

Title: “Antibacterial Potential of *Emilia coccinea* against multidrug resistant human pathogenic bacteria”

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Abstract

Background: Diverse plant extracts with antibacterial activities can be of great help in the control of multidrug resistant bacterial infection (MDR) pathogens.

Objectives: Investigating the antimicrobial potency and phyto-active chemicals edible plant *Emilia coccinea* against multidrug resistance pathogenic bacteria in combination with conventional antibiotics against multi-drug-resistant pathogens.

Methods: The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) determination was done using INT (iodonitrotetrazolium) colorimetric assay. Antibacterial activity of the hydroethanolic plant extract of *Emilia coccinea* against MDR pathogenic strains of *S. aureus*, *Pseudomonas aeruginosa*, *Shigella*, *Salmonella species* and *Escherichia coli* was carried out by the broth micro diffusion method with the use of Mueller Hinton broth. Checkerboards testing for combination between the edible plant extract of *Emilia coccinea* and conventional antibiotics were carried out. Qualitative analysis of phytochemicals was conducted using qualitative chemical method.

Results: There was a significant increase in antibiotic efficacy when in combination with the hydroethanolic plant extracts with results <0.5 synergy, >0.5 but <1 indifference/additive and >4

antagonistic. Plant Extracts show the presences of phytochemical which supports the traditional use of this plant as an antimicrobial.

Conclusion: The present study showed antimicrobial properties of *E. coccinea*, against *S. aureus*, *Pseudomonas aeruginosa*, *Shigella*, *Salmonella species* and *Escherichia coli*. Hence, poses a pace for further investigations on the mechanism of action of *E. coccinea*.

Key words: checkerboard combination, *Emilia Coccineae*, MDR pathogenic strains, antimicrobial, MIC and MBC, phytochemical.

1.0. Introduction

The discovery of penicillin in 1928 was followed by the discovery and commercial production of many other antibiotics. We now take for granted that any infectious disease is curable by antibiotic therapy. Large amounts of antibiotics used for human therapy, as well as for farm animals and even for fish in agriculture, resulted in the selection of pathogenic bacteria resistant to multiple drugs. Multidrug Medicines produced from plants have become more important as alternative medicines (conventional antibiotics) in treating a broad spectrum of ailments, and researchers are continuing to pay attention to the use of phytochemicals materials in the treatment of many diseases [1]. The majority of the developing world believes that these plant-based products (edible plant extracts and their phytochemicals) are safer and more cost-effective [2]. With the emergence of new diseases and MDR pathogens, the usage of these plant products has increased in developed, developing, and underdeveloped countries [3] Ethnopharmacology and medicinal discovery employing plant-based products are still critical in healthcare delivery worldwide.

Salmonella enterica causes gastroenteritis, enteric (typhoid and paratyphoid) fevers in humans and animals [4]. *Salmonella* infections were initially treated with first line drugs ampicillin, sulphamethoxazole-trimethoprim, and chloramphenicol. Due to emergence of multidrug resistant (MDR) *Salmonella* strains, these drugs have become less effective [5]. This led to a shift to third generation cephalosporins (ceftriaxone) and fluoroquinolones (ciprofloxacin). However,

resistance to ciprofloxacin has also emerged with reported decreased susceptibility to the drug [6]. The World Health Organization recommends use of fluoroquinolones as optimal treatment choice in areas showing resistance to first line drugs and use of third generation cephalosporins in severe MDR cases [7], [8]. The prevalence of MDR bacteria continues to rise worldwide leading to increased incidence of treatment failure, increased morbidity, mortality as well as increased healthcare cost [9]. In Cameroon, typhoid fever is highly prevalent due to poor sanitation and fecal contamination of water sources [10]. Also, increasing levels of antimicrobial resistance in *Salmonella* and other MDR bacteria has been reported in a study in the Northwest region of Cameroon showed a significant decrease in sensitivity to ciprofloxacin and all first line antibiotics [11]. Also, a five years retrospective study of medical records reported a high degree of multidrug resistance in *Salmonella* and other gram-negative bacteria in Buea health district in the Southwest region [12]. This has been attributed to indiscriminate use of antibiotics, inadequate dosing and poor compliance to treatment regimens, circulation of low-quality drugs, no susceptibility testing in some health facilities and irrational use of the antibiotics.

Edible plants have been an essential source of therapeutic medications over the past years with distinct features that make them suitable as an alternative treatment for MDR infections that pose medical challenges [13]. In order to protect themselves from microbial, herbivore, and insect predators, edible plants have an almost infinite capacity to mix aromatic molecules, primarily phenolic compounds, polyphenols, alkaloids, flavonoid, terpenoids, ketones, and essential oils [14]. Many bioactive substances derived from plants, known as phytochemicals have been studied and found to be relatively safer than synthetic counterparts [15]. These compounds also have various therapeutic effects due to their high potency [16]. These phytochemicals derived from edible plants have various mechanisms of action, including the inhibition of efflux pumps and

target altering and drug degrading enzymes [17]. When these edible plants are used alone or in combination with other antibiotic compounds, edible plants phytochemicals have been found to have antimicrobial activities against clinically significant pathogens like *Salmonella species*, *E. coli*, *Staphilococcus species*, *pseudomans*, *Shigella* and some species listed below lowering the risk of developing a variety of diseases [18]. A successful method for modifying resistance is to use conventional antibiotics and edible plants phytochemicals in combination that will eliminate the resistance mechanism and still allow the medicine to be effective against resistant microorganisms [19]. Edible Plants such as *Caesalpinia bonducella* Flem (*Caesalpinaceae*), *Rhododendron spiciferum* Franch (*Ericaceae*), *Curcuma longa* Linn (*Zingiberaceae*), *Azadiracta indica*, Juss (*Meliaceae*), *Boerhaavia diffusa* Linn (*Nyctaginaceae*) and *Ocimum sanctum* Linn (*Lamiaceae*) among others, are known to possess antibacterial properties.

2.0. Research methodology

2.1. Study design

An experimental study design was carried out to determine the antimicrobial property, combination and phytochemical screening of ethno-medicinal plants use in South West of Cameroon against MDR bacteria pathogens.

2.2. Study area

Medicinal plants were collected from Fako Division in the Southwest Region, by the botanist at the Limbe herbarium unit. SW is situated between 5° 12' 00.0" N and 9° 18' 00.0" E it located about 255km from Yaoundé the capital city of Cameroon, and Fako located between 4.0972° N and 9.2786° E, the division is further divide into 7 subdivisions. With a population of 466,412 [20].

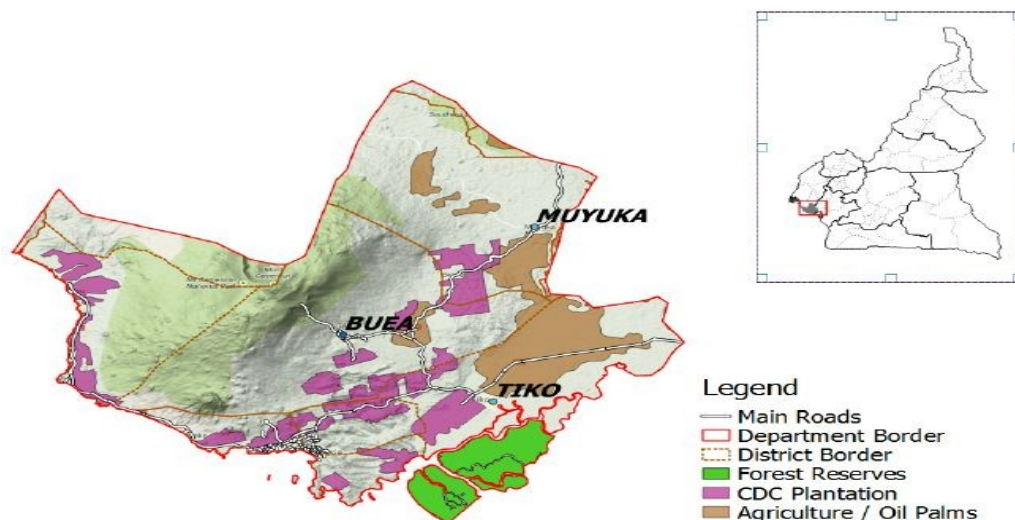


Figure 1 Map of Fako Region of Cameroon

2.3. Plant material and identification

Plants parts of *E. coccinea* (stem and leaf) that are healthy were gotten from Limbe respectively, Fako South West Region of Cameroon. The herbarium department of the Botanic Garden Limbe identified and authenticated the plant samples. It was discovered in sunder land T.C.H. 1233 14 April 1992. The voucher number was given as SCA 2423. Collection was done by botanist.

2.3.1. Plant material collection and identification

The healthy plant materials were washed thoroughly with running tap water and then with sterile distil water to remove all dust and sand particles. The plant parts were dried under shade, and powered by Mechanical blender to fine homogenous powder. The powdered samples were hermetically sealed and stored in air tight bottles at room temperature. These were used as the raw material for the extraction of antimicrobial compounds used to test the edible plants potential against the pathogenic MDR bacteria strains.

2.3.2. Plant preparation and extraction

The air-dried plant powder for *E. coccinea* [stem (36g), leaf (20,8g)] were macerated into hydro ethanol (1:10 w/v) for 48 h. followed by filtration using double coffee paper number 4 then Watman filter paper grade 1. The overall filtrate was dried using an oven at temperatures of 35°C which yielded [stem (3.1g), leaf (8.8g)] *E. coccinea* crude hydroethanolic extract, partitioning was prosecuted. The resulting hydroethanolic extract was stored in good glass containers in figure 3 bellow. The Extracts and fractions of *E. coccinea* were kept at 4 °C until further use [20].

2.4. Microbial strains and culture media

The studied microorganisms included sensitive and resistant strains of *Staphylococcus aureus*, *salmonella typhimurium*, *salmonella enterica*, *salmonella paratyphi*, *E. coli*, *Shigella dysenteriae*, *Shigella flexneri* and *Pseudomonas eurogenisa* obtained from the American Type Culture Collection (ATCC), Centre Pasteur Yaounde, and regional hospital Bamenda (Table1). MHD agar was used for activation of the tested gram-negative bacteria. While the MHB was used for antimicrobial assays and stored at 40°C.

Table 1 Bacterial isolates, sources and codes for research strains

s/n	Bacterial Codes	Name of strain	Source	Type
1	SANR 46003	<i>Staphylococcus aureus</i> 46003	BEI Resource	Strains
2	SENR 13555	<i>Salmonella enteritidis</i> 13555	BEI Resource	Strains
3	STM 14028	<i>Salmonella typhimurium</i> ATCC 14028	American Type Culture Collection	Strains
4	STM cpc	<i>Salmonella typhimurium</i>	Centre Pasteur of Cameroon	Isolates

5	SE cpc	<i>Salmonella enteritidis</i>	Centre Pasteur of Cameroon	Isolates
6	SPB cpc	<i>Salmonella paratyphi</i>	Centre Pasteur of Cameroon	Isolates
7	<i>S.typhi</i> cpc	<i>Salmonella typhi</i>	Centre Pasteur of Cameroon	Isolates
8	EC ATCC 25922	<i>Escherichia coli</i> 259922	American Type Culture Collection	Isolates
9	SD cpc	<i>Shigella dysenteriae</i>	Centre Pasteur of Cameroon	Isolates
10	SFNR 518	<i>Shigella flexneri</i> NR518	BEI Resource	Strain
11	<i>S. typhi</i>	<i>Salmonella typhi</i>	regional hospital Bamenda	Isolates
12	<i>S. aureus</i>	<i>Staphylococcus aureus</i>	regional hospital Bamenda	Strains
13	<i>E.coli</i>	<i>Escherichia coli</i>	Regional hospital Bamenda	Strains
14	<i>Pseudomonas</i>	<i>Pseudomonas aeruginosa</i>	Regional hospital Bamenda	Strains

BEI=Biodefense and Emerging Infection Research Resources Repository;
ATCC= American Type Culture Collection, CPC= Centre Pasteur of Cameroon,
RHB= Regional Hospital Bamenda

2.5. Chemicals

Pure molecules of antibiotics including; amoxicillin (30mg)-CAS (61336-70-7), ciprofloxacin (30mg) CAS (85721-33-1), tetracycline (60mg)-CAS (64-75-5) purchased from Shanghai Macklin Biochemical Shanghai China, hydrochloric acid, lead acetate, sodium chloride, 1% ferrous sulphate solution, acetic anhydrite, INT (iodonitrotetrazolium), DMSO <2.5%, chloroform and potassium iodide [21].

2.6. INT Colorimetric Assay for MIC and MBC Determination

2.6.1. MIC determination

The antibacterial assay of extract and fractions was carried out in triplicate using a broth micro dilution method for plant extracts in 96-wells micro plates, using INT as the bacterial growth indicator [21]. Test extracts and reference antibiotics were dissolved in DMSO and MHB to obtain the final working solution. The final concentration of DMSO in the assay was less than 2.5%, a concentration innocuous to bacterial growth. The solution obtained was added to the culture media 100ul of MHB, followed by a two-fold serial dilution in a 96-wells micro plate. Subsequently, bacterial suspension initially prepared at the McFarland standard of 0.5 (1.5×10^8 CFU/mL) from previously activated strains 18 to 24hours (fig. 3), as mentioned, (0.266ul) was diluted in 20ml of MHB, and 100 μ L of bacterial inoculum then was seeded in the wells of plates containing test extracts, antibiotics and a negative control. After the final test preparation was done, the plates were covered with a sterile plate sealer, then it was shaken to mix the contents of the wells using a plate shaker, followed by incubation at 37 °C for 18h-24h. Wells containing 100ul of MHB and 100 μ L of inoculum, served as negative controls. The final concentration of the extracts was recorded. The minimal inhibitory concentration (MIC) of test samples was recorded after 18 h incubation at 37 °C, following the addition (50 μ L) of INT 0.02% (w/v) (test indicator) and incubated at 37^oc for 30 min. The viable bacteria reduced the yellow dye to a preferred color (pink) corresponding to MIC. In figure 4 Wells with test samples only (blank control) was used to ensure that the extracts are not producing color with INT [22].

2.6.2. MBC determination

The minimal bactericidal concentration (MBC) was assessed by adding aliquots (50ul) of the preparations which did not show any growth after incubation during MIC testing, and of adequate broth (150ul). These preparations were incubated at 37 °C for 48 h. The MBC was considered as the lowest concentration of extracts that prevented the color change of the medium after the addition of INT as above mentioned. The assays were performed in triplicate and test was repeated thrice.



Figure 2MH agar 37oc 18h activated bacterial culture of Escherichia coli

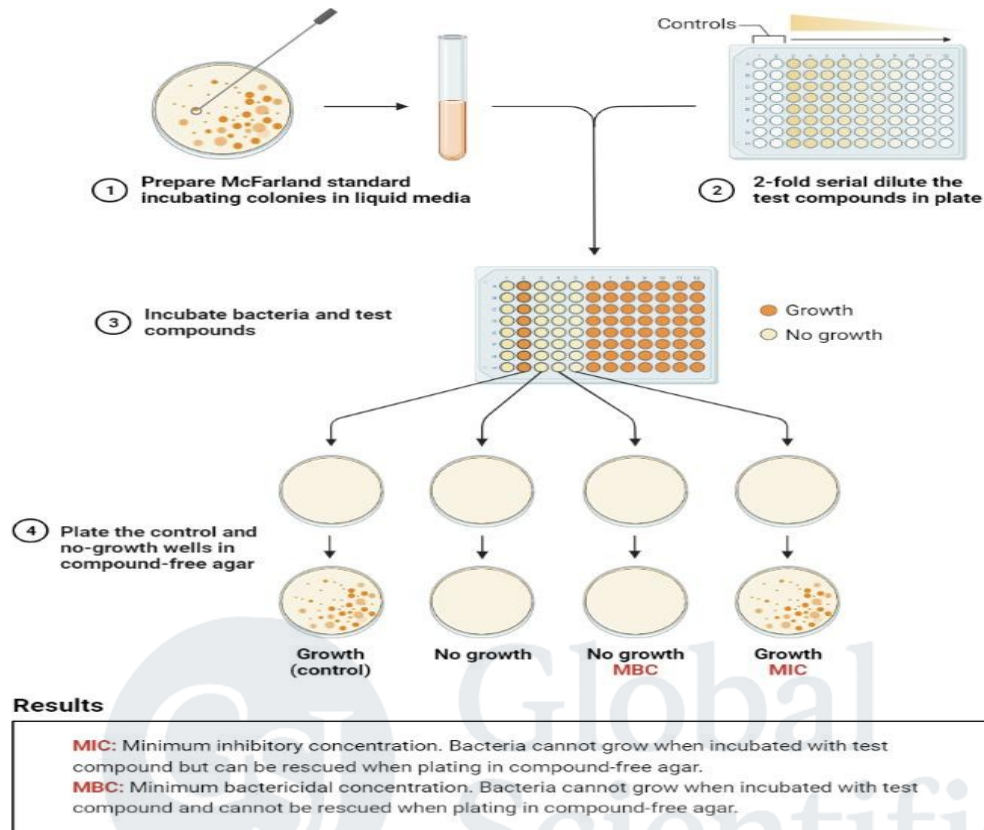


Figure 3 INT colorimetric assay

2.7. Checkerboard testing for combination between test extract and conventional antibiotics

The checkerboard assay is a laboratory test used to evaluate the effectiveness of combining two or more antibiotics against a specific microorganism [23]. In this study, the assay was used to investigate the combined effects of plant extracts and antibiotics against drug-resistant microorganisms. One extract was serially diluted along the abscissa, while antibiotic was serially diluted along the ordinate. The total volume of the combination was 100µL per well. Then 100µL of each bacterial suspension (1 × 10⁶ CFU/mL) was added to each well of a 96-well micro plate. The micro plates were incubated at 37 °C for 18 h. The OD (optical density) was measured at 595nm with a spectrophotometer and the use of INT to determine the checkerboards combination. The 96-well plate was set up with varying concentrations of extracts and antibiotics, and the minimum

amount of each substance needed to inhibit microbial growth (MIC) was determined. The fractional inhibitory concentration index (FICI) was calculated to assess the interaction between the extracts and antibiotics. Synergism is preferred to treat infections associated with multidrug-resistance (MDR) or those at risk of treatment failure with a single drug because plant extracts in combination provide more benefits than what is generally available alone [24]. Combined use of plant extracts could improve the effectiveness of pharmacological action by obtaining synergism, acting on multiple targets simultaneously, reducing the doses of individual extracts, and minimizing side effects [25]. The results were interpreted in figure 5 as follows:

- ✓ Synergistic effect (FICI < 0.5): The combination of substances enhances their individual inhibitory effects.
- ✓ Additive or indifferent effect (FICI 0.5-4): The combination has no significant effect or a slight enhancement of inhibitory activity.
- ✓ Antagonistic effect (FICI > 4): The combination reduces the inhibitory activity of the substances [26].

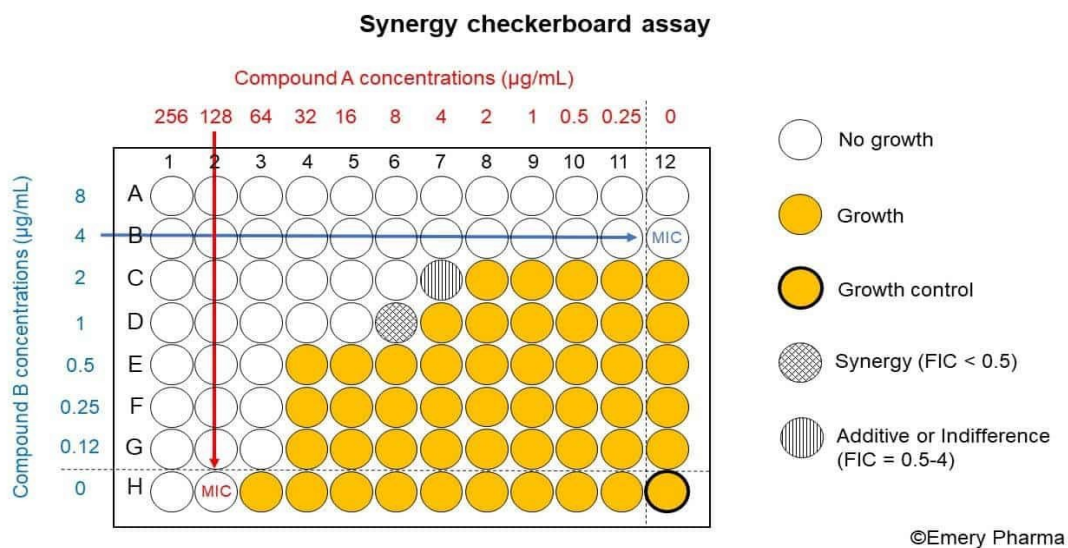


Figure 4 Checkerboard combination assay

2.8. Qualitative phytochemical testing

Phytochemical screening was carried out using quantitative tests as described by [27]. Aliquots of all the extracts were diluted with DMSO and some with distilled water in the ratio of 1:100 (w/v). For the alkaloid detection, 5 ml of the extract was added to 2 ml HCl this acidic medium was heated, 1 ml of Mayer’s reagent was added. An orange precipitate was observed immediately indicating the presence of alkaloids. To test the presence of flavonoids, 2 ml diluted of the extract, a few drops of lead acetate was added to the extract. The appearance of an intense yellow color that becomes colorless was observed it indicated the presence of flavonoids. For saponins detection, the extract was diluted with 20 ml of distilled water and agitated in a graduated cylinder for 15 min. There was formation of 1-2cm layer of foam hence indicated the presence of saponins. Phenols were detected by warming 2 ml of the diluted extract to 45–50°C followed by the addition of 3-4drops of 10% FeCl₃. There was the formation of dark green to black color hence indicating the presence of phenols. The table2 below describes the different qualitative phytochemical tests that here carried out.

Table 2 A table showing the methods for qualitative phytochemical testing

Secondary metabolite	Test type and reagents	Expected outcome
Tannins	5mg of extract dissolved in methanol then added 5 drops of 0.5% H ₂ SO ₄	Observed a green to bluish black color
Saponins	5mg of raw extract + 5ml of distil water heat for 5mins then allow to cool then shake vertically for 15s left to rest.	Observed the formation of persistent foam of ≥1cm indicates the presence of saponins.
Phenols	2mls of diluted extract in DMSO was placed in a clean test tube and 3-4 drops of 10% FeCl ₃ was added	Formation of a dark green to black color indicates the presence of phenols
Flavonoid	2ml of diluted extract in DMSO	The appearance of a golden

	was placed in a clean test tube and few drops of lead acetate was added	yellow precipitate indicated the presence of flavonoids
Alkaloids	1ml of diluted extract in DMSO was placed in a clean test tube + 1ml of 1% H ₂ SO ₄ the boiled for 5min then allowed to cool + 5drops of Mayer's reagent	The appearance of white precipitate indicates the presence of alkaloids
Anthocyanin	2ml of diluted extract in DMSO was placed in a clean test tube + 1% H ₂ SO ₄ and was heated for 5mins	The formation of a deep yellow to orange color indicated the presence of anthocyanin's
Terpenoids.	2ml of diluted extract in DMSO was placed in a clean test tube + 3 to 5 drops of 5% conc. H ₂ SO ₄ were added then 3drops of methyl chloride (CH ₂ CH ₂) was added and stared.	The formation of green to golden yellow indicated the presence of terpenoids.

2.9. Data analysis

Average values of MIC and MBC ($\leq 1024 \mu\text{g/ml}$) are presented on tables and figures. Antibacterial activity of plant extract as single was compared to plant extract-drug combination at 5% using FICI values of FIC: ≤ 0.5 synergy, FIC: $>0.5 \leq 1$ additive or indifference, FIC: $>2 \leq 4$ antagonism.

2.10. Ethical consideration

Ethical clearance was sort from the institutional review board hosted by the faculty of health science, University of Bamenda (2024/0228H/UBa/IRB). A letter of authorization was requested from the botanist at the herbarium center of the Limbe Botanic Garden for plant authentication.

3.0. Results

3.1. Minimum inhibitory concentrations (MICs) of *Emilia coccinea* on different classes of multidrug resistant pathogenic bacteria

The hydroethanolic extract of the stem and leaf of *E. coccinea* was tested for the potential of antimicrobial properties on a panel of different bacteria strains/isolate and the results were represented in (table 3-7). For the MIC results the hydroethanolic extract from the leaf and stem of *E. coccinea* inhibited the growth of 10/14 (71%) tested MDR bacteria strains with MIC ranging from 16 to 2048µg/ml (table: 3-11). The renowned antibiotic/ antibacterial drug tetracycline showed MIC values ranging from 4 to 512µg/ml on 14/14 (100%). The best activity was obtained from the hydroethanolic leaf extract with values of 128µg/ml being recorded for 1/14 (7.1%) tested bacterial (table 3). Furthermore, this lowest MIC value of 128µg/ml obtain by OECL was recorded for the bacteria strain *S. typhi* cpc showing a difference in tetracycline which is the conational antibiotic of 4 µg/ml (table 3). Also, it was observed that some MDR. Strains were highly resistance to the edible plant extract like *Pseudomonas* (table7). It was also noted that the bacterial strains where greatly resistant to extract OECL (table 6 and table7).

a. MICs of *Emilia coccinea* extract on *salmonella* species of multidrug resistant pathogenic bacteria.

With lowest MIC values (128µg/ml) gotten from OECL on *salmonella typhi* strain from CPC with initial concentration of extract (2048µg/ml) and lowest MIC value for antibiotic (4µg/ml) with initial concentration of antibiotic (512µg/ml).

Table 3 MICs values (µg/ml) of *Emilia coccinea* extracts on *salmonella* species of multidrug resistant pathogenic bacteria

Bacterial strain	OECL MIC	OECS MIC	Tetracycline MIC
<i>Salmonella strains</i>			

<i>SENR 1355</i>	512	-	4
<i>STM ATCC14</i>	512	-	4
<i>STM cpc</i>	1024	2024	16
<i>SE cpc</i>	256	1024	4
<i>SPB cpc</i>	1024	-	4
<i>S. typhi cpc</i>	128	512	4
<i>S. typhi RHB</i>	-	-	128

Minimum inhibition concentration (MIC), Bacteria: *salmonella different strains*, Extract: OECL: leaf of *Emilia coccinea*, OECS: stem of *Emilia coccinea*, ATB: tetracycline

b. MICs of *Emilia coccinea* extracts on *Staphylococcus* class of multidrug resistant pathogenic bacteria.

With lowest MIC values (512µg/ml) gotten from OECL on *Staphylococcus aureus 46003* strain from BIE source with initial concentration of extract (2048µg/ml) and lowest MIC value for antibiotic (64µg/ml) on *S. aureus* from RHB with initial concentration of antibiotic (512µg/ml).

Table 4 : MICs of *Emilia coccinea* extracts on *Staphylococcus* class of multidrug resistant pathogenic bacteria

Bacteria strain	OECL MICs	OECS MICs	TETRACYCLINE
<i>Staphylococcus</i>			
<i>SANR 46003</i>	512	256	128
<i>S. aureus RHB</i>	2048	2048	64

Minimum inhibition concentration (MIC), Bacteria: *Staphylococcus strains*, Extract: OECL: leaf of *Emilia coccinea*, OECS: stem of *Emilia coccinea*, ATB: Tetracycline

c. MICs of *Emilia coccinea* extracts on *Shigella* class of multidrug resistant pathogenic bacteria.

With lowest MIC values ($512\mu\text{g/ml}$) gotten from OECL on *Shigella dysenteriae* strain from Centre Pasteur of Cameroon with initial concentration of extract ($2048\mu\text{g/ml}$) and lowest MIC value for antibiotic ($4\mu\text{g/ml}$) on both strains with initial concentration of antibiotic ($512\mu\text{g/ml}$).

Table 5 MICs values ($\mu\text{g/ml}$) of *Emilia coccinea* extracts on *Shigella* class of multidrug resistant pathogenic bacteria

Bacteria strain	OECL MICs	OECS MICs	TETRACYCLINE MICs
<i>Shigella</i>			
SD CPC	512	1024	4
SFNR 518	1024	1024	4

Minimum inhibition concentration (MIC), bacteria: *Shigella strains*, extract: OECL: leaf of *Emilia coccinea*, OECS: stem of *Emilia coccinea*, ATB: Tetracycline

d. MICs value ($\mu\text{g/ml}$) of *Emilia coccinea* extracts on *Escherichia coli* class of multidrug resistant pathogenic bacteria.

With lowest MIC values ($1024\mu\text{g/ml}$) gotten from OECL on *Escherichia coli* strain from American type isolate culture with initial concentration of extract ($2048\mu\text{g/ml}$) and lowest MIC value for antibiotic ($4\mu\text{g/ml}$) on *Escherichia coli* with initial concentration of antibiotic ($512\mu\text{g/ml}$).

Table 6 MICs values ($\mu\text{g/ml}$) of *Emilia coccinea* extracts on *Escherichia coli* class of multidrug resistant pathogenic bacteria

Bacteria strain	OECL MICs	OECS MICs	TETRACYCLINE MICs
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<i>E. coli</i>			
<i>EC ATCC 25922</i>	1024	<2048	4
<i>E. coli RHB</i>	2048	<2048	128

Minimum inhibition concentration (MIC), bacteria: *Escherichia coli* strains (EC), Extract: OECL: leaf of *Emilia coccinea*, OECS: stem of *Emilia coccinea*, ATB: Tetracycline

e. MICs of Emilia coccinea extracts on Pseudomonas eurogenisa class of multidrug resistant pathogenic bacteria.

With MIC values (>2048µg/ml) gotten from OECL on *Pseudomonas eurogenisa* strain from RHB with initial concentration of extract (2048µg/ml) and MIC value for antibiotic (256µg/ml) on *S. aureus* from RHB with initial concentration of antibiotic (512µg/ml).

Table 7 MICs of Emilia coccinea extracts on Pseudomonas eurogenisa class of multidrug resistant pathogenic bacteria

Bacteria strain	OECL MICs	OECS MICs	TETRACYCLINE MICs
<i>Pseudomonas RHB</i>			
<i>Pseudomonas</i>	>2048	>2048	256

Minimum inhibition concentration (MIC), *Pseudomonas* (PA), Extract: OECL: leaf of *Emilia coccinea*, OECS: stem of *Emilia coccinea*, ATB: Tetracycline

3.2. Minimum bactericidal concentrations (MBCs) of Emilia coccinea extracts on different classes of multidrug resistant pathogenic bacteria

The hydroethanolic extract was tested for the potential of bactericidal effect it was recorded that only *Salmonella species* showed and MBC lesser than 2048 with values (1024µg/ml) as a moderate effect by OECL with lesser MBC for antibiotic of up to (4µg/ml) on *Salmonella species* (table 8),

values of 1024µg/ml was also recorded by OECS on *Staphylococcus* species with antibiotic MBC value of 64µg/ml (table 9). It was shown that all extracts of *E. coccinea* had no potential bactericidal effect on *E. coli species and Pseudomonas* (table 10 and table 11).

a. MBCs of *Emilia coccinea* extracts on *salmonella* species of multidrug resistant pathogenic bacteria.

With lowest MBC values (1024µg/ml) gotten from OECL on *salmonella typhi* strain from CPC and *Salmonella paratyphi* with initial concentration of extract (2048µg/ml) and lowest MBC value for antibiotic (4µg/ml) with initial concentration of antibiotic (512µg/ml).

Table 8 MBCs values (µg/ml) of *Emilia coccinea* extracts on *Salmonella* species of multidrug resistant pathogenic bacteria

Bacterial strain	OECL MBC	OECS MBC	Tetracycline MBC
<i>Salmonella strains</i>			
<i>SENR 1355</i>	2048	>2048	32
<i>STM ATCC14</i>	>2048	>2048	4
<i>STM cpc</i>	2048	>2048	16
<i>SE cpc</i>	2048	2048	64
<i>SPB cpc</i>	1024	>2048	4
<i>S. typhi cpc</i>	1024	>2048	4
<i>S. typhirrRHB</i>	>2048	>2048	256

Minimum Bactericidal Concentration (MBC), Bacteria: *salmonella different strains*, Extract:

OECL: leaf of *Emilia coccinea*, OECS: stem of *Emilia coccinea*, ATB: tetracycline

b. MBCs of *Emilia coccinea* extracts on *Staphylococcus* class of multidrug resistant pathogenic bacteria.

With lowest MBC values ($1024\mu\text{g/ml}$) gotten from OECS on *Staphylococcus aureus* 46003 strain from BIE source with initial concentration of extract ($2048\mu\text{g/ml}$) and lowest MBC value for antibiotic ($64\mu\text{g/ml}$) on *S. aureus* from BIE source with initial concentration of antibiotic ($512\mu\text{g/ml}$).

Table 9 MBCs Value ($\mu\text{g/ml}$) of Emilia coccinea extracts on Staphylococcus class of multidrug resistant pathogenic bacteria

Bacteria strain	OECL MBCs	OECS MBCs	TETRACYCLINE MBCs
<i>qStaphylococcus</i>			
<i>SaANR 46003</i>	>2048	1024	64
<i>S. aureus</i>	>2048	>2048	512

Minimum Bactericidal concentration (MBC), Bacteria: *Staphylococcus strains*, Extract: OECL: leaf of *Emilia coccinea*, OECS: stem of *Emilia coccinea*, ATB: Tetracycline

c. MBCs of Emilia coccinea extracts on Shigella class of multidrug resistant pathogenic bacteria.

With MBC values ($>2048\mu\text{g/ml}$) gotten from OECL on all strain of *Shigella* with initial concentration of extract ($2048\mu\text{g/ml}$) and lowest MBC value for antibiotic ($16\mu\text{g/ml}$) on *Shigella dysenteriae* strains with initial concentration of antibiotic ($512\mu\text{g/ml}$).

Table 10 MBCs values ($\mu\text{g/ml}$) of Emilia coccinea extracts on Shigella class of multidrug resistant pathogenic bacteria

Bacteria strain	OECL MBCs	OECS MBCs	TETRACYCLINE MBCs
<i>Shigella</i>			
SD CPC	>2048	2048	16
SFNR 518	>2048	>2048	32

Minimum Bactericidal concentration (MBC), bacteria: *Shigella strains*, extract: OECL: leaf of *Emilia coccinea*, OECS: stem of *Emilia coccinea*, ATB: Tetracycline

d. MBCs value ($\mu\text{g/ml}$) of *Emilia coccinea* extracts on *Escherichia coli* class of multidrug resistant pathogenic bacteria.

With MBC values ($>2048\mu\text{g/ml}$) gotten from on all *Escherichia coli* strain with initial concentration of extract ($2048\mu\text{g/ml}$) and lowest MBC value for antibiotic ($32\mu\text{g/ml}$) on *Escherichia coli* from American type isolate culture with initial concentration of antibiotic ($512\mu\text{g/ml}$).

Table 11 MBCs values ($\mu\text{g/ml}$) of *Emilia coccinea* extracts on *Escherichia coli* class of multidrug resistant pathogenic bacteria

Bacteria strain	OECL MBCs	OECS MBCs	TETRACYCLINE MBCs
<i>E. coli</i>			
<i>EC ATCC 25922</i>	>2048	>2048	32
<i>E. coli RHB</i>	>2048	>2048	256

Minimum Bactericidal concentration (MBC), bacteria: *Escherichia coli* strains (EC), Extract: OECL: leaf of *Emilia coccinea*, OECS: stem of *Emilia coccinea*, ATB: Tetracycline

e. MBCs of *Emilia coccinea* extracts on *Pseudomonas eurogenisa* class of multidrug resistant pathogenic bacteria.

With MBC values ($>2048\mu\text{g/ml}$) gotten from OECL on *Pseudomonas eurogenisa* strain from RHB with initial concentration of extract ($2048\mu\text{g/ml}$) and MBC value for antibiotic ($256\mu\text{g/ml}$) on *S. aureus* from RHB with initial concentration of antibiotic ($512\mu\text{g/ml}$).

Table 12 MBCs of *Emilia coccinea* extracts on *Pseudomonas eurogenisa* class of multidrug resistant pathogenic bacteria

Bacteria strain	OECL MICs	OECS MICs	TETRACYCLINE
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				MICs
Pseudomonas RHB				
Pseudomonas	>2048	>2048		256

Minimum Bactericidal concentration (MBC), *Pseudomonas* (PA), Extract: OECL: leaf of *Emilia coccinea*, OECS: stem of *Emilia coccinea*, ATB: Tetracycline

3.3. Antibacterial efficacy conventional antibiotics in combination with *Emilia coccinea* in the treatment of multidrug resistant pathogenic bacteria

The MICs of the different antibiotics alone recorded in this study (08–128 µg/mL) confirming multidrug resistance for the different strains of isolate used. The antibacterial interactions were determined following the values of the FICI mentioned above [28]. A total of 24 combination assays were carried out for both extracts of which, (09) were synergistic based on FICI ranging from 0.162-0.35, with the lowest MIC and most synergistic interactions being (table 4). The MICs of antibiotics in combinations with the extracts were considerably reduced compared to the MICs of the antibiotics alone. The reductions were much greater in the synergistic than the additive interactions. The highest synergistic interaction was recorded from the interaction of both extracts and Ciprofloxacin with a FICI (0.162)

There was a reduction in the MICs in the interaction of OECS/antibiotics and OECL/ antibiotics with different MD resistant strains starting with OECL/ciprofloxacin on isolate *S. aureus* from (128-16µg/ml), *S. typhi* had OECL/tetracycline (128-32µg/ml) and ciprofloxacin (128-16µg/ml), *pseudomonas* and OECL/ciprofloxacin (128-16µg/ml) and *E. coli* and OECL/ciprofloxacin (34-4µg/ml). *S. typhi* and OECS/ciprofloxacin (128-16µg/ml), *Pseudomonas eurogenisa* and OECS/ciprofloxacin (32-4µg/ml) and *E. coli* and OECS/tetracycline (64-16µg/ml), (table 6). Synergic interaction was recorded for two antibiotics out of three antibiotics tested. Additive interaction was recorded for all three antibiotics with amoxilline showing and additive only with

extract OECL and isolate *Pseudomonas eurogenisa RHB* with FICI (0.75) and the in table 5, the highest proportion of additive interaction was recorded against all 4 isolates.

Antibacterial efficacy of OECL in combination with conventional antibiotics for the treatment of multidrug resistant pathogenic bacteria.

Table 13 MICs values (µg/ml) and FICIs of antibiotics in combination with OECL/ isolates

(OECL/ antibiotics/ Isolate)	Mic ATB	ATB EXT	FIC of ATB	MIC of EXT	EXT/ ATB	FIC of EXT	FICI	Out com e
<i>S. aureus RHB</i>								
Amoxilline	128	128	1	2048	2048	1	2	IND
Tetracyclin	64	128	2	1024	32	0.03	2.03	IND
Ciprofloxacin	128	16	0.125	2048	256	0.125	0.25	SYG
<i>S. typhi RHB</i>								
Amoxicillin	128	128	1	2048	2048	1	2	IND
Tetracyclin	128	32	0.25	1024	128	0.125	0.37	SYG
Ciprofloxacin	128	16	0.125	2048	128	0.06	0.18	SYG
<i>Pseudomonas RHB</i>								

Amoxicillin	128	64	0.5	2048	512	0.25	0.75	AD
								D
Tetracycline	32	16	0.5	2048	32	0.01	0.51	AD
						5	5	D
Ciprofloxacin	32	4	0.1	2048	128	0.06	0.16	SYG
						2	2	
<i>E. coli RHB</i>								
Amoxilline	128	128	1	2048	2024	1	2	IND
Tetracycline	64	16	0.25	2048	64	0.03	0.28	SYG
Ciprofloxacin	8	2	0.25	2048	128	0.06	0.31	SYG

FIC= MIC of test compound in combination ÷ MIC of test compound alone, FICI= FIC (Antibiotic) +FIC (Extract), FICI= ≤0.5= synergy (SYG), >0.5 ≤ 1= additive interaction (ADD), 1-4= indifference (IND), > 4= antagonism (ANT), Isolate include MDR strain of: *S. aureus RHB*, *S. typhi RHB*, *Pseudomonas RHB* and *E. coli RHB* and extract (OECL): leaf of *Emilia coccinea*

a. Antibacterial efficacy of OECS in combination with conventional antibiotics for the treatment of multidrug resistant pathogenic bacteria

Table 14 MICs values (µg/ml) and FICIs of antibiotics in combination with OECS/ isolates

(OECS/ antibiotics/ Isolate)	Mic ATB	ATB / EXT	FIC of ATB	MIC of EXT	EXT/ ATB	FIC of EXT	FICI	Outco me
<i>S. aureus RHB</i>								
Amoxilline	128	128	1	2048	2048	1	2	IND

Tetracyclin 32 16 0.5 2048 32 0.01 0.51 ADD
 e

Ciprofloxa 2 1 0.5 2048 16 0.00 0.50 ADD
 cin 7 7

S. typhi RHB

Amoxicilli 128 128 1 2048 2048 1 2 IND
 ne

Tetracyclin 128 128 1 2048 2048 1 2 IND
 e

Ciprofloxa 128 16 0.12 2048 256 0.12 0.25 SYG
 cin 5 5

Pseudomonas RHB

Amoxicilli 128 128 1 2048 512 0.25 1.25 IND
 ne

Tetracyclin 32 32 1 2048 64 0.03 1.03 IND
 e

Ciprofloxa 32 4 0.1 2048 128 0.06 0.16 SYG
 cin 2 2

E. coli RHB

Amoxilline 128 128 1 2048 2048 1 2 IND

Tetracyclin	64	16	0.25	1024	128	0.1	0.35	SYG
e								
Ciprofloxa	16	16	1	2048	32	0.01	1.01	1ND
cin								

FIC= MIC of test compound in combination ÷ MIC of test compound alone, FICI= FIC (Antibiotic) +FIC (Extract) FICI= ≤0.5= synergy (SYG), >0.5 ≤ 1= additive interaction (ADD), 1-4= indifference (IND), > 4= antagonism (ANT). ATB: Tetracycline, amoxicillin and ciprofloxacin; Isolate include MDR strain of; *S. aureus RHB*, *S. typhi RHB*, *Pseudomonas RHB* and *E. coli RHB* and extract (OECS): stem of *Emilia coccinea*

3.4. Results on phytochemical analysis of secondary metabolites *Emilia coccinea*

The phytochemicals tested in this study were; tannin, saponin, phenols, flavonoids, terpenoids, anthocyanin and alkaloid (table: 6), which were identified from the leaf of *E. coccinea* (OECL) and stem of hydroethanolic extract of *E. coccinea* (OECS). Compared to the study carried out by [29]. indicating the presence of the various phytochemicals

All the hydroethanolic extract of plant parts of *E. coccinea* showed to contain anthocyanin and tannins. Also, it was observed that, all parts of *E. coccinea* contain saponin and phenols. It was again observed that the hydroethanolic extract of the stem of *E. coccinea* contained flavonoids except for the hydroethanolic leaf extract of *E. coccinea* which showed no presence of flavonoids. Using the shalkonski test, the hydroethanolic leaf extract of *E. coccinea* shows the presence of terpenoids. Also, all *E. coccinea* plant extract part showed the presence of alkaloids.

a. Phenols and phenolic phytochemicals present in the different parts of *Emilia coccinea*

Table 15 Phenols and phenolic phytochemical constituents detected in *Emilia coccinea* extracts

Phytochemical constituents	OECL	OECS
TANNIN	+++	+

PHENOL	+++	+
FLAVANOIDS	-	+
ANTHOCYANINS	++	+

(+) indicates the presence of the phytochemical. (-) indicates the absence of the phytochemical; OECL (leaf extract of *Emilia coccinea*); OECS (stem extract of *Emilia coccinea*)

b. Non- phenolic phytochemicals present in the different parts of *Emilia coccinea*

Table 16 non-phenolic phytochemical constituents detected in *Emilia coccinea* extracts

Phytochemical constituents	OECL	OECS
SAPONINS	+++	+
TERPENOIDS	+++	-
ALKALOIDS	+++	++

(+) indicates the presence of the phytochemical. (-) indicates the absence of the phytochemical. OECL (leaf extract of *Emilia coccinea*). OECS (stem extract of *Emilia coccinea*)

4.1. Discussion

The results above showed the moderate and significant activity of *E. coccinea* from highly multidrug resistance this shows the antimicrobial potency of *E. coccinea*. Confirming the results of [30]. which stated that *E. coccinea* has antimicrobial activities against gastrointestinal microorganisms such as *E. coli*, *S. aureus* and *Salmonella species*. From the findings above *Staph*, *Salmolla taphi*, *Pseudomonas* strains from BRH Indicates an urgency to investigate the causative and mechanism of action of these MDR bacterial strains which showed great level of resistance

with the plant extract and even the antibiotic of choice, hence, justifying the WHO bacteria priority pathogens list of 2024.

Salmonella species has MBC of $1024\mu\text{g/ml}$ which was considered a moderate effect; this value is significantly higher than the MBC of the control conventional antibiotics (up to $4\mu\text{g/ml}$) for *Salmonella species*, indicating a lesser potency of the extract compared to the control conventional antibiotics. *Staphylococcus species* with MBC of $1024\mu\text{g/ml}$ was recorded, which is similar to the value observed for *Salmonella species*. This justifies the WHO recommendation of using the MBC values to evaluate the bactericidal activity of antimicrobial agents [31]. The antibiotic MBC value of $64\mu\text{g/ml}$ is lower than the extract's MBC values, suggesting that antibiotics are more effective against *Staphylococcus species*. The MBC values mentioned in the statement are within the acceptable range for evaluating bactericidal activity [31]. While on *E. coli species* and *Pseudomonas*, the extracts showed no potential bactericidal effect, indicating that they are not effective against these species. Also, it was stated that the comparison between the MBC values of the extracts and antibiotics provides insight into their relative potencies [32]. This also indicates the importance of a combination test with different and potent conventional antibiotics with the edible plant extract *E. coccinea*.

All *E. coccinea*-antibiotic combinations tested showed synergistic interactions against at least one isolate. Leaf of *E. coccinea* was the most synergistic in combination with ciprofloxacin with FICI $0.162\mu\text{g/ml}$. Furthermore, there was a great reduction in the MIC of antibiotics, ciprofloxacin and tetracycline in combination with OECL, to a great level recorded against the different MDR pathogenic strains. This high reduction of the MIC of the treatment antibiotic further demonstrates the strong synergism in the combinations and indicates that OECL is a potential partner antibacterial which could be used in combination with some treatment antibiotics in the

management of MDR *E. coli*, *pseudomonas*, *staphylococcus* and *Salmonella* infections which may lead to resultant reduction in morbidity and mortality. However, further studies are required to establish this.

The stem of *E. coccinea* (OECS), showed lower levels of synergism against the MDR pathogenic strains in various combinations with ciprofloxacin and tetracycline. The observed synergistic antibacterial activity could be due the action of the antibiotic and the *E. coccinea* at different target sites in the bacterial cell. These differences in the level of synergism of OECL and OECS could be due to their structures. When OECS was combined with conventional antibiotic ciprofloxacin a synergistic effect was observed. The likely mechanism of action for OECS could involve the inhibition of cell wall synthesis or some essential process in the bacteria while ciprofloxacin acts by its known mechanism of inhibiting DNA replication.

Additivity was also recorded in several combinations of the plant extracts with the 3 classes of antibiotics. Great number of additive interactions were recorded for OECS and with the three classes of antibiotics. This interaction suggest that the molecules in the combination compounds may be sharing the same target sites, hence acting by the same mechanism in the bacterium, in which case both *E. coccinea* and amoxilline may be acting by inhibiting the bacterial cell wall synthesis [33]. OECL also showed lesser number of additive interactions for two antibiotic classes (amoxicillin and tetracycline) on isolate *Pseudomonas aerogenosa*, with which its combination with tetracycline was a synergistic reaction on *S. typhi* and *E. coli* respectively, further indicating that OECL may be acting at two different target sides.

OECS showed the highest indifference in combination with all antibiotics and MDR isolates. The likely mechanism of action here could be blocking of the site of action of the antibiotics by the OECS in the cell wall for amoxilline, protein synthesis for tetracycline and DNA replication for

ciprofloxacin. Overall, when all the interactions are considered, OECL was the most synergistic and also showed relatively lower additive interaction, no antagonism, and indifference

This study carried out on *Emilia coccinea* revealed the presence of medicinal active constituents. The preliminary phytochemical content of *E. coccinea* leaf reviewed the presence of alkaloids, tannins, saponin, phenols, anthocyanin, and terpenoids. From a study carried out by [30], indicated the presence of flavonoids and the absence of terpenoids in the stem of *E. coccinea*. This viable difference could be possible due to the difference in extract as it was a methanolic extract of *E. coccinea*. It was stated by [34], that there is significant action of phytochemicals in treating various diseases. Confirming the antimicrobial potential *E. coccinea*

5.1. Conclusion

The present study clearly shows that the hydroethanolic extract of edible plant *E. coccinea* has antimicrobial properties against MDR bacteria strains with the leaf having the highest activity. There was a significant increase of antibiotic efficacy in combination with edible plant extract *E. coccinea*. It was also noted that the hydroethanolic extract of *E. coccinea* showed the presence of phytochemical constituents which supports the traditional use of this plant as due to its antimicrobial properties. The results obtained from this study provided evidence that *E. coccinea* exhibited beneficial antibacterial activity against *S. aureus*, *Pseudomonas aeruginosa*, *Shigella*, *Salmonella species* and *Escherichia coli*.

6.1. Acknowledge

The authors thank Redemption Higher Institute of Biomedical and Management Sciences and the University of Buea, the University of Bamenda for providing the facilities needed and Atem Enoworock Besong for the critical review, drafting and correction of manuscript.

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