofen might be as a result of their inhibitory effect on the cyclooxygenase I (COX-I) enzyme via metabolic exploit in effort to suppress inflammatory infection [29], the responsiveness over severe side effects of drugs resulted to the discovery of novel anti-ulcer agent derived from plants source without any minute toxicity and adverse properties [30].

Effect from the leave extract of *E. coccinea* showed the presence of saponin, flavonoids, glycosides, tannins and phenolic responsible for the management or treatment of ulcer. The result obtained from the treatment groups elicited a significant decreased in ulcer index compared to the untreated control control. A better effect was observed in 400 mg/kg of the aqueous. This study is in line with the report from Cho and Ogle [31] with Pharmacological differences and similarities between stress and ethanol-induced mucosal damage. The mean ulcer index showed significant decreased across the graded doses of the extract when compared with the negative control.

Histological report from the rat stomach treated with ethanol alone shows patchy funnel shaped mucosal ulceration, brings about leisure when compared with cimetidine groups and the treatment groups that elicited normal architectural structure of the stomach with a well mucosa linning stomach preventing gastric juice from interfering with the linning [32]. The lesions associated with ethanol induced ulcer as shown in the negative control, displayed a severe and widespread erosions with mild stomach lesion had pronounced haemorrhage indicating the present of ulcer as shown in Plate 1. Cimetidine presented impartially protection against gastric mucosa linning revealed by visible mucosa, which looks objectively normal lined having columnar epithelium as shown in Plate 1. The results from the *in-vivo* antiulcer designated the effect of the extract specifically at 200 and 400 mg/kg, which is comparable to cimetidine having a healthy revealed mucosa surrounded with less or no patchy ulceration in the mucosa linning. Hence, the action of the aqueous extract showed reduction in acid discharge irrespective of the source of secretory stimulus. This showed the potency in treating peptic ulcer disease and gastroesophageal reflux either for a short or longer period uses. This is similar to Schneeweiss et al. [32] work. Ethanol is known to be a chemical drug interrupts with gastric mucosal wall thereby causing an intense micro-vascular variations with sturdy vasocontractility complemented by arteriolar relaxation liable for the distension of mucosal capillaries [31]. The possible pathogenesis of the stomach mucosal impairment involved the regeneration of reactive oxygen species (ROS) liable to interplay an essential character in lipid peroxides formation subsequent by injurious anti-oxidative enzyme action of vital cells. This concurred with Konturek et al. [33] report. *Emilia coccinea* crude extracts inhibited ulcerogenic propensities of ethanol induced ulcer treated with 100, 200 and 400 mg/kg, displayed an effective redolent of antioxidant latency.antioxidant property consist of vitamins, flavonoids and polyphenols.

This study has shown vibrant information on the acute toxicity of the aqueous extract with protective effects and less or no adverse effect. The acute toxicity study revealed that the plant is toxic and the observed LD_{50} of the aqueous extract after 14 days of thorough examination of lethality, and pathological adverse effect as recorded

[34]. The sub-chronic study showed that the haematological parameters aid in the determination of the level of lethal exposure to eccentric agents presented in the body via means in which most plant material altered normal blood functioning. More so, it elucidates blood related defect prone to chemical component. Such evaluation have been exceedingly responsive, particular, and dependable which rests on the beginning for lucid and ethical investigation, disease diagnosis, prophylatic and curative [35, 36]. The physiological values of the indexes can be biased by the ingestion of definite toxic plant materials [34]. *Emilia coccinea* extract at graded doses exhibited no significant alteration in leucocytes cells, erythrocytes cells and thrombocytes after 28 days of oral management. With slight significant upsurge in MCH (mean corpuscular haemoglobin) as shown in Table 10. These report are in contrast to previous study of Omodamiro and Nwankwo [37] whose reports showed significant increase in MCH MCV, PCV, red blood cells and total haemoglobin.

4. Conclusion

In conclusion to this study carried out, the aqueous and ethyl acetate extract of *E. coccinea* potentiate prophylactic effect of stomach linings against ulceration induced by ethanol in rats. Thereby shown that aqueous and ethyl acetate extract of *E. coccinea* displayed antimicrobial effect against gram positive and gramnegative microorganism with dosage dependant. These investigations provide a scientific point for the usefulness of this plant in treating ulcer and microbial infections.

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Conflict of interests No conflict of interest

References

1. Abah ES, Egwari LO, Mosaku TO. *In vitro* antimicrobial screening of *Anchomanes difformis* (Blume) Engl. leaves and rhizomes against selected pathogens of public health importance. *Adv. Bio. Res.,* 2011, 5: 221-225.
2. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. *Nirali Prakashan, Pune.* 2006, 15-38pp.
3. Ozumba UC. Antibiotic sensitivity of Isolates of Pseudomonas aenigenosa in Enugu, Nigeria. *African Journal Clinical and Experimental Microbiology.* 2003, 44:48-51.
4. Olorode O. Taxonomy of West African Flowering Plants. London Longman Publishing Company. 1984, 121.
5. Kumar S, Paul S, Walia Y, Kumar A, Singhal P. Therapeutic Potential of Medicinal Plants: *A Review on Journal of Biological Chemical Chronicles,* 2015, 1:46-54.
6. Ayitey-Smith E. Prospects and Scope, Plant Medicin in Health Care. Ghana University Press. 1989, 29p.
7. World Health Organization. Programme on Traditional Medicine. General guidelines for methodologies on research and evaluation of traditional medicine. Geneva: World Health Organization. 2000. http://www.who.int/iris/handle/10665/66783.
8. Ogbebor N, Adekunle AT. Ingibition of conidial germination and mycelia growth of Corynespora caiicola (Berk and Curt) of rubber (Hevea brasiliensis muell. Arg.) Using extracts of some plants. *African Journal of Biotechnology.* 2005, 4:996-1000.
9. Ndip RN, Tarkang AEM, Mbulla SM, Luma HN, Malenque A. In vitro Anti- helicobacter phylori activity of extract of selected medicinal plants from North West Cameroon. 2007, 245p
10. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituent of some Nigeria medicinal plants. *African Journal Biotechnology.* 2005, 4;7: 685-688.
11. World Health Organization. WHO traditional medicine strategy: World Health Organization, Geneva Switzerland, 2013.
12. Atindehou KK, Kone M, Terreaux C, Traore D, Hostettmann K, Dosso M. Evaluation of the antimicrobial potential of medicinal plants from the Ivory Coast. *Phytotherapy Research* 2002; 16;5: 497–502.
13. Odugbemi T. Outlines and pictures of Medicinal plants from Nigeria. University of Lagos press, Nigeria. 2006, 283-285.
14. Kerharo J, Bouquet A. Plants medicinales et toxiques de la Cote-d' ivoire-Haute- Volta. *Vigot Freres Paris.* 1950, 295.
15. Milosavljevic T, Kostić-Milosavljević M, Jovanović I, Krstić M. Complications of peptic ulcer disease. Digestive diseases (Basel, Switzerland). 2011, 29;5: 491–3.
16. Najm WI. Peptic ulcer disease. *Primary care.* 2011, 38;3: 383–94,
17. Steinberg KP. Stress-related mucosal disease in the critically ill patient: risk factors and strategies to prevent stress-related bleeding in the intensive care unit. *Critical Care Medicine.* 2002, 30;6: 362–364.
18. Harborne JB. Phytochemical methods. London Chapman and Hall, Ltd. 1973, 49-188.
19. Ellof JN. A sensitive and quick microplate method to determine the minimum inhibitory concentration of plants extracts for bacteria. *Planta Medica.* 2008, 64:711-713.
20. Emeruwa KC. Antimicrobial substances from *Carica papaya* fruit extracts. *Journal of Natural Products.* 2009, 45;2: 123-127.
21. Lorke D. A new approach to practical acute toxicity testing. Archives of Toxicology. 1983, 54:275-287.
22. Karau D, Aly S, Antonella C, Carla M, Jacques S, Vittorio C, Alfred ST. Antibacterial activity of alkaloids from *Sida acuta*. *African Journal of Biotechnology.* 2005, 4;12:1452-1457.
23. Sies H. Oxidative stress: Oxidant and anti-oxidant. *Experimental Physiology*. 1997, 82;2: 291–295.
24. Cadenas E. Basic mechanisms of antioxidant activity. *Biofactors.* 1997, 6:391–397.

25. Oyewole IO, Magaji ZJ, Awoyinka OA. Biochemical and toxicological studies of aqueous extract of *Tithonia diversifolia* leaves in Wister albino rats. *Journal of Medicinal Plants Research.* 2007, 1:30-33.
26. O'Hara MA, Kiefer D, Farrell K, Kemper K. A review of 12 commonly used medicinal herbs. *Archive of Family Medicine.* 1998, 7: 523-536.
27. Idu M, Omogbai EKI, Aghimien GEI, Amaechina F, Timothy O, Omonigho SE. Preliminary phytochemistry, antimicrobial properties and acute toxicity of*Stachytarpheta jamaicensis* (L.) Vahl. Leaves. *Trends in Medical Research.* 2007, 2:193- 198.
28. Ogbebor N, Adekunle AT. Inhibition of conidial germination and mycelia growth of *corynespoa casiicola* (Berk and Curt) of rubber (Hevea brasilieusis Muella Arg). Using extract of some plants. *African Journal Biotechnology.* 2005, 4:996-1000.
29. Agrawal NM, Dajani EZ. Prevention and treatment of ulcers induced by non-sterioddal anti-inflammatory drugs. *Journal on Gastroenterology.* 1993, 3;4:142-148.
30. Curtis WD, Griffin JW. Non-steroidal anti-inflammatory drug induced gastroduodenal injury: therapeutic recommendations. *Journal on Medical College Georgia Augusta Ailment Pharmarcology.* 1991, 1:99-109.
31. Cho CH, Ogle CW. The Pharmacological differences and similarities between stress and ethanol-induced mucosal damage. *Life Sciences.* 1992, 51:1833-1842.
32. Schneeweiss S, Maclure M, Dormuth CR, Glynn RJ, Canning C. Avorn, *Journal Clinical pharmacology & therapeutics.* 2006, 79;4: 379-386.
33. Konturek PCH, Duda A, Brzozowski T. Activation of genes for superoxide dismutase, interleukin-1ß), tumour necrosis factor-α) and intercellular adhesion molecule-1 during healing of ischaemia-reperfusion gastric injury. *Scand. J. Gastroenterol.* 2000, 35: 452-463.
34. Adedapo AA, Abatan MO, Olorunsogo OO. Toxic effects of some plants in the genus *Euphorbiceae* on haematological and biochemical parameters of rats. *Veterinarski Arhiv.* 2004, 74;1:53–62**.**
35. Okonkwo JE, Iyadi KC, Effiong CO. Effect of chronic administration of haematological parameters of rats. *Nig J Physiol Sc.* 2004, 19;1–2:10–13
36. Yakubu MT, Akanji MA, Oladiji AT. Hematological evaluation in male albino rats following chronic administration of aqueous extract of *Fadogia agretis* stem. 2007, pp. 34–38.
37. Omodamiro OD, Nwankwo CI. The effect of Voacanga africana leaves extract on serum lipid profile and haematological parameters on albino wistar rats. *European Journal of Experimental Biology.* 2013, 3;3:140-148.

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