Research Square

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Formulation of Improved Traditional Drugs against virulent species of Salmonella spp in Benin: assessment of the properties from Uvaria chamae, Lantana camara and Phyllantus amarus in Benin, West Africa

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Abstract

Background Uvaria chamae (Annonaceae), Phyllantus amarus (Phyllantaceae) and Lantana camara (Verbenaceae) are empirically alleged to be used as Beninese medicinal plants in the treatment of salmonellosis. This study aimed to produce scientific data on in vitro and in vivo efficacy of Uvaria chamae, Lantana camara and Phyllantus amarus on multiresistant Salmonella spp isolated in Benin.

Results After in vitro tests on aqueous and ethanolic extracts of Uvaria chamae, Lantana camara and Phyllantus amarus , only the aqueous extract of Uvaria chamae (leaves) showed the best anti- Salmonella 's activity. It has been used for the following experiments. The induction of salmonellosis revealed 9.0 10 8 CFU/ml was optimal concentration for triggering and maintaining the symptoms in chicks. This infective concentration has been used for in vivo assessment. 24 hours post inoculation later, the symptoms of salmonellosis (wet cloaca, diarrhea stool and somnolence) were observed in infected groups. After seven days of treatment, the rate of reduction of bacterial load at 100 mg / L, 200 mg / L, 400 mg / L of this extract was 85%, 52.38% and 98% respectively in the chicks groups infected with Salmonella Typhimurium ATCC 14028. About the groups infected with Salmonella spp (virulent strain), the rate of reduction of bacterial load at 100 mg / L, 200 mg / L, 400 mg / L of this extract was 0%, 98.66% and 99.33%. The toxicity tests did not show any significant effect of the Uvaria chamae 's extract on the biochemical and hematological parameters of the chicks.

Conclusion The aqueous extract of Uvaria chamae is active in vitro and in vivo on multiresistant strains of Salmonella spp . This plant is a good candidate for the development of an improved traditional medicine for the management of salmonellosis.

Background

Salmonella spp is the most common foodborne pathogens habitually isolated from food-producing animals. This germ is responsible for zoonotic infections in humans and animal species including birds. *Salmonella spp* can be transmitted to humans along the farm-to-fork continuum, commonly through contaminated foods of animal origin, namely poultry and poultry-related products, pork, fish [1].

It is Gram negative, rod-shaped bacteria, and facultative anaerobes belonging to the Enterobacteriaceae's family and divided into three main species: *Salmonella enterica, Salmonella bongori* and *Salmonella subterranean* [2]. It isone of the top four causes of diarrheal diseases worldwide [3]. Recent studies estimated that there were approximately 94 million cases of non-typhoid *Salmonella* gastroenteritis resulting in 155,000 deaths globally each year [4]. Out of these cases, 80.3 million were estimated as foodborne origin [5]. *Salmonella Typhimurium, Salmonella Enteritidis, Salmonella Heidelberg* and *Salmonella Newport* are the epidemiologically important serotypes with poultry and have been associated with the majority of human salmonellosis burden worldwide [6–9].

Increasing prevalence of multidrug resistant *Salmonella* such as resistance towards clinically important antimicrobials like fluoroquinolones and third-generation cephalosporins has become an emerging problem worldwide [10–13]. As an alternative to antimicrobial resistance, exploration of medicinal plants with anti-*Salmonella* activity is becoming very common in West Africa. *In vitro* antibacterial activity data of medicinal plant extracts on *Salmonella* strains do exist [14] but there is a few data about *in vivo* efficacy of medicinal plants against this bacterium. The difficulty of choosing a suitable study model, the complexity of such research work, and the still limited data on the physiology of *Salmonella* strains could explain this situation. This study is then devoted to the experimental induction of salmonellosis in chicks as animals' models. These data can be a good starting point for *in vivo* efficacy testing of herbal extracts on *Salmonella spp.* Indeed, promoting medicinal plants used in the traditional treatment of salmonellosis involves a structured approach. At this time, an ethnopharmacological survey revealed 57 species of medicinal plants used in the treatment of salmonellosis in Benin [15]. Based on quotation frequency and literary data, *Phyllantus amarus, Senna siamea, Uvaria chamae and Lantana camara* have been selected. Toxicological, chemical and antibacterial (to ten enteropathogens) characterization

have been done. This study showed interesting contents in polyphenols and flavonoids and an effective antibacterial activity at 100 mg/mL with MIC between 100 and 25 mg/mL and inhibition diameters between 7.5 and 21 mm [16].

With these results, it was found necessary to evaluate the *in vitro* and *in vivo* efficacy of extracts of *Uvaria chamae, Phyllantus amarus* and *Lantana camara* on multiresistant *Salmonella spp* isolated in Benin.

Results

In vitro anti-*Salmonella* activity of *Uvaria chamae, Phyllantus amarus* and *Lantana camara*

Salmonella Typhimurium ATCC 14028 was sensitive to aqueous extract of *Uvaria chamae (*leaves), ethanolic extractof *Phyllantus amarus (*leaves), ethanolic and aqueous extract of *Lantana camara (*leaves). The aqueous extract of *Uvaria chamae (*leaves)showed the best inhibition diameter (9.33 ± 2.08 mm) (Figure 1). There is no significant difference between inhibition diameters (P = 0.6885).

Figure 2 presents antibacterial activity of aqueous and ethanolic extracts of *Uvaria chamae, Phyllantus amarus* and *Lantana camara* on multiresistant strains of *Salmonella spp* isolated in Benin. Out of ethanolic extract (roots) of *Uvaria chamae,* all extracts showed variable susceptibility to multiresistant strains of *Salmonella spp.* Leaves aqueous extract of *Uvaria chamae* was active on 90 % of *Salmonella* spp. Root aqueous extract of *Uvaria chamae* showed the best inhibition diameter (13±1 mm on P19 *Salmonella spp).* Leaves aqueous extract of *Lantana camara* and ethanolic leaves extract of *Phyllantus amarus* showed the smallest inhibition diameters (6 mm). The Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (CMB) and the antibiotic potency (ap) of the plant extracts studied on the tested strains are summarized in Table 1. MIC range from 1.625 (Leaves ethanolic extract of *Uvaria chamae* on P17 *Salmonella spp)* to 100 mg/ml. Leaves aqueous extract of *Uvaria chamae* was bacteriostatic on *Salmonella* Typhimurium ATCC 14028 and *Salmonella spp* P19.

In vivo anti-*Salmonella* activity of aqueous extracts (leaves) of *Uvaria chamae* using chick models

Preliminary test

This step aimed to choose the optimal effective concentration of inoculum for Salmonella's induction. 24 hours after infection, wet cloaca and diarrhea stools were detected in groups 1, 2 and 3 infected with inoculum concentrations 1, 2 and 3 respectively whereas these symptoms were absent in group 4, which received distilled water only. These symptoms were present until the 10th day of observation in the group 3 while they disappeared between the fifth and the 7th day in the group 2 (Table 2). However, no deaths were recorded. Salmonella spp was investigated in faecal samples to support clinical observations. The strains were detected in chicks of group 2 and 3, three days after salmonellosis induction. They were present till the ninth day for group 3 whereas they disappeared from feces on the sixth day for group 2 (Table 3). On the ninth day, a bacterial count was made on the faeces samples to assess the bacterial load. The results are shown in Table 4.

Clinical observations suggest that only 9.0.10^{8} UFC/ml could trigger and maintain the symptoms of salmonellosis.

In vivo efficacy of leaves aqueous extract of *U. chamae* on *Salmonella Typhimurium* ATCC 14028 (reference strain) and *Salmonella spp* P19

(Virulent strain)

24 hours after induction, symptoms were detected in all infected chicks. Diarrheal stools were abundant. The cloacae of the chicks were wet and somnolence was noticed in some chicks (Tables 5–10). During the 9 days of monitoring, including 7 days of treatment, these symptoms evolved considerably according to the groups. They remained persistent in infected and untreated chicks (group 2). However, diarrhea was slightly reduced at the last days of monitoring, indicating a progression of the disease to asymptomatic carriage (Tables 7 and 8).

Throughout the monitoring, there was a progressive increase in the weight of the chicks of all the groups (figures 3 and 4). For chicks infected by *Salmonella Typhimurium* ATCC 14028, there was no significant difference in weight change compared to other groups (P = 0.9783) (figure 3). Same observation was done in chicks infected with *Salmonella spp* P19 (virulent strain) (P = 0.9492) (figure 4).

3 days after infection with *Salmonella Typhimurium,* the bacterial load of *Salmonella* increased in all infected chicks, but differentially according to the groups. The bacterial load at day 3 was in the range 2000–30000 CFU/g. Treatment of the chicks with the extracts and colistin started at day 3 and continued until day 9. During treatment, the bacterial load gradually decreased in group 3 treated with colistin and disappeared on Day 9. In group 2 (infected and untreated), the bacterial load decreased slightly between the third and sixth day (11000 to 10000 CFU/g) before undergoing a considerable increase on the ninth day (10000 to 16000 CFU/g). In groups 4, 6 respectively treated with 100 and 400 mg/l of the extract, the bacterial load was first increased before falling down on day 9. In group 5 (treated with the extract at 200 mg/l), a gradual decline from the 3rd to the 9th day (21000 to 10.000 CFU/g) (figure 5) was observed.

Bacterial load reduction between the 3rd and the 9th day after the infection (7 days of treatment) was assessed globally and summarized in the figure 6. The inoculum increased by 45.45% in group 2 (infected and untreated). It was reduced by 100% in lot 3 (treated with colistin). Treatment with leaves aqueous extract of *Uvaria chamae* also showed remarkable efficacy (bacterial load reduction between 52.38% and 98%).

3 days after infection with *Salmonella spp* (virulent strain) (figure 7), the bacterial load of *Salmonella* increased in all infected chicks. The bacterial load at day 3 was in the range 3000–15000 CFU/g. Treatment of the chicks with the extracts and colistin started on day 3 and continued until day 9. During treatment, the bacterial load gradually decreased from 1500 to 1000 CFU/g in group 3 treated with colistin. In group 2 (infected and untreated), the bacterial load decreased slightly between the third and sixth day (9000 to 3000 CFU/g) before undergoing a considerable increase on the ninth day (3000 to 52,000 CFU/g). In groups 4, 6 respectively treated with 100 and 400 mg/l, the bacterial load was first increased before falling on day 9, whereas in group 5 treated with the extract at 200 mg / l, bacterial load were constant to 15000 CFU/g between day 3 and day 6 and decreased to 200 CFU/g at day 9.

Bacterial load reduction between the 3rd and the 9th day after the infection (7 days of treatment) was assessed globally and summarized in the figure 8. The inoculum increased by 477.77 % in group 2 (infected and untreated). It was reduced by 33.33 % in lot 3 (treated with colistin), 98.66% in group 5, 99.33 % in group 6. The extract at 100 mg / l did not allow a reduction of the bacterial load (group 4).

Effects of Leaves aqueous extract of *Uvaria chamae* on biochemical and hematological parameters

Effect of Leaves aqueous extract of *Uvaria chamae* on hematological and biochemical parameters was investigated to evaluate whether as a biologically active substance, this extract did not have a pathological effect on certain biochemical and hematological parameters. The results are shown in Figures 9 and 10.

Uremia increased insignificantly (p>0.05) at day 4 in chicks which received 400 mg/l (4.66 g / l to 4.99 g / l) and 100 mg/l (4, 49 g / l at 5.27 g/l) of extract. As for those which received 200mg/l of extract, their uremia increased at day 7 (4.23g / l to 4.67g / l).The creatinine concentration at day 4 increased insignificantly in chicks having 100 mg of extract (0.17g / l to 0.21g / l) and those which received the antibiotic (0.16g / l to 0.21g / l). In chicks which received 200mg (0.16g / l to 0.18g / l) and 400mg (0.17g / l to 0.19g / l) of extract, their creatinine increased at day 7 (p>0.05). Same observation was done with AST and ALT (no significant variation) (figure 9). With hematological parameters, the same observations are made. There was no significant difference in all groups. However, the number of blood cells increased from the 1st to the 4th day and from the 4th to the 7th day (figure 10).

Discussion

This study aimed to produce scientific data on *in vitro* and *in vivo efficacy* of extracts of *Uvaria chamae, Lantana camara* and *Phyllantus amarus* on multiresistant *Salmonella spp* isolated in Benin. *In vitro* anti-salmonella tests were used to assess the activity of the extracts of the three plants and to select the extract to be used for the *in vivo* tests.

The performance of the *in vivo* efficacy test was preceded by an experimental infection which made it possible to choose the optimal concentration of the inoculum.

In vitro anti-*Salmonella* activity of *Uvaria chamae, Phyllantus amarus* and *Lantana camara*

The aqueous and ethanolic extracts of the leaves and bark of *U. chamae* showed an inhibition of *Salmonella spp* with the exception of the ethanolic extract of *U. chamae's* roots. The aqueous extract of *Uvaria chamae* was active on 90% of the virulent *Salmonella spp* and on *Salmonella Typhimurium* ATCC 14028. These results can be compared to those obtained by Ogueke [17]. This author showed that at a concentration varying between 150–250 mg / ml, aqueous and ethanolic extracts of bark and the ethanolic extract of leaves of *Uvaria chamae* inhibited *Salmonella Typhi.*

Aqueous and ethanolic extracts of *Phyllantus amarus* inhibited *Salmonella spp* with maximal inhibition diameter of 12 mm. The activity of *Phyllantus amarus* extracts on *Salmonella spp* were reported in 2008. Using agar cup diffusion method, the authors showed that ethanolic extracts of *P. amarus* were active on *Salmonella Typhi* [18]. In our study, only leaves ethanolic extract of *Phyllantus amarus* inhibit *Salmonella Typhimurium* ATCC 14028 (9.33±1.53 mm). The inhibitory power of the extract on the reference strain was greater than those obtained in a previous study. For concentrations ranging from 200 to 1000 µg / ml, the inhibition diameters varied between 7 and 9 mm on *Salmonella Typhimurium* ATCC 6539 [19].

The ethanolic leaves extract of *Lantana camara* had an inhibition diameter of 8 mm on *Salmonella Typhimurium* ATCC 14028. The inhibitory power of extracts of *Lantana camara* on *Salmonella spp* has already been reported in the literature. Lyumugabe *et al.* [20] obtained an inhibition diameter of 11 mm on *Salmonella Typhimurium.*

Experimental infection of three-day-old chicks with Salmonella Typhimurium ATCC 14028: Preliminary test

To achieve an experimental infection requires that certain experimental conditions be met. The most important are the virulence of the strain used, the choice of the appropriate study model, the choice of the dose and the optimal infective concentration. The choice of chicks as a study model was motivated by two main reasons.

Firstly, *Salmonella Typhimurium* is known for his ability to infect birds, contaminate eggs [21] and be transmitted to humans. It is the main agent of salmonellosis in humans. Secondly, chicks have been used successfully for experimental

infections of non-typhoid salmonellosis : for investigation of the invasiveness of *Salmonella enterica* in chickens [22] ; for investigation of the dynamics of egg contamination over an extended time course [23]; for investigation of pathogenicity of some avian *Salmonella* Serovars [24]. In this study, we choose three-day old chicks for various reasons. One had to choose an age of susceptibility, an age when the animal's immune system is not mature enough to prevent infection. Such an age guarantees the establishment of the infection. Also it is known that in poultry, the signs of the disease are rarely observed after the first two weeks of life [25]. These strategic choices appear to be optimal since, 24 hours after the infection, the animals showed signs of salmonellosis, particularly in groups 2 and 3, which received the concentrations of inoculum 2 and 3 respectively. It was observed wet cloaca, diarrhea stool and somnolence. Clinical signs have been associated with the detection of salmonella in feces. The chosen model has therefore made it possible to reproduce the disease. Several studies have focused on the use of chicks at an age of susceptibility to optimize infection. Osman *et al.* (2010) used 1-day-old SPF White Leghorn chicks for inoculation with *Salmonella Typhimurium.* Beal *et al.* (2004) confirmed that *Salmonella Typhimurium* is a non-pathogenic commensal in chickens greater than three days of age and can colonize the tract sub-clinically for 8–9 weeks after experimental infection. However, it is possible to use older birds and have interesting results. It all depends on the nature of the study, the virulence of the strain, the concentration and the infective dose. For example, Pande *et al.* (2016) used with success henses of 14 weeks for oral induction with *S. Typhimurium* PT 9. The virulence of *Salmonella Typhimurium* ATCC 14028 was a guarantee because these virulence factors have been characterized by PCR by Deguenon *et al.*[[27]. This study showed that the strain has 5 virulence genes: invA, spvR, SpvC, FimA and Stn. Spv genes are responsible for the systemic infection and multidrug resistance in humans and animals [28]. SpvC gene is able to inhibit the activation of macrophages [29]. Presence of fimA gene indicates the presence of fimbriae which is important for *Salmonella spp* to adhere to epithelial cells [27]. Stn gene is suspected to contribute to enterotoxigenic potency [30]. The presence of all these genes therefore guarantees the pathogenicity of *Salmonella Typhimurium* and its ability to infect chicks.

This virulence explains why salmonellosis symptoms were observed in infected animals. Three infective concentrations were chosen because we had no assurance of sufficient bacterial load to induce salmonellosis in chicks of this age. We had to expand the possibilities. The results showed that only concentrations 2 and 3 could trigger the symptoms of salmonellosis. These symptoms were present until the 10th day of observation in the group 3 chicks while they disappeared between the fifth and the 7th day for the group 2. *Salmonella spp* were investigated in faecal specimens to support clinical observations. *Salmonella spp* were detected in chicks of group 2 and 3, three days after infection. Chickens infected with concentration 3 of inoculum still host *Salmonella.*

By relating microbiological data to clinical observations, it seems obvious that only the infective 3 concentration was able to keep the 3-day-old chicks sicked for 10 days. This observation was reinforced by the count at day 9. The disappearance of *Salmonella* in group 2 could be explained by a positive reaction of the immune system of the birds.

In vivo anti-*Salmonella* activity of *Uvaria chamae* using chick model

Leaves aqueous extract of *U. chamae* inhibit *Salmonella Typhimurium* ATCC in chicks at 100, 200 and 400 mg/L but the bacterial load was not canceled. This confirms the *in vitro* anti-*Salmonella* tests results which showed that this extract has a bacteriostatic effect on *Salmonella Typhimurium* ATCC 14028. On the other hand, at 200 and 400 mg / L, the extract showed a better *in vivo* activity than Colistin on virulent *Salmonella spp* P19. There is no scientific data on the *in vivo* activity of extracts of *Uvaria chamae* on *Salmonella spp* using chicks model. But interesting data exist about other natural substances. In a last study, Five-months-old local chickens, free of antibodies against fowl typhoid were used for challenge with *Salmonella Gallinarum.* Administration of extract of *Aloe secundiflora* showedincrease in the levels of interleukin 6 (IL−6) [31]. In a recent study, *Piper beetle* L. leaves extract when used in supplementation in drinking water helped to decrease the colonies of *Salmonella sp.* in small intestine of quails [32]. Mice model were also used to assess *in vivo* anti-

Salmonella activity of natural substances. *Punica granatum* extract had significant effects on mortality and the numbers of viable S. Typhimuriumrecovered from feces after experimental infection [33].

Conclusions

This study demonstrated the *in vitro* inhibitory potency of aqueous and ethanolic extracts of *Uvaria chamae, Phyllantus amarus and Lantana camara* on *Salmonella spp.* Leaves aqueous extract of *U. chamae* inhibit *Salmonella Typhimurium* ATCC 14028 and *Salmonella spp* in chicks at 200 and 400 mg/L. The extract showed no toxicity at the concentrations tested. This extract could be enhanced by the development of an improved traditional medicine for the management of non-typhoid salmonellosis.

Methods

Leaves of *Lantana camara* (Verbenaceae), Leaves and roots of *Uvaria chamae* (Annonaceae), Leaves of *Phyllanthus amarus* Schumach.&.Thonn were collected from the wild. In Benin, no permission was necessary to collect these samples.

Identification was done at the Beninese National Herbarium (University of Abomey Calavi) by Professor Hounnankpon YEDOMONHAN (https://chercheurs.inrab.org/details/173).

Reference numbers in the Herbarium are AA6686/HNB for *Phyllantus amarus,* AA6687/HNB for *Uvaria chamae,* AA6688/HNB for *Lantana camara.* This experimental research has been done in compliance with our Institution (University of Abomey-Calavi), national and international guidelines. All the plants collected in this study have been replaced by young ones in order to maintain the species survival.

210 three-day old and 90 three-week old *Isa Brown* male chicks were used for the experimentation. The birds were taken from a commercial hatchery "Terre et Associés", Abomey-Calavi (Benin). The birds were kept in an enclosure carefully cleaned and disinfected. During the experiment, the animals took water and feed. All experiments were conducted according to the protocol approved by Ethical committee of Research Unit of Applied Microbiology and Pharmacology of natural substances. After the procedure, animals were killed in compliance with the Beninese code for the care and use of animals for scientific purposes. All animal restraint for killing was ethically carried out carefully to avoid fear, distress or pain. In order to limit fear, distress or pain related to restraint, ketamine was administered to animals.

Eleven bacterial strains were used:

- Salmonella Typhimurium ATCC 14028 were acquired from Research Unit in Applied Microbiology and Pharmacology of natural substances, University of Abomey-Calavi, Benin.
- Ten multiresistant strains of Salmonella spp: they were isolated by Deguenon et al.[27]. The strains were multidrug-resistant to penicillins, first generation cephalosporins and some aminoglycosides.

Production of aqueous and ethanolic extracts

Method described by Legba et al. (2018) has been used. The organs were dried in the laboratory at a temperature of 16 ° C for. The dried material was then powdered using a Retsch SM 2000/1430 / Upm / Smf type mill. Fifty grams of powder were macerated in 500 ml of distilled water or ethanol on a Stuart Bioblock Scientific Fisher stirrer for 72 hours at room temperature. Homogenate was filtered with hydrophilic cotton and Wattman No. 1 paper. This filtrate was then dried at 40 ° C for ethanolic extract and 50°C for aqueous extract in the Pasteur oven.

In vitro anti-*salmonella* assessment of aqueous and ethanolic extract of *Uvaria chamae, Phyllantus amarus* and *Lantana camara*

Salmonella Typhimurium 14028 and the ten multiresistant *Salmonella spp* were used. A 24-hour pure colony of portion from the Mueller Hinton medium of each strain was emulsified in 5 ml of physiological water to give a turbidity of 0.5 Mc Farland [34].

Each inoculum was seeded by swab on Petri dishes containing Mueller Hinton agar. Wells of 6 mm diameter were hollowed out using sterile Pasteur pipette tip. 50 µl of each extract was deposited in the wells. A well containing sterile distilled water served as a negative control. After 1 hour pre-diffusion at room temperature, the petri dish was incubated at 37 ° C in an oven for 24 hours. After incubation period, the dishes were examined for the measurements of the zones of inhibition. [34].

Method used by Legba et al. (2018) were used. 100 µl of the stock solution of each extract prepared at 200 mg / ml were added to 100 µl of Mueller-Hinton Broth. A series of two-fold dilutionfrom well to well was made then 100 µl of different bacterial suspensions were added. Positive and negative controls were prepared respectively by adding 100 µl of MH broth to 100 µl of bacterial suspension and 100 µl of MH broth to 100 µl of the extracts. The plates were incubated at 37°C for 24 hours. The MIC was estimated using Tetrazolium. The content of each well was cultured on Agar MH Agar and incubated at 37 ° C for 24 hours for the determination of CMB. CMB is the lowest concentration of extract to which no colony of bacteria can be observed. The antibiotic potency (a.p) of each extract was then calculated with the formula CMB/CMI.

Experimental infection of three-day-old *Isa Brown* male chicks with *Salmonella* Typhimurium ATCC 14028

A preliminary microbiological examination of the cloaca of the chicks helped to check if chicks are exempt from *salmonella.* A cloaca swab was performed on all chicks and *salmonella spp* were searched according to the method described by Deguenon et al.[27].

Salmonella inoculums were prepared from a pure isolate of the bacterial strain in distilled water at three selected concentrations: 3.010^8 CFU (Concentration 1), 6.010^8 CFU (Concentration 2), $9.0\ 10^8$ CFU (Concentration 3).

- Groups 1 (n = 3): 2 ml of Inoculum concentration 1
- Groups 2 (n = 3): 2 ml of Inoculum concentration 2
- Groups 3 (n = 3): 2 ml of Inoculum concentration 3
- Groups 4 (n = 3): 2 ml of Distilled water

Oral inoculation was performed using 20-gauge feeding needle and disposal syringe. To confirm the viability of the strain used for inoculation, a sample of each inoculum (about 100 microliters) was taken before and after oral administration for culture at 37 ° C for 24–45 hours.

The birds were observed for 10 days and the symptoms of salmonellosis were recorded. On days 3, 6, and 9, the feces from each lots of chicks were collected. Salmonella was sought using method described par Deguenon *et al.[*[27]. Five (5) grams of fecal samples were transferred immediately following collection in 45 ml of buffered peptone water. Samples were homogenized in the broth either by vortex mixer and then incubated for 18–24 h at 37 °C. 1 ml of pre-enrichment was then inoculated in 9 ml of selenite cystine broth for 24 h. Isolation was done on petri-dishes containing Xylose Lysine Decarboxylase (XLD) and incubated for 24h. API 20E Gallery was used for positive identification of all suspicious colonies from fecal material. *Salmonella* counts were performed on Day 9 samples to assess bacterial load. The method described by Pande *et al.[*[23].

In vivo anti-*salmonella* assessment of Leaves aqueous extract of *Uvaria chamae*

Salmonella Typhimurium 14028 and *Salmonella spp* (P19) were used for this step. After inoculation, chicks were treated with leaves aqueous extract of *Uvaria chamae* and colistin.

For each strain, 90 three-day-old chicks were randomly assorted into six groups:

- Group 1: non-infected and non-treated (G1, n = 18)
- Group 2: infected and untreated (G2, n = 18)
- Group 3: infected and treated with 200 mg/l of Colistin (G3, n = 18)
- Group 4: infected and treated with 100 mg/l of Uvaria chamae leaves aqueous extract (G4, n = 18)
- Group 5: infected and treated with 200 mg/l of Uvaria chamae leaves aqueous extract (G5, n = 18)
- Group 6: infected and treated with 400 mg/l of Uvaria chamae leaves aqueous extract (G6, n = 18).

Preliminary examination and inoculation were performed as previously but a single concentration of inoculum were used: 9.0 10^8 UFC/ml. The birds were observed for 9 days and the symptoms of salmonellosis were recorded. From the third day after infection, the chicks are subjected to oral treatment with the aqueous leaf extract of *Uvaria chamae* and colistin as reference antibiotic. The treatment was done for 7 days. On days 3, 6 and 9 after infection, faeces from each group were collected and *Salmonella* counts were performed [35].

Effects of Leaves aqueous extract of *Uvaria chamae* on biochemical and hematological parameters

In order to evaluate the toxicity of *Uvaria chamae* leaves aqueous extracts for chicks, the different concentrations of extracts tested and Colistin were administered for 7 days to three-week-old chicks.

90 three-week-old Isa Brown male chicks were divided in five groups:

- Group 1: oral administration of 100 mg/L of Leaves aqueous extract of U. chamae (G1, n = 18)
- Group 2: oral administration of 200 mg/L of Leaves aqueous extract of U.chamae (G2, n = 18)
- Group 3: oral administration of 400 mg/L of Leaves aqueous extract of U.chamae (G3, n = 18)
- Group 4: oral administration of 200 mg/L of Leaves aqueous extract of U.chamae (G4, n = 18) of Colistin (G4, n = 18)
- Group 5: water

The hematological (white cell Number (NB); Blood red Cell (NR), Hemoglobin (Hb), Hematocrit (The)) and biochemical (Uremia, creatinine, AST and ALT) data were recorded on Days 0, 4 and 7.

Statistical analysis

Microbiological data were analyzed using ANOVA test with Graph Pad Prism 7.0 Software. Hematological and Biochemical data were analyzed by the SPSS 17.0 Software.

Abbreviations

ALT: alanine aminotransferase

ANOVA: Analysis of Variance

a.p: antibiotic potency

API: Analytical Profile Index

AST: aspartate transaminase

ATCC: American Type Culture Collection

CFU: colony forming unit,

G: Group

HNB: National Herbarium of Benin

MIC: Minimum Inhibitory Concentration

MBC: Minimum Bactericidal Concentration

MH: mueller hinton

PCR: Polymerase chain reaction

P9, P70, P16, P14, P15, P19, P362, P368, P17, P29: *Salmonella* samples identification code

SPF: Specific-Pathogen-Free

SPSS: Statistical Package for the Social Sciences

ST: *Salmonella* Typhimurium

TWAS: The World Academy of Sciences

UNESCO: United Nations Educational, Scientific and Cultural Organization

XLD: Xylose Lysine Decarboxylase

Declarations

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Authors' contributions:

BBL, VD, CG, AA, ED, LB-M and JD wrote the protocol.

VD got the funding.

BBL, VD, CG, AA, and ED processed laboratory works.

VD did the statistical analyses.

VD, BBL wrote the draft of the manuscript.

VD, JD and LB-M reviewed the manuscript.

All authors have read and approved the manuscript.

Authors' Information

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Availability of data and material

All data generated or analysed during this study is included in this published article and supplementary information files.

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Consent for publication

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Competiting interests

Authors declare no competing interest

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Tables

Table 1 : MIC (mg/ml), MBC (mg/ml) and a. p. of the aqueous and Ethanolic extracts of the plants on Salmonella spp.

Extracts	Parameters	*P9*	*P70*	*P16*	*P14*	*P15*	*P19*	*P362*	*P368*	*P17*	*P29*	*ST*
U.Chamae Leaves aqueous extract	MIC	12.5	25	6.25	6.25	6.25	12.5	-	12.5	3.25	12.5	6.25
	MBC	100	>100	>100	>100	>100	100	-	50	100	>100	100
	a.p.	8	-	-	-	-	8	-	4	32	-	8
U.Chamae Leaves ethanolic extract	MIC	3.125	-	-	-	-	3.125	-	-	1.5265	-	-
	MBC	>100	-	-	-	-	>100	-	-	100	-	-
	a.p.	-	-	-	-	-	-	-	-	65.51	-	-
U.Chamae Roots aqueous extract	MIC	-	-	50	-	6.25	-	12.5	-	-	25	-
	MBC	-	-	>100	-	>100	-	>100	-	-	>100	-
	a.p.	-	-	-	-	-	-	-	-	-	-	-
U.Chamae Roots ethanolic extract	MIB	-	-	-	-	-	-	-	-	-	-	-
	MBC	-	-	-	-	-	-	-	-	-	-	-
	a.p.	-	-	-	-	-	-	-	-	-	-	-
P. amarus Leaves aqueous extract	MIC	-	-	50	-	-	-	100	-	-	50	-
	MBC	-	-	>100	-	-	-	>100	-	-	>100	-
	a.p.	-	-	-	-	-	-	-	-	-	-	-
P.amarus Leaves ethanolic extract	MIC	6.25	-	-	25	-	-	-	-	-	12.5	12.5
	MBC	>100	-	-	>100	-	-	-	-	-	>100	>100
	a.p.	-	-	-	-	-	-	-	-	-	-	-
L.camara Leaves aqueous extract	MIB	-	-	-	-	-	-	-	-	-	-	-
	MBC	-	-	-	-	-	-	-	-	-	-	-
	a.p.	-	-	-	-	-	-	-	-	-	-	-
L.camara Leaves ethanolic extract	MIB		6.25			3.125					12.5	12.5
	MBC		>100			>100					>100	>100
	a.p.	-	-	-	-	-	-	-	-	-	-	-

ST= *Salmonella* Typhimurium ATCC 14028

Table 2 : Salmonellosis Symptoms in three-day-old chicks inoculated with S*almonella Typhimurim* ATCC 14028(n = 3 for each concentration of inoculum)

Groups	Salmonellosis symptoms	Days Post infection									
		1	2	3	4	5	6	7	8	9	10
1 (*3.0 10^8 UFC/ml*)	*Wet cloacal diarrheal stool*	-	-	-	-	-	-	-	-	-	-
		+	-	-	-	-	-	-	-	-	-
	Somnolence	-	-	-	-	-	-	-	-	-	-
2 (*6.0 10^8 UFC/ml*)	*Wet cloacal*	+	+	+	+	+	-	-	-	-	-
	diarrheal stool	+	+	+	+	+	+	+	-	-	-
	Somnolence	-	+	-	-	-	-	-	-	-	-
3 (*9.0 10^8 UFC/ml*)	*Wet cloacal*	+	+	+	+	+	+	+	+	+	+
	diarrheal stool	+	+	+	+	+	+	+	+	+	+
	Somnolence	-	-	+	+	-	-	-	-	-	-
4 (Distilled Water)	*Wet cloacal*	-	-	-	-	-	-	-	-	-	-
	diarrheal stool	-	-	-	-	-	-	-	-	-	-
	Somnolence	-	-	-	-	-	-	-	-	-	-

Legend: Absence (-), Presence (+)

Table 3 : Salmonella detection in fecal samples of three-day-old chicks inoculated with Salmonella Typhimurim ATCC 14028(n = 3 for each concentration of inoculum)

Groups	Detection of *Salmonella* at Days Post infection			
	0	3	6	9
G1 (*3.0 10^8 UFC/ml*)	-	-	-	-
G2 (*6.0 10^8 UFC/ml*)	-	+	-	-
G3 (*9.0 10^8 UFC/ml*)	-	+	+	+
G4 (Distilled Water)	-	-	-	-

Legend: Absence (-), Presence (+)

Table 4 : Salmonella count in fecal samples of three-day-old chicks inoculated with Salmonella Typhimurim ATCC 14028

Groups	Bacterial load at Day 9 (CFU/g)
G1 (*3.0 10^8 UFC/ml*)	0
G2 (*6.0 10^8 UFC/ml*)	0
G3 (*9.0 10^8 UFC/ml*)	$1.67.\ 10^3$
G4(Distilled Water)	0

Table 5 : Salmonellosis Symptoms in three-day-old chicks inoculated with S*almonella* Typhimurim ATCC 14028 and treated with Leaves aqueous extract of *U.Chamae* and Colistin (n = 18 for each group)

Groups	Salmonellosis symptoms	Days Post infection								
		1	2	3	4	5	6	7	8	9
G1	*Wet cloacal*	-	-	-	-	-	-	-	-	-
(*non-infected and non-treated*)	*diarrheal stool*	-	-	-	-	-	-	-	-	-
	Somnolence	-	-	-	-	-	-	-	-	-
G2	*Wet cloacal*	+	+	+	+	+	+	+	+	+
(infected and untreated)	*diarrheal stool*	+	+	+	+	+	+	+	+	+
	Somnolence	-	+	+	+	+	+	-	-	-
G3	*Wet cloacal*	+	+	+	+	+	-	-	-	-
(*Infected and treated with 200 mg/l of Colistin*)	*diarrheal stool*	+	+	+	+	+	+	-	-	-
	Somnolence	+	+	+	-	-	-	-	-	-
G4	*Wet cloacal*	+	+	+	+	+	+	-	-	-
(infected and treated with 100 mg/l of Uvaria chamae leaves aqueous extract)	*diarrheal stool*	+	+	+	+	+	+	+	+	-
	Somnolence	+	+	+	-	-	-	-	-	-
G5	*Wet cloacal*	+	+	+	+	+	+	+	-	-
(infected and treated with 200 mg/l of Uvaria chamae leaves aqueous extract)	*diarrheal stool*	+	+	+	+	+	+	-	-	-
	Somnolence	+	+	-	-	-	-	-	-	-
G6	*Wet cloacal*	+	+	+	+	+	+	-	-	-
(infected and treated with 400 mg/l of Uvaria chamae leaves aqueous extract)	*diarrheal stool*	+	+	+	+	+	+	+	-	-
	Somnolence	+	+	+	+	-	-	-	-	-

Table 6 : Salmonellosis Symptoms in three-day-old chicks inoculated with P19 S*almonella spp* strain and treated with Leaves aqueous extract of *U.Chamae* and Colistin (n = 18 for each group)

Groups	Salmonellosis symptoms	Days Post infection								
		1	2	3	4	5	6	7	8	9
G1	*Wet cloacal*	-	-	-	-	-	-	-	-	-
(*non-infected and non-treated*)	*diarrheal stool*	-	-	-	-	-	-	-	-	-
	Somnolence	-	-	-	-	-	-	-	-	-
G2	*Wet cloacal*	+	+	+	+	+	+	+	+	+
(infected and untreated)	*diarrheal stool*	+	+	+	+	+	+	+	+	+
	Somnolence	+	+	+	+	-	-	-	-	-
G3	*Wet cloacal*	+	+	+	+	+	+	-	-	-
(*Infected and treated with 200 mg/l of Colistin*)	*diarrheal stool*	+	+	+	+	+	+	-	-	-
	Somnolence	+	+	+	+	-	-	-	-	-
G4	*Wet cloacal*	+	+	+	+	+	+	+	-	-
(infected and treated with 100 mg/l of Uvaria chamae leaves aqueous extract)	*diarrheal stool*	+	+	+	+	+	+	+	+	+
	Somnolence	+	+	+	-	-	-	-	-	-
G5	*Wet cloacal*	+	+	+	+	-	-	-	-	-
(infected and treated with 200 mg/l of Uvaria chamae leaves aqueous extract)	*diarrheal stool*	+	+	+	+	+	+	-	-	-
	Somnolence	+	+	+	-	-	-	-	-	-
G6	*Wet cloacal*	3	-	-	-	-	-	-	-	-
(infected and treated with 400 mg/l of Uvaria chamae leaves aqueous extract)	*diarrheal stool*	+	+	+	+	+	+	-	-	-
	Somnolence	+	+	-	-	-	-	-	-	-

Legend: Absence (-), Presence (+)

Table 7: Evolution of faeces aspect from three-day-old chicks inoculated with Salmonella *Typhimurim ATCC 14028 and treated with Leaves aqueous extract of* U.Chamae *and Colistin*

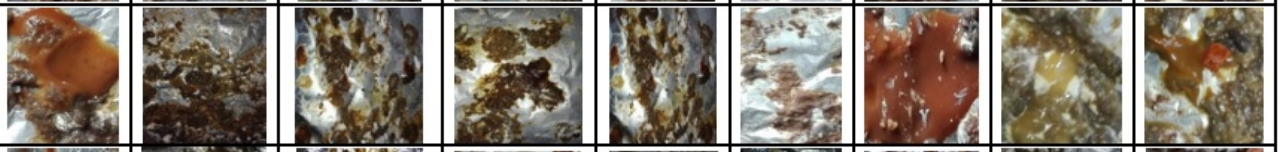
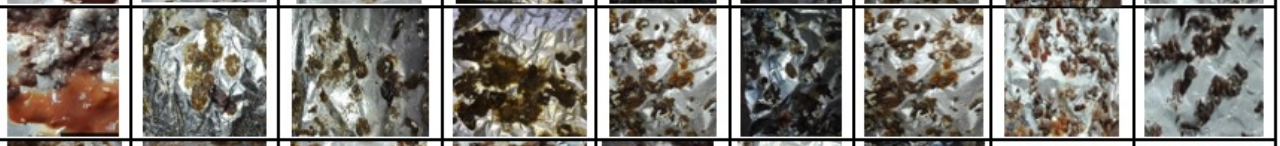
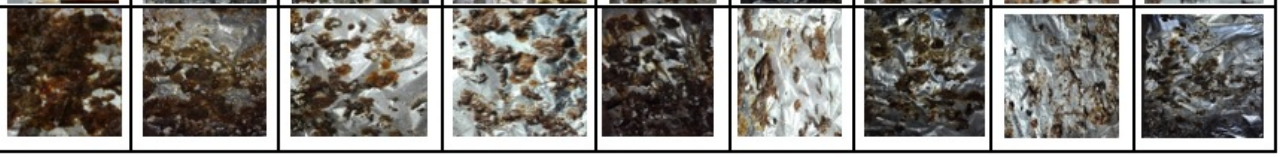
Groups	Days Post infection								
	1	2	3	4	5	6	7	8	9
G1 *(non-infected and untreated)*									
G2 *(infected and untreated)*									
G3 *(infected and treated with 200 mg/l of Colistin)*									
G4 *(infected and treated with 100 mg/l of Uvaria chamae leaves aqueous extract*									
G5 *(infected and treated with 200 mg/l of Uvaria chamae leaves aqueous extract*									
G6 *(infected and treated with 400 mg/l of Uvaria chamae leaves aqueous extract)*									

Table 8: Evolution of faeces aspect from three-day-old chicks inoculated with P19 *Salmonella* strain *and treated with Leaves aqueous extract of* U.Chamae *and Colistin*

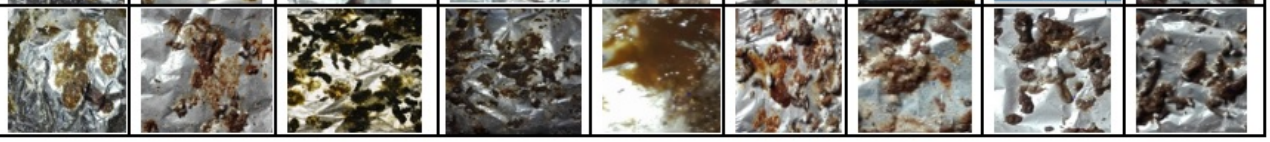
Groups	Days Post infection								
	1	2	3	4	5	6	7	8	9
G1 *(non-infected and untreated)*									
G2 *(infected and untreated)*									
G3 *(infected and treated with 200 mg/l of Colistin)*									
G4 *(infected and treated with 100 mg/l of Uvaria chamae leaves aqueous extract*									
G5 *(infected and treated with 200 mg/l of Uvaria chamae leaves aqueous extract*									
G6 *(infected and treated with 400 mg/l of Uvaria chamae leaves aqueous extract)*									

Table 9: Evolution of cloacal aspect from three-day-old chicks inoculated with Salmonella Typhimurium *and treated with Leaves aqueous extract of* U.Chamae *and Colistin*

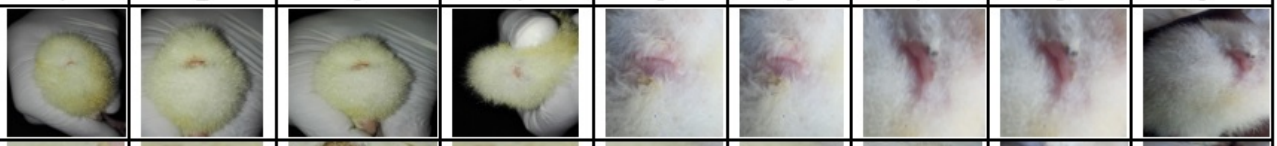
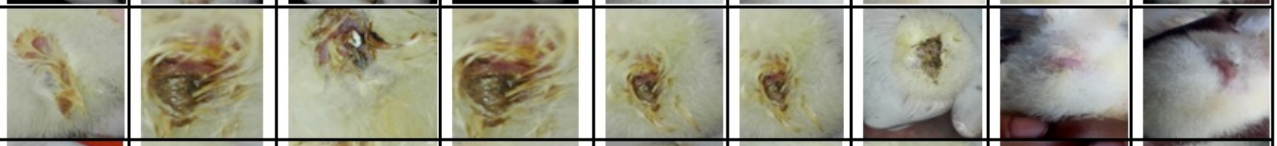
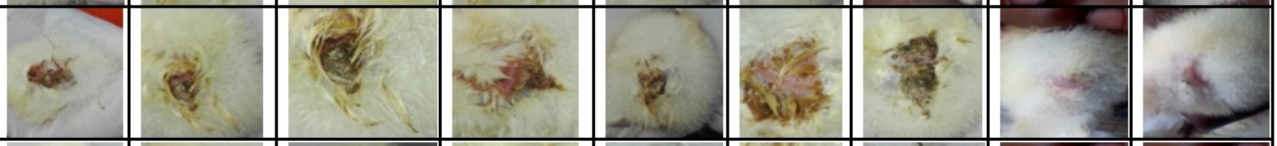
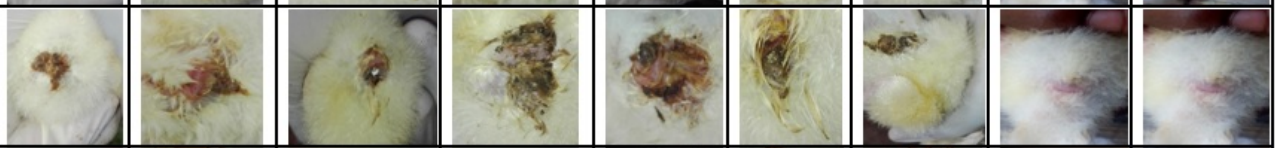
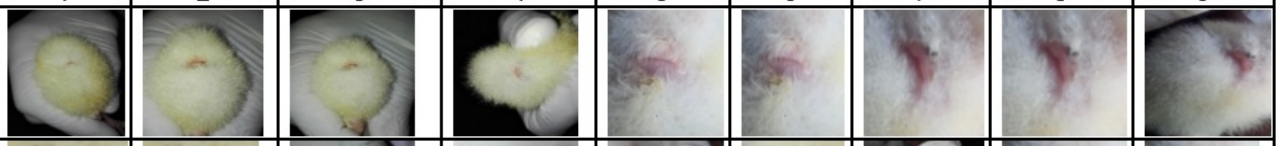
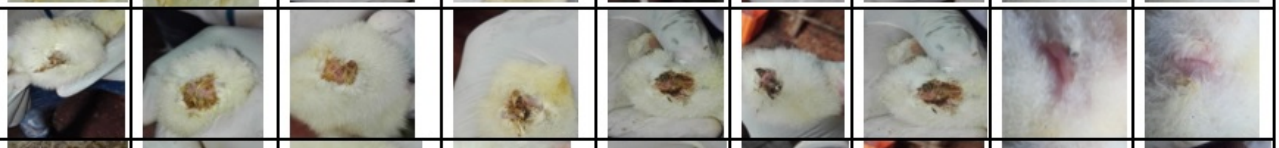
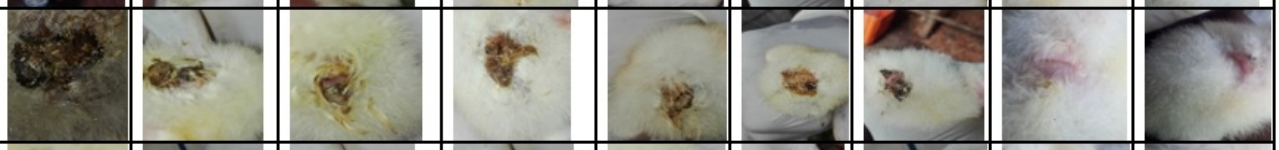
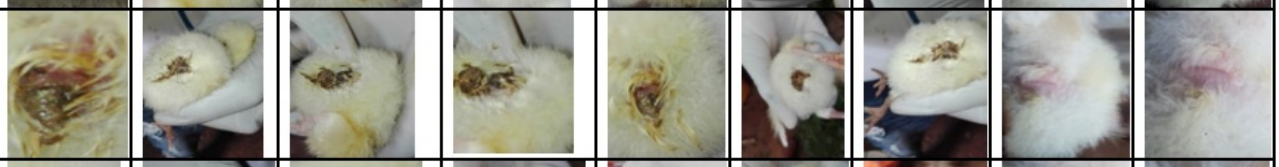
Groups	Days Post infection								
	1	2	3	4	5	6	7	8	9
G1 *(non-infected and untreated)*									
G2 *(infected and untreated)*									
G3 *(infected and treated with 200 mg/l of Colistin)*									
G4 *(infected and treated with 100 mg/l of Uvaria chamae leaves aqueous extract*									
G5 *(infected and treated with 200 mg/l of Uvaria chamae leaves aqueous extract*									
G6 *(infected and treated with 400 mg/l of Uvaria chamae leaves aqueous extract)*									

Table 10: Evolution of cloacal aspect from three-day-old chicks inoculated with P19 Salmonella and treated with Leaves aqueous extract of U Chamae and Colistin

Groups	Days Post infection								
	1	2	3	4	5	6	7	8	9
G1 *(non-infected and untreated)*									
G2 *(infected and untreated)*									
G3 *(infected and treated with 200 mg/l of Colistin)*									
G4 *(infected and treated with 100 mg/l of Uvaria chamae leaves aqueous extract*									
G5 *(infected and treated with 200 mg/l of Uvaria chamae leaves aqueous extract*									
G6 *(infected and treated with 400 mg/l of Uvaria chamae leaves aqueous extract)*									

Figures

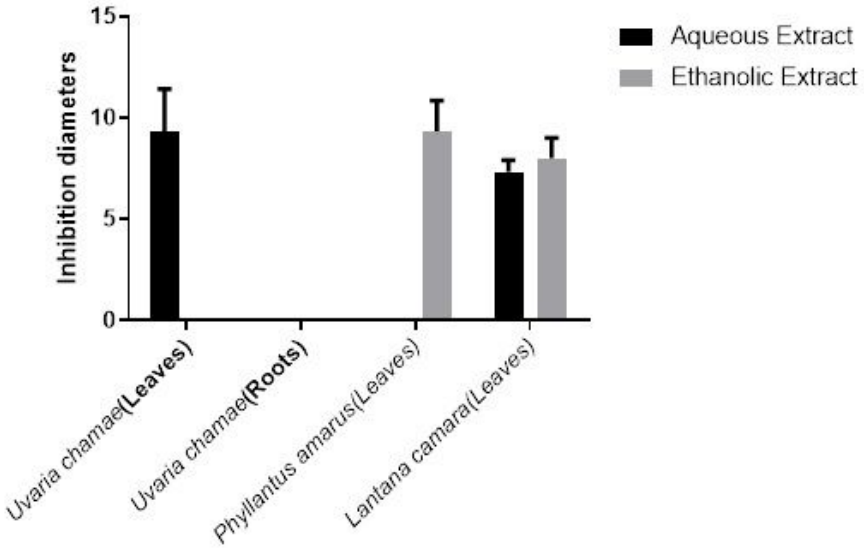


Figure 1

Antibacterial activity of aqueous and ethanolic extracts of Uvaria chamae, Phyllantus amarus and Lantana camara on Salmonella Typhimurium ATCC 14028

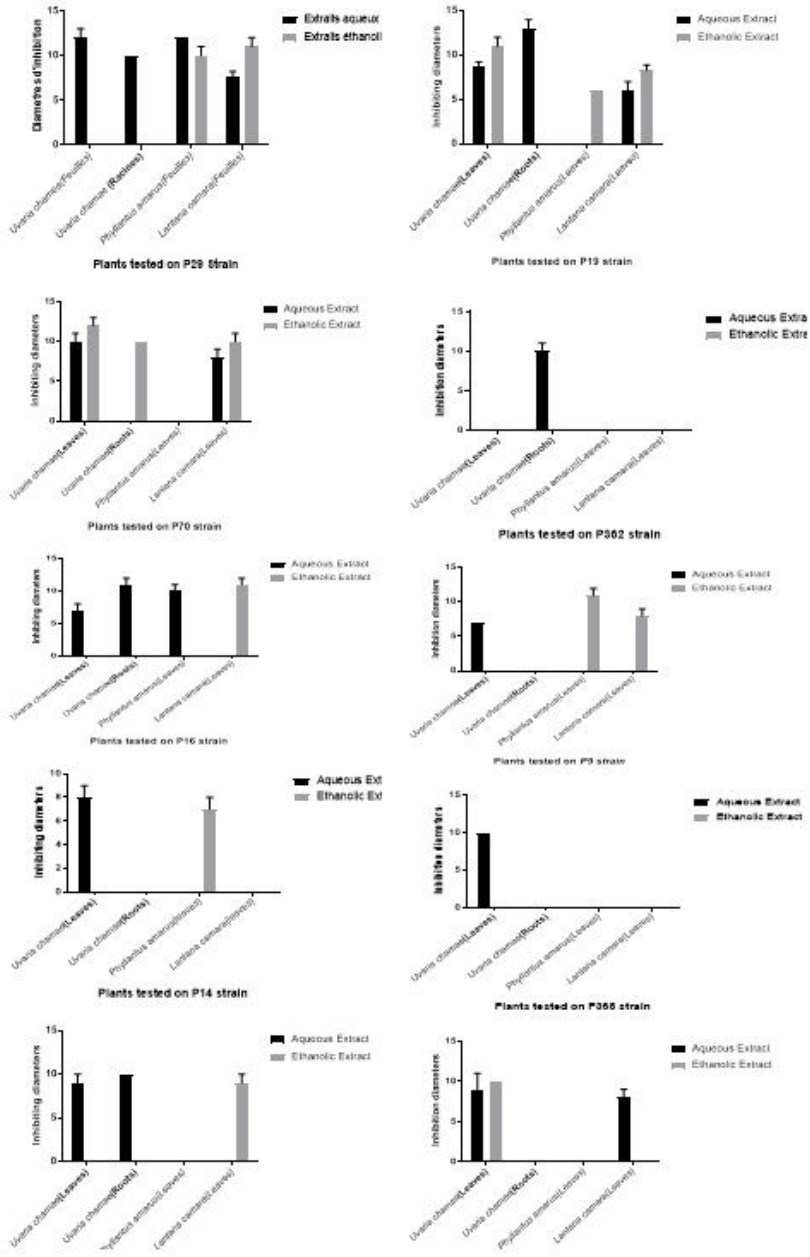


Figure 2

Antibacterial activity of aqueous and ethanolic extracts of Uvaria chamae, Phyllantus amarus and Lantana camara on multiresistant Salmonella spp isolated in Benin

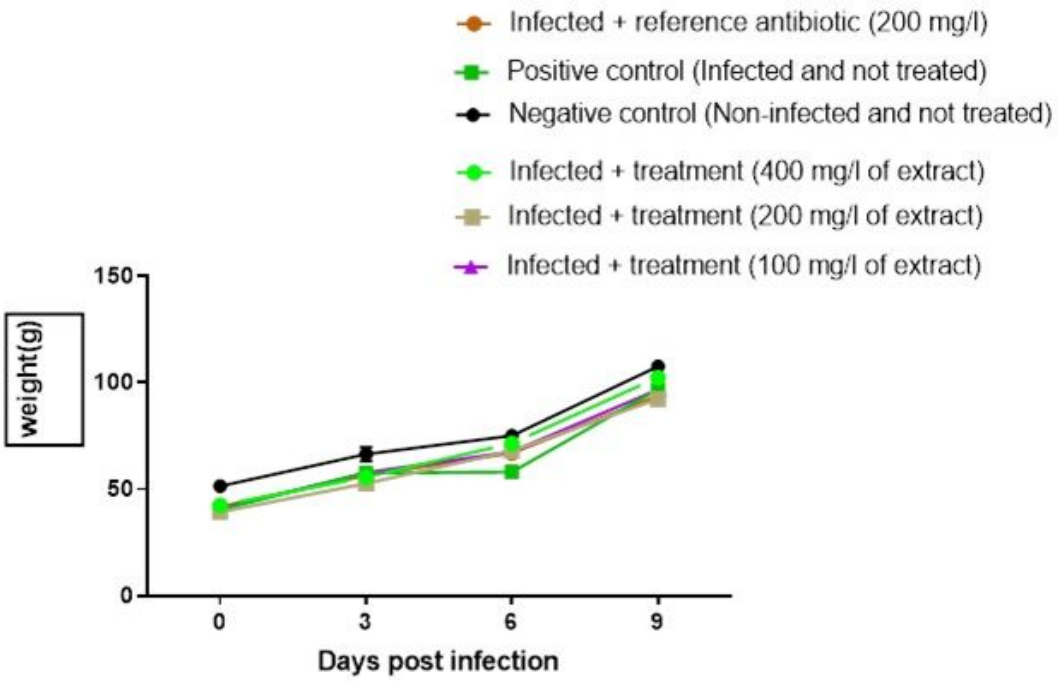


Figure 3

weight changes of chicks orally infected with Salmonella Typhimurium 14028 and treated with leaves aqueous extract of U.chamae and Colistin

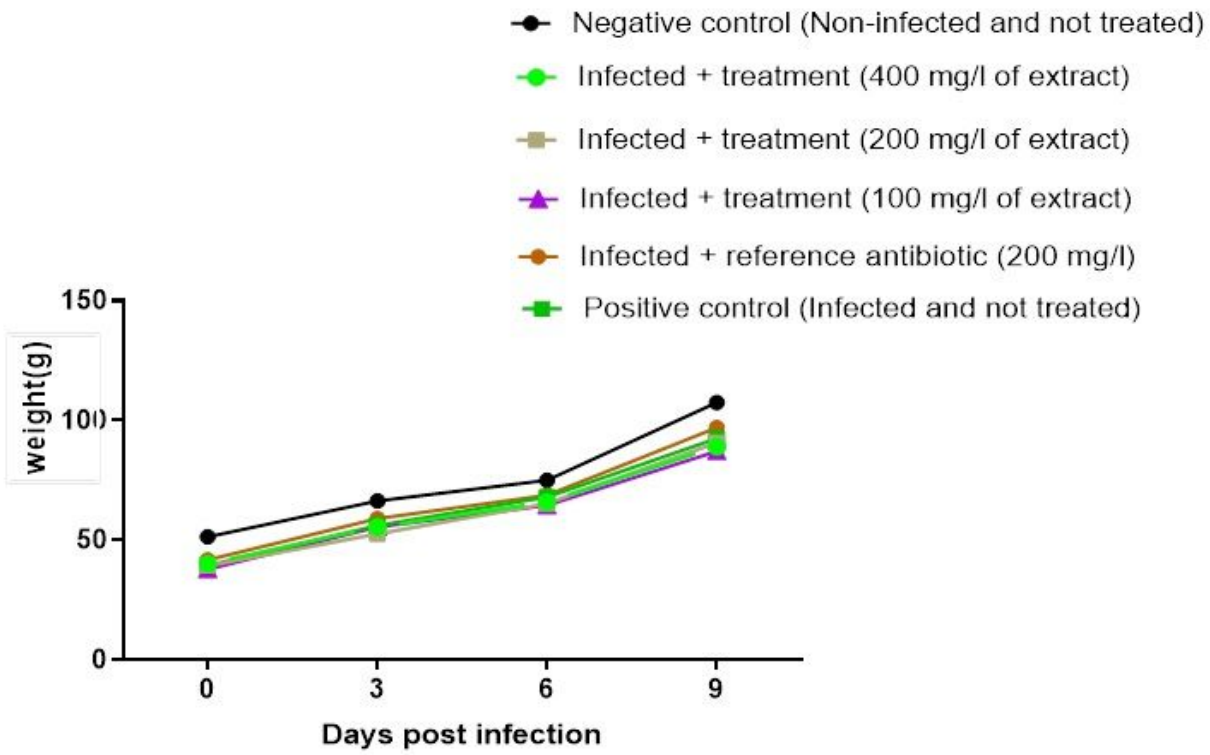


Figure 4

Weight changes of chicks orally infected with Salmonella spp (P19) and treated with leaves aqueous extract of U.chamae and Colistin

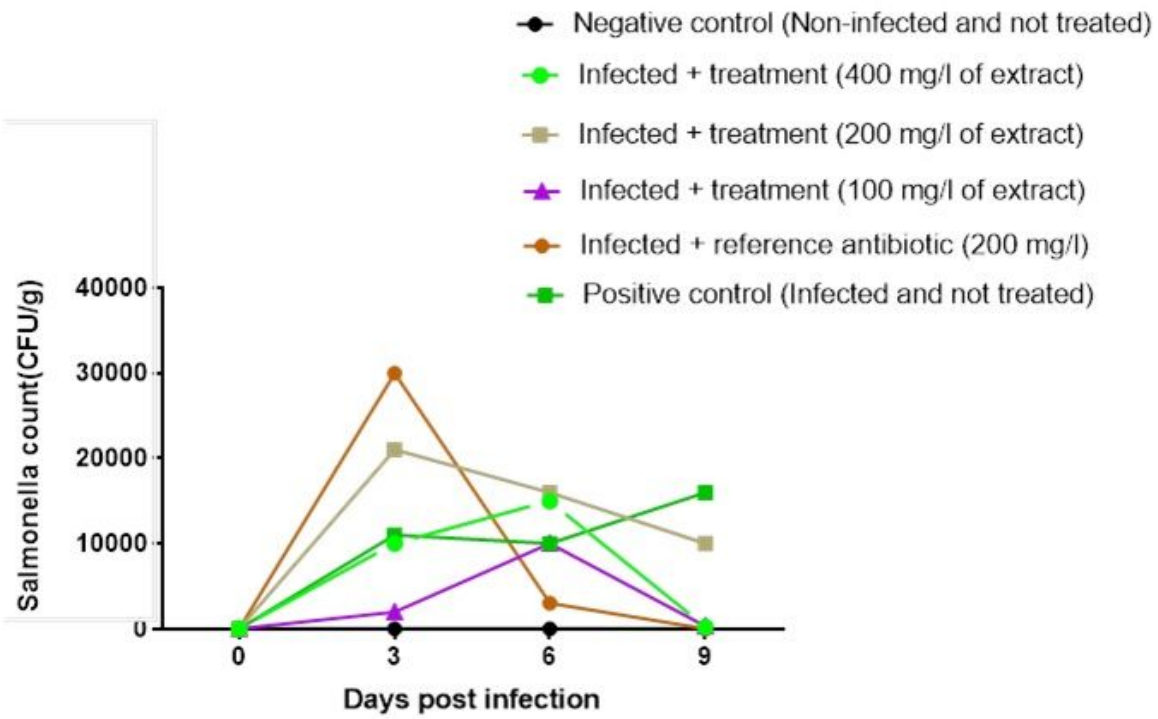


Figure 5

Enumeration of Salmonella from feces of chicks orally infected with S. Typhimurium 14028 and treated with leaves aqueous extract of U.chamae and Colistin

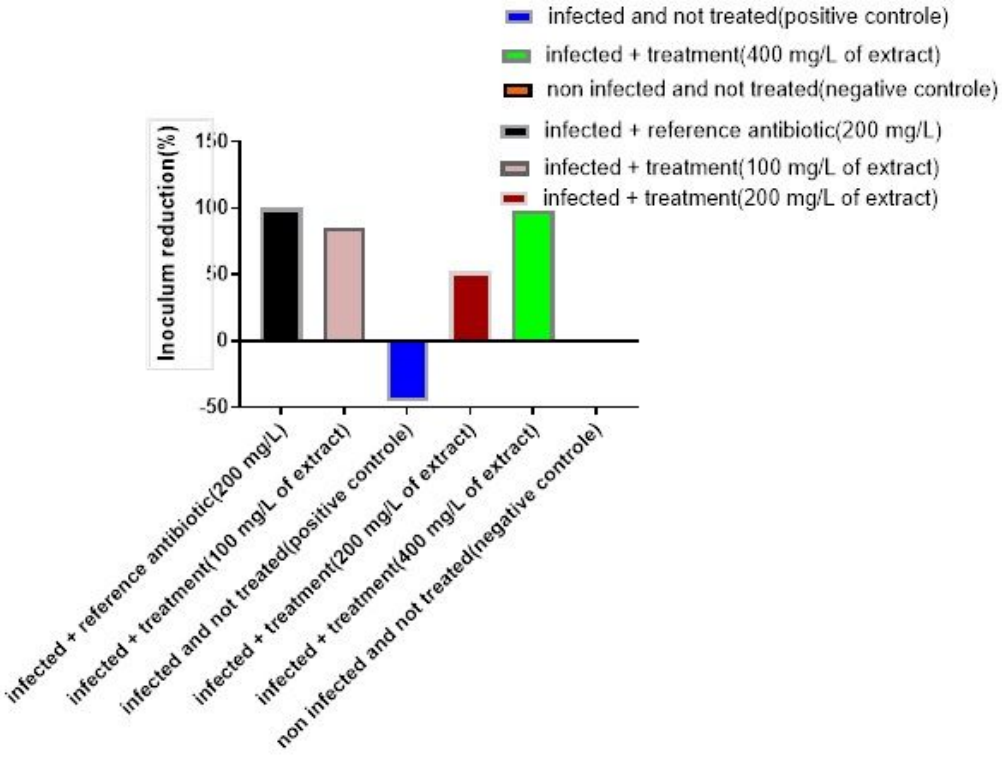


Figure 6

Inoculum reduction of Salmonella Typhimurium 14028 between Day 3 and Day 9(Seven-day treatment)

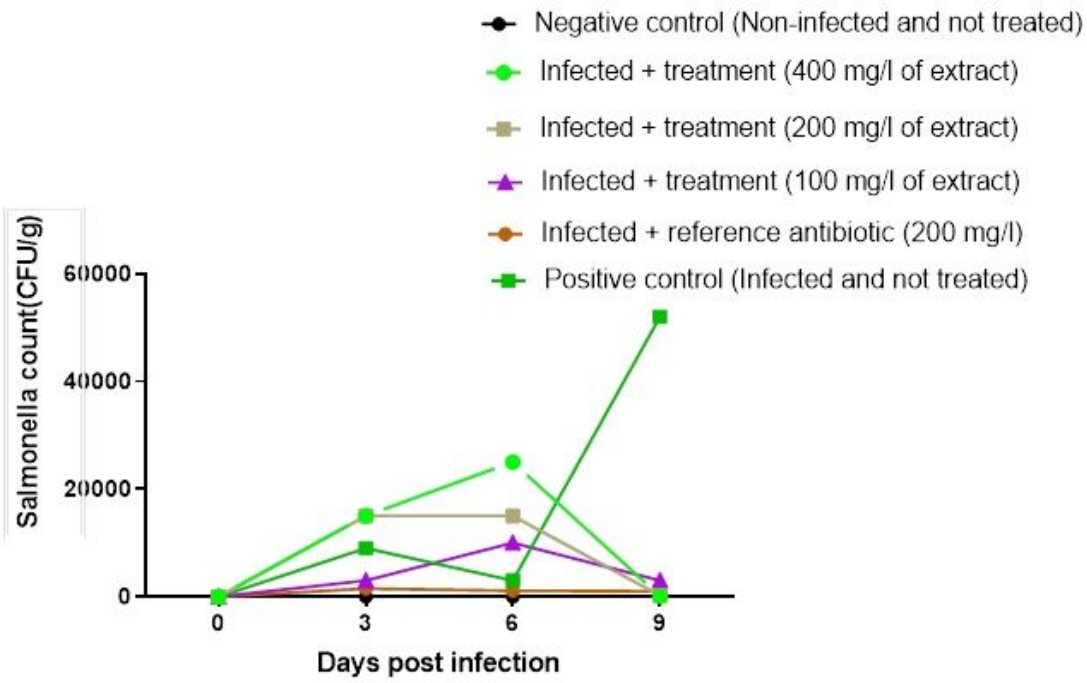


Figure 7

Enumeration of Salmonella from faeces of chicks orally infected with Salmonella spp (P19) and treated with leaves aqueous extract of U.chamae and Colistin

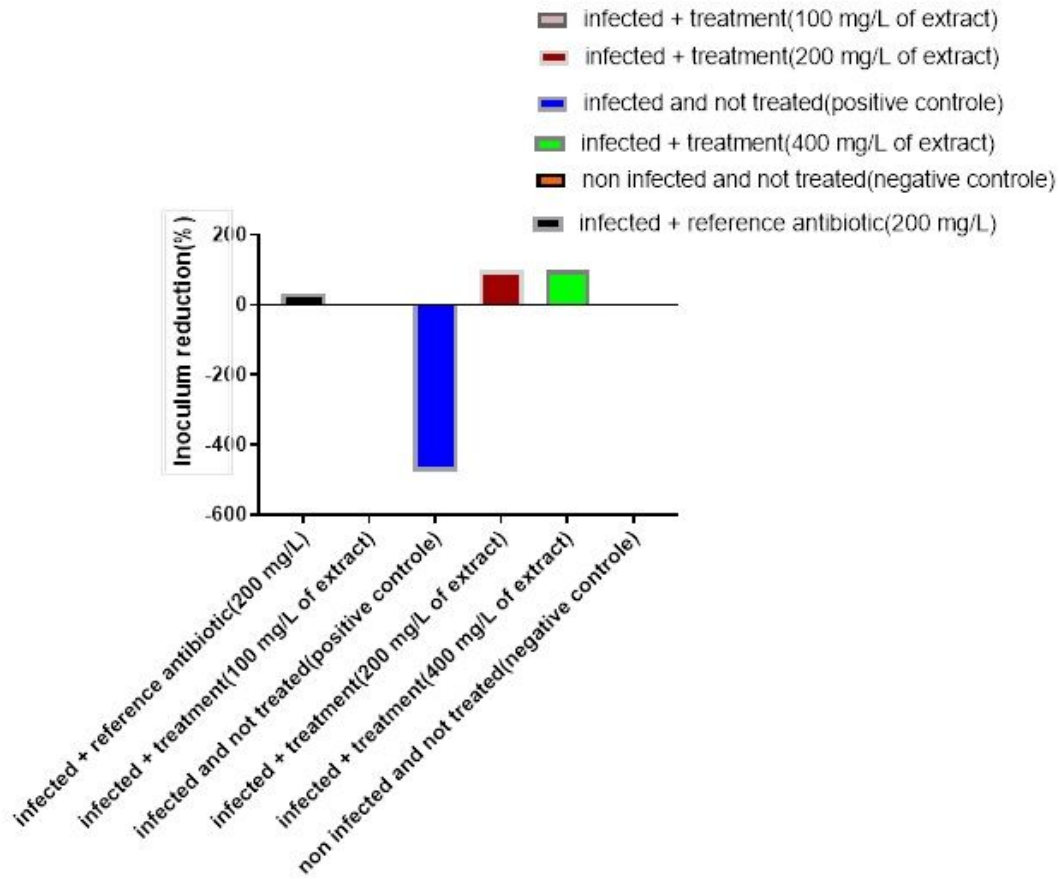


Figure 8

Inoculum reduction of Salmonella spp (P19) between Day 3 and Day 9(Seven-day treatment)

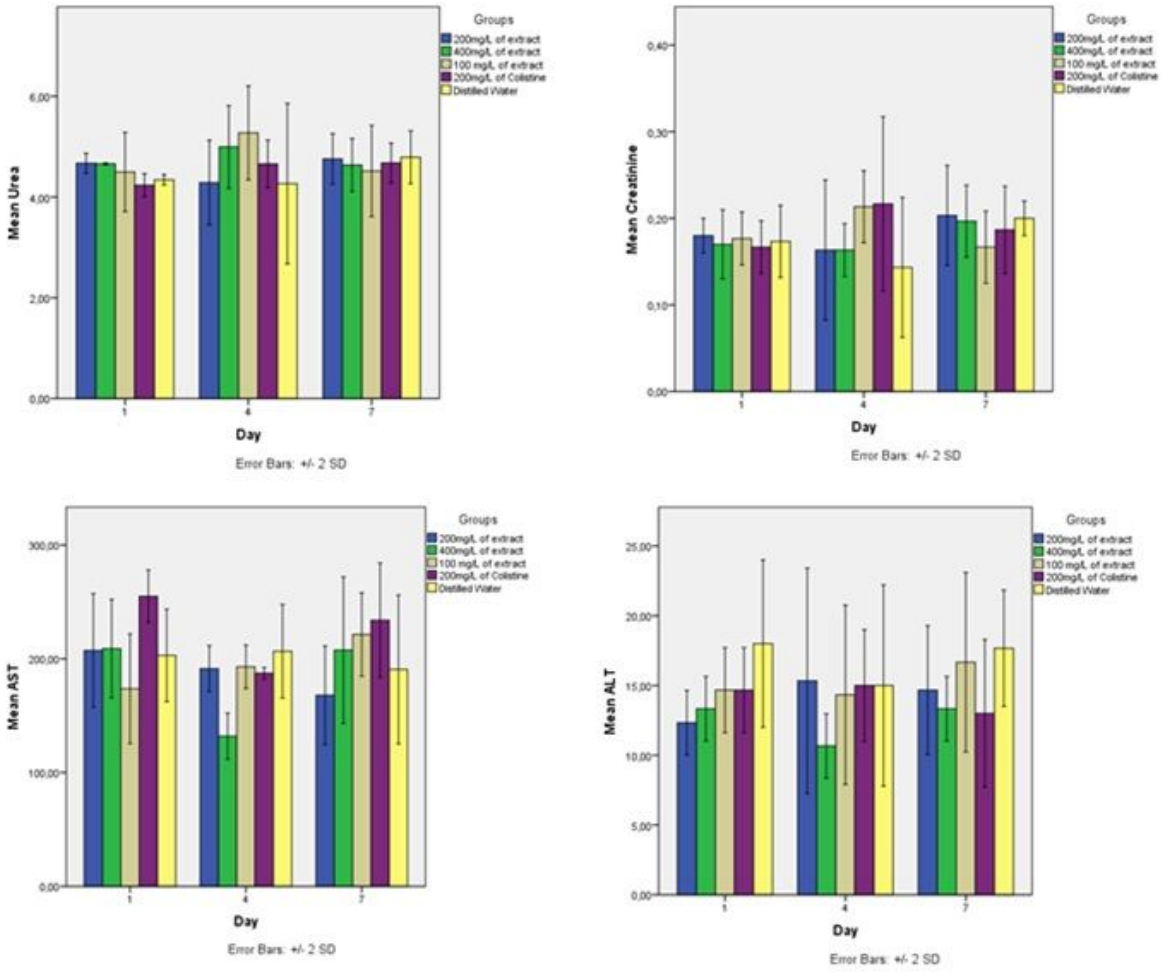


Figure 9

Effect of Leaves aqueous extract of Uvaria chamae on Biochemical parameters of Three-week-old chicks

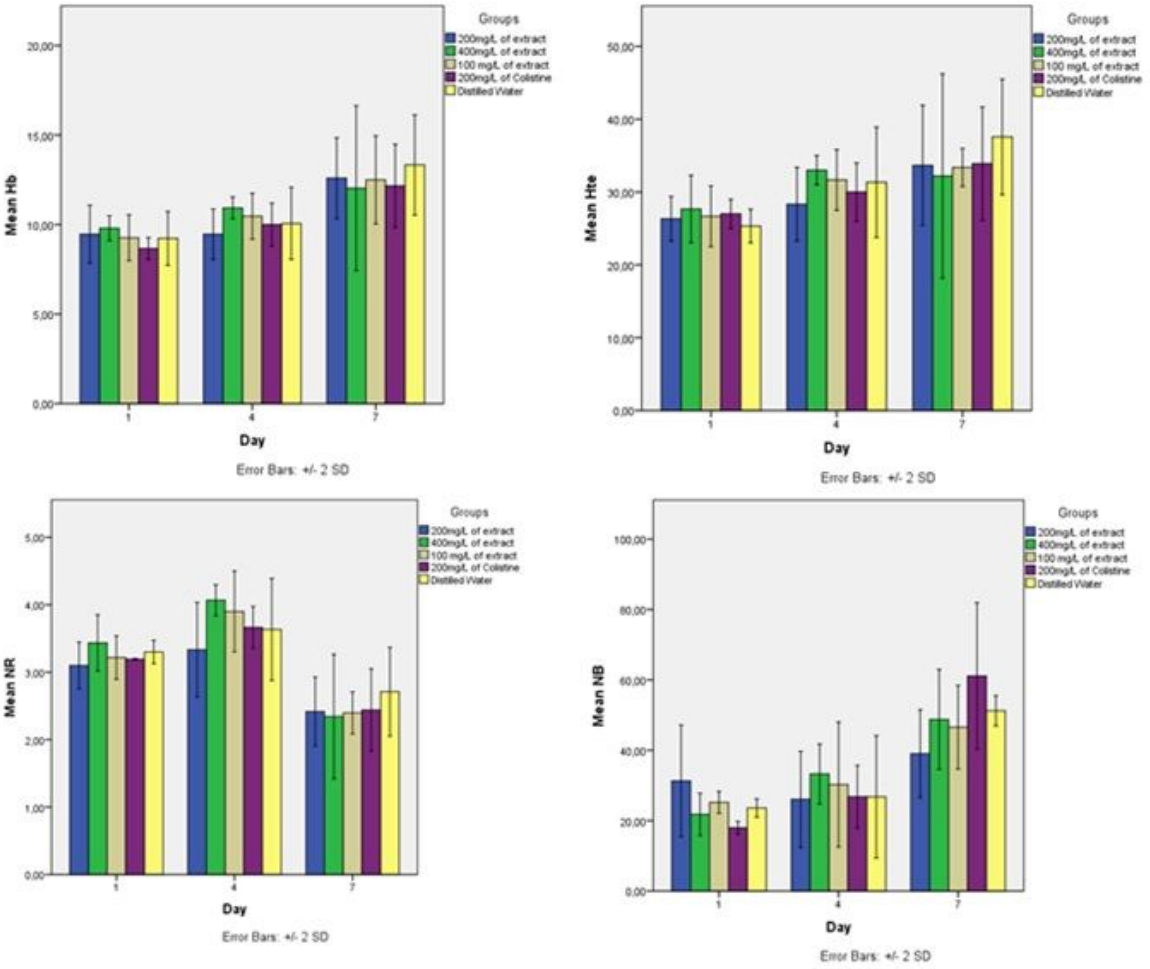


Figure 10

Effect of Leaves aqueous extract of Uvaria chamae on Haematological parameters of Three-week-old chicks

Supplementary Files

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