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PROSPECTS FOR THE USE OF MEDICINAL PLANTS EXTRACTS (*Mallotus oppositifolius* AND *Kalanchoe crenata*) AS ANTIMICROBIALS AGAINST SALMONELLOSIS IN POULTRY

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ABSTRACT: This study is a contribution to the search for alternatives to combat antibiotic resistance in *Salmonella* strains in poultry farming. The objective of this work is to highlight the main phytochemical compounds of 2 Ivorian medicinal plants (*Mallotus oppositifolius* and *Kalanchoe crenata*) and to evaluate their acute oral toxicity with a view to their use in the poultry sector, to fight against certain avian pathologies, including Salmonellosis. The phytochemical compounds of the extracts of the plants used in this study were highlighted by colouring and precipitation methods. Acute oral toxicity was adapted to broilers according to the guideline OECD 425, 2008. The phytochemical screening carried out showed that aqueous extract of *Mallotus oppositifolius* possesses polyphenols and catechical tannins. The ethanolic extract also has anthraquinones, saponosides, sterols, and terpenes. The ethanolic extract of *Kalanchoe crenata* only owns polyphenols and catechical tannins. At the end of the acute oral toxicity study, no mortality was observed in all batches of experimentation and the biochemical analysis of the subject's blood showed creatinine values ≤ 10 mg/L.; aspartate aminotransferase (ASAT) ≤ 275 IU/L; alanine aminotransferase (ALT) ≤ 50 IU/L, urea=0.01g/L, CRP≤6 mg/L; and blood sugar was between 2 and 5 g/L in subjects from different batches. Ultimately, the aqueous and ethanolic extracts of *Mallotus oppositifolius* and the ethanolic extract of *Kalanchoe crenata* can be used as an antibacterial in broiler farming.

Keywords: Antimicrobial supplements, Medicinal plants, Phytochemicals, Poultry, Salmonella.

INTRODUCTION

Infectious diseases represent a significant concern in livestock farming (especially in poultry) because of their frequency of appearance, the economic losses caused and the risk to the health of consumers (Traoré et al., 2012; Chota et al, 2021). The agents responsible for these infections are diverse and varied. They include fungi, viruses, protozoa and bacteria. Among the pathogenic bacteria, those multi-resistant to antibiotics such as bacteria of the *Salmonella* genus are encountered in most poultry farms (Cardinale et al., 2004). Thus, the consumption of poultry, including chicken, could constitute an essential vector in the transmission of multi-resistant *Salmonella* strains to humans (Carole et al, 2022; Yamba, 2023). Indeed, in Côte d'Ivoire, numerous scientific studies have reported the presence of these multi-resistant strains in poultry as well as in chickens (Karou et al., 2013; Bonny et al., 2014) than in native and commercial quails (Bonny et al., 2019). Faced with the problem posed by the spread of microorganisms resistant to antibiotics in general and more particularly by *Salmonella*, it is essential to consider the use of alternative routes to the use of antibiotic molecules, frequently used in the Ivorian poultry sector. Among them, the use of medicinal plants containing bioactive molecules used in the composition of certain pharmaceutical drugs constitutes a promising avenue (Zirintunda et al, 2024).

Indeed, in Africa, for treatment, nearly 80% of patients resort to traditional practitioners (WHO, 2002), holders of an impressive quantity of plant-based recipes with proven active ingredients on certain microorganisms. This study aims to highlight the main antimicrobial compounds of 2 Ivorian medicinal plants and to evaluate their toxicity with a view to their use in the poultry sector, to fight against certain avian pathologies, including Salmonellosis.

MATERIAL AND METHODS

Plant materials

The plants used in this study (Figure 1) were collected from traditional therapists, on the markets selling medicinal plants in the commune of Bingerville (Abidjan, Ivory Coast) (Figure 2). These plants were selected on the basis of their antibacterial activities expressed against *Salmonella* strains of avian origin in the work of Carole et al. (2021). Once

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collected, the leaves of *Kalanchoe crenata* and *Mallotus oppositifolius* plants, known for their antibacterial properties, were dried away from the sun at 21°C for 96 hours and reduced to powder to obtain aqueous and ethanolic extracts.

Production of aqueous and ethanolic extracts of the different plants used

The aqueous and ethanolic extracts of the different plants were obtained according to the protocol described by Bene et al (2015) One hundred grams of powder were macerated in 1 L of distilled water (or in a 70% ethanolic solution) in a blender. The homogenate obtained was filtered successively once through a sieve, then through a cloth and twice through hydrophilic cotton. The filtrate obtained was dried in an oven at 50°C for 48 hours, to obtain the aqueous extract (EA) or the hydroethanolic extract (EE70) (Figure 3). The different extracts obtained are stored at 4°C.

Phytochemical screening of aqueous and ethanolic extracts of plant extracts

This study carried out according to the work of Bagre et al. (2007), allowed the demonstration of the presence of families of secondary metabolites, namely alkaloids, polyphenols, tannins, flavonoids, saponin, polyterpenes or sterols and anthraquinones.

- ✓ Polyphenols: One drop of 2% alcohol solution of ferric chloride was added to 2 mL of extracts.
- ✓ Flavonoids: The extracts are taken up in 5 mL of hydrochloric alcohol (mixture of 10 mL of 96° ethanol, 10 mL of distilled water and 10 mL of concentrated hydrochloric acid). Subsequently, two to three magnesium shavings were added.
- ✓ Sterols and polyterpenes: Liebermann's reagent was used for this demonstration. A mass of 0.1 g of dry extract was dissolved hot in 1 mL of acetic anhydride and collected in a test tube. Then, 0.5 mL of concentrated sulfuric acid (H_2SO_4) was added to it.
- ✓ Alkaloids: A mass of 1 g of dry extract was dissolved in 6 mL of ethanol at 60°. A volume of 2 mL of the alcoholic solution thus obtained was distributed into a test tube. 2 drops of DRAGENDORFF reagent (aqueous solution of potassium iodobismuth) are added to the tube.
- ✓ Catechic tannins: A volume of 1 mL of hydrochloric alcohol (equivolume mixture of alcohol, distilled water and hydrochloric acid) was added to 5 mL of dissolved extract. The mixture was brought to a boil for 15 min.
- ✓ Detection of saponins: A mass of 0.1 g of dry extract was dissolved in 10 mL of distilled water. The resulting solution was stirred vigorously for 45 seconds. After stirring, the solution was allowed to stand for 15 minutes.
- ✓ Detection of anthraquinones: To 3 mL of extract, an equivalent volume of 10% aqueous potassium hydroxide (KOH) is added.

Study of the acute oral toxicity of different extracts on broiler chickens

This experimental study, which is the very first adapted to broiler chickens, was carried out with the agreement of the National Ethics Committee for Life and Health Sciences (CNESVS) of the Pasteur Institute of Côte d'Ivoire (N/ Ref: 037-23/MSHPCMU/CNECVS–km). Acute oral toxicity was evaluated in broilers according to the guideline 425 of the OECD (2008), for testing chemical substances. It consisted of testing plant extracts at a dose of 2000 mg/kg.

The test was carried out on 9 broiler chickens and their behaviour was observed as well as the number of deaths over a period of 3 days. After 18 hours of fasting, the chickens were distributed as follows: a control group consisting of 3 chickens receiving distilled water, at a rate of 3 mL per subject; a batch having received an aqueous extract (AE) consisting of 3 chickens, at a rate of 2000 mg/kg of live weight per subject, a batch having received an ethanolic extract (EE) consisting of 3 chickens, at a rate of 2000 mg/kg live weight per subject. The test substances were administered orally by gavage using a 5 cc syringe, without the needle, in a volume of three millilitres (3 mL). A behavioural observation was carried out 3 hours after administration of the substances. Then, hydration and nutrition were carried out daily for 3 days. During this period, signs of toxicity including motility, tremors, mobility as well as mortalities were noted.

The individual weight of each animal was determined shortly before administration of the test substance and then on the 2nd day after administration of the test substance. Weight change over the three days was also recorded. After the experiment, blood samples collected from the subjects of each experimental group were processed to obtain serum for the determination of biochemical parameters such as transaminases (ALT, AST), urea and creatinine. The dosage was carried out using the Cobas c311 automaton (Hitachi, Japan). Afterwards, all test animals were euthanized and then subjected to macroscopic necropsy. Organs such as the kidneys, liver, heart and lungs were the subject of histological analyses to observe potential cellular damage (Figure 4).

Statistical analysis

At the end of the different tests, all data residuals were analyses by using Microsoft Excel (spreadsheet) and XLSTAT software and significance was considered at $P<0,05$ for analysis of variance. Normality test was applied on all data residuals. Subsequent test used by Tukey's for comparing mean values of the variables (for more than two means). The results obtained were expressed as mean ± standard deviation.

RESULTS

Phytochemical compounds with potential antibacterial activities are alkaloids, anthraquinones, flavonoids, polyphenols, saponosides, sterols, catechin tannins and terpenes. Phytochemical screening of active plant extracts for these compounds showed the presence of at least one compound. Polyphenols and catechic tannins are the compounds most found in aqueous extracts of *Mallotus oppositifolius* and aqueous extracts of *Kalanchoe crenata*. The ethanolic extract of *Mallotus oppositifolius* is the one which contains the most molecules among the extracts analyzed, these are terpenes, sterols, catechic tannins, saponosides, anthraquinones and polyphenols (Table 1).

The study of the acute oral toxicity of the extracts by oral route on broiler chickens revealed no deaths, as well as no clinical signs of toxicity after the administration of the extracts, at a dose of 2000 mg/kg of weight body (pc). All animals survived after 48 hours of observation, which implies that the LD50 is greater than 2000 mg/kg bw. Thus, according to the Globally Harmonized System of Classification and Labelling of chemicals (GHS, 2003), the aqueous and ethanolic extracts of the leaves of *Mallotus oppositifolius* and *Kalanchoe crenata* are non-toxic orally in broilers.

At necropsy, the organs of the test animals showed coloring, appearance, and conditions identical to those observed with control animals. Analysis of histological sections of the organs (heart, kidney, liver and lung) of the test animals revealed no abnormalities related to the proposed treatment (Figure 5).

Table 2 show the variations in serum creatinine in chickens having received different extracts at a concentration of 2000 mg/Kg of body weight and in the control batch during the test period is less than 10 mg/L. The creatinine level observed with the subjects of the ME group is higher than those observed with the other groups. The rate observed with the control batch is the lowest of the different experimental groups. The variations in transaminases (ASAT and ALT) in chickens having received different extracts at a concentration of 2000 mg/Kg body weight and the control batch during the test period vary from 201.2±8.34 IU/L at257.2±8.76 IU/L for aspartate aminotransferase (ASAT) and 7±4.10 IU/L at 9.3±2.82 IU/L for alanine aminotransferase (ALT). In short, the administration of the different active extracts to the test chickens had no effect on their blood biochemical parameters.

Table 1 - Phytochemical screening of plant extracts.

Phytochemical compound	*Mallotus oppositifolius*		*Kalanchoe crenata*
	Aqueous extract	Ethanolic extract	Ethanolic extract
Alkaloids	-	-	-
Anthraquinones	-	+	-
Flavonoids	-	-	-
Polyphenols	+	+	+
Saponosides	-	+	-
Sterols	-	+	-
Catechical tannins	+	+	+
Terpenes	-	+	-

+: presence; - : absence

Table 2 - Biochemical parameters of the different test groups having received 2000 mg/Kg/Pc of extract

Batches	Creatinine (mg/L)	ASAT (IU/L)	ALAT (IU/L)	Urea (g/L)	CRP (mg/L)	Blood sugar (g/L)
Control	0.035±0.03 ^{d}	201.2±8.34 ^{a}	7.6±7.7 ^{b}	0.01±0	0.24±0.02 ^{c}	2.25±0.04 ^{b}
MA	0.045±0.02 ^{d}	215.5±8.06 ^{a}	7.85±0.7 ^{b}	0.01±0	0.215±0.14 ^{c}	2.01±0.08 ^{b}
ME	0.08±0.02 ^{d}	257.2±8.76 ^{a}	9.3±2.82 ^{b}	0.01±0	0.155±0.14 ^{c}	2.15±0 ^{b}
KE	0.05±0.042 ^{d}	205.75±7.99 ^{a}	7±4.10 ^{b}	0.01±0	0.09±0.11 ^{c}	2,225±0.205 ^{b}
Reference values	≤ 10	≤ 275	≤ 50	0.01	≤ 6	Between 2 & 5

$^{a, b, c \text{ and } d}$: Means with different superscripts in the same row were significantly different at P<0.05. MA: batch exposed to the ethanolic extract of *Mallotus oppositifolius*; ME: batch exposed to ethanolic extract of *Mallotus oppositifolius*; KE: batch exposed to the ethanolic extract of *Kalanchoe crenata*: control: batch exposed to no extract.

DISCUSSION

The valorization of natural antimicrobials has been the subject of numerous investigations. In this study, plant extracts were shown to possess antibacterial activity against strains of *Salmonella* multi-resistant (Carole et al., 2021).

Thus, phytochemical screening revealed the presence in plants of the main families of chemical compounds likely to confer antimicrobial properties to a plant. These include terpenes, polyphenols, sterols and anthraquinones. Obviously,

terpenoids are known to have antifungal properties (Merghache et al., 2012). The works of Tené et al. (2009) showed that butelin and 12-oxohardwickic acid, isolated from *Croton macrostachyus* both belonging to this family, had antifungal and antimicrobial activities. Finally, numerous studies have shown that phenols and phenolic derivatives have antimicrobial activities (Tabopda et al., 2008). Phytochemical screening also allows us to observe variations in the chemical composition of different extracts. The data obtained in this study suggest that it would be possible to produce drugs with antibacterial activities, from the aqueous and ethanolic extracts of *Mallotus oppositifolius* and the ethanolic extract of *Kalanchoe crenata*, for livestock such as chickens.

The administration of so-called toxic substances causes undesirable physiological and biochemical disorders, affecting the structural and functional integrity of tissues. The results of the histological analyses obtained in this work show the maintenance of the tissue integrity of the architecture of the organs analysed, with a remarkable absence of central hemorrhagic necrosis on the histological sections produced. In addition, the subjects who received the different extracts appear to have normal organs with intact cellular architecture, less vacuole formation and an absence of necrosis in the tissues, which reveals their harmless action on the tissues.

On the other hand, the results of biochemical analysis of sera do not indicate any abnormal changes in biochemical parameters such as creatinine, urea, transaminases (alanine aminotransferase and aspartate aminotransferase) and total bilirubin. Transaminases or aminotransferases are tissue enzymes catalysing the transport of alpha-amino radicals from alanine and aspartic acid to alpha-ketoglutaric acid. Transaminases are present in the liver, but also in muscle and ASTs in the kidney, pancreas, and other tissues. They are synthesized in the cytoplasm of the cells of these organs and released into the circulation when these cells are damaged (Peirs, 2005). These enzymes increase in cases of myopathy, rhabdomyolysis or myocardial infarction and AST, particularly in cases of haemolysis. Serum urea and creatinine are considered the main markers of nephrotoxicity.

Figure 1 - Plants of *Kalanchoe crenata* (a, b), *Mallotus oppositifolius* (c, d), Plant stems (a, c); plant leaves (b, d).

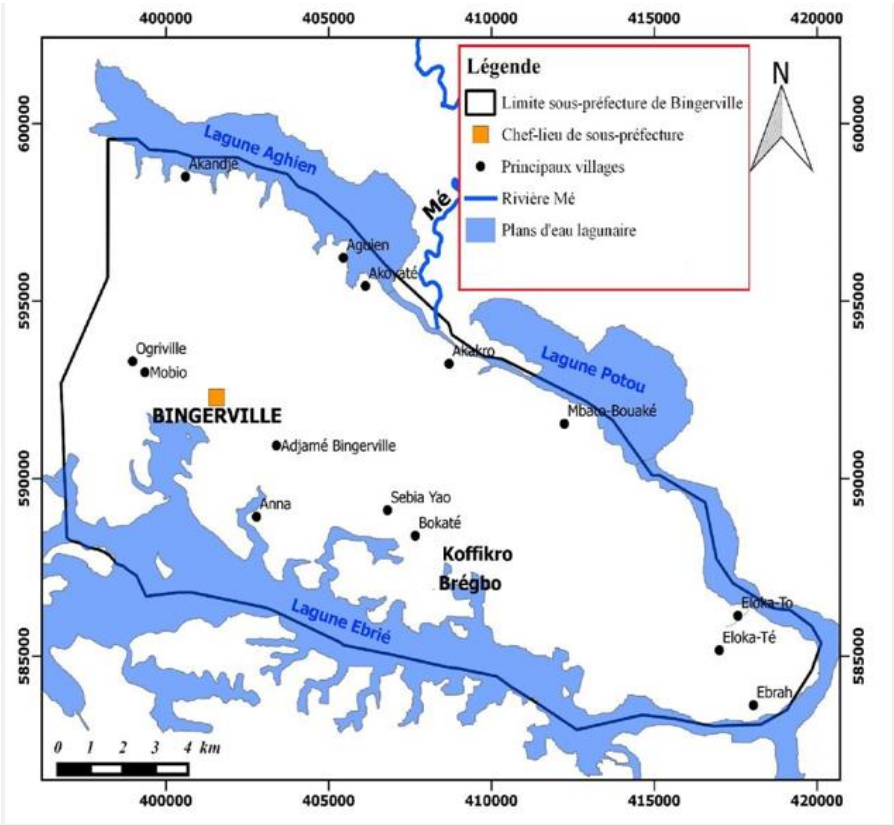
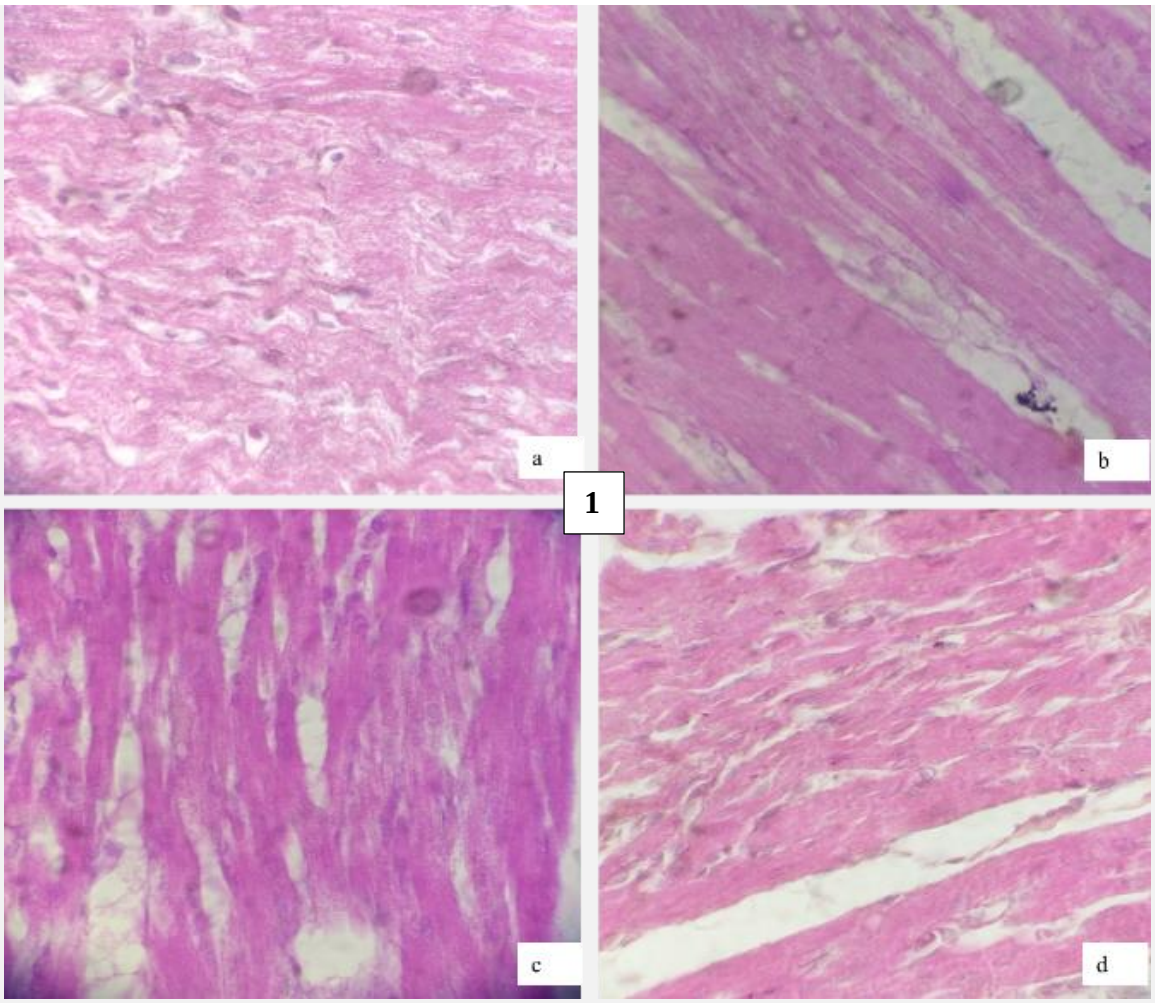
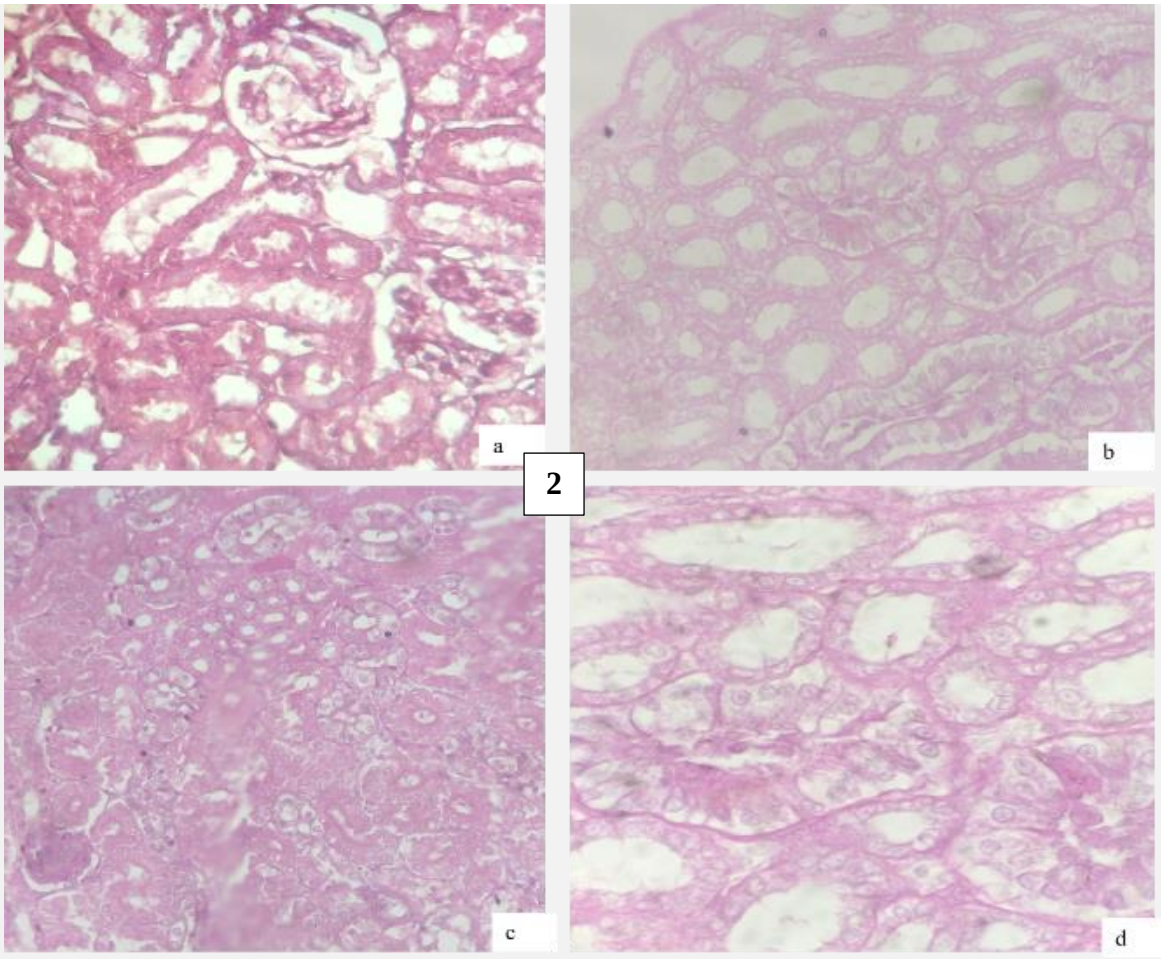


Figure 2 - Geographical location of Bingerville. *Source: Kanohin et al. (2017).




a
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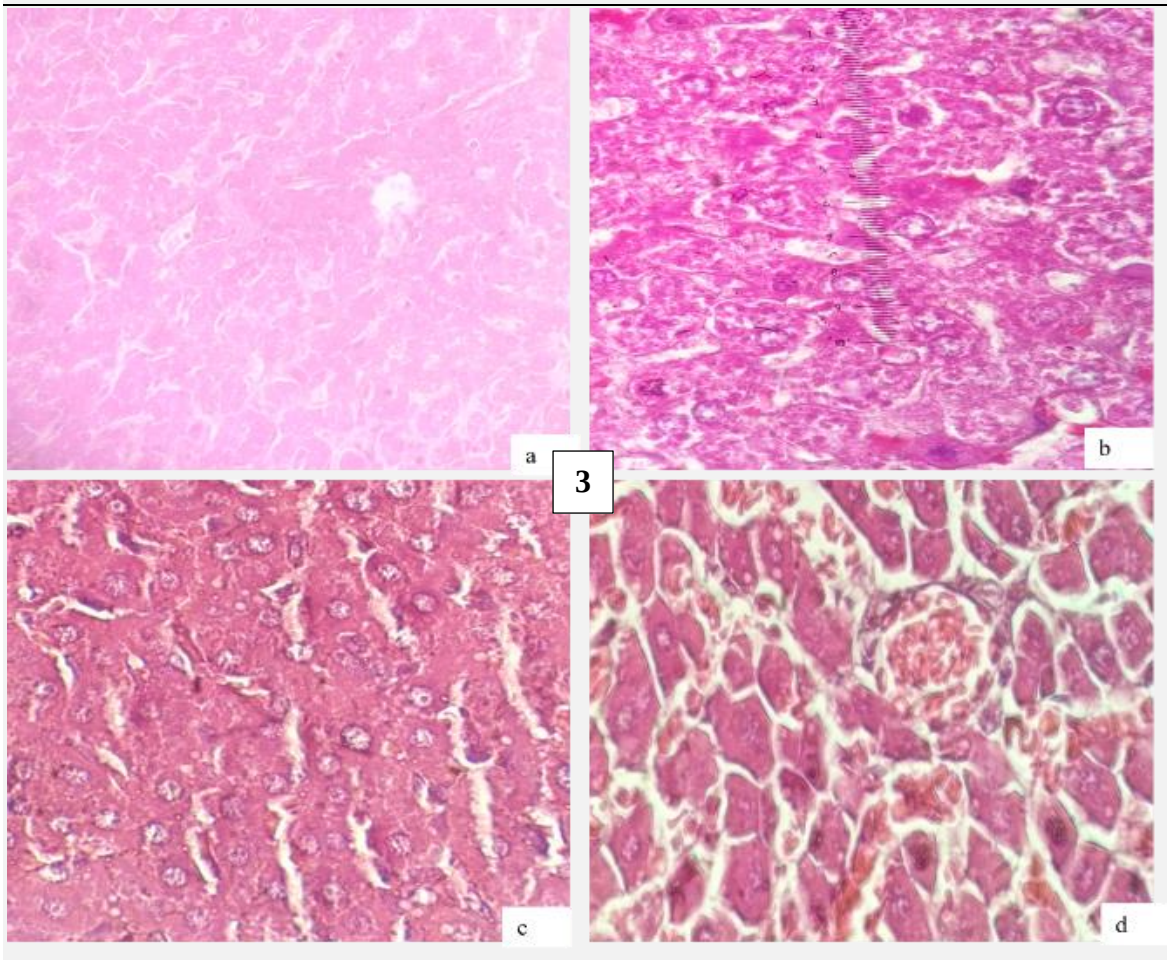
a
b
3
c
d

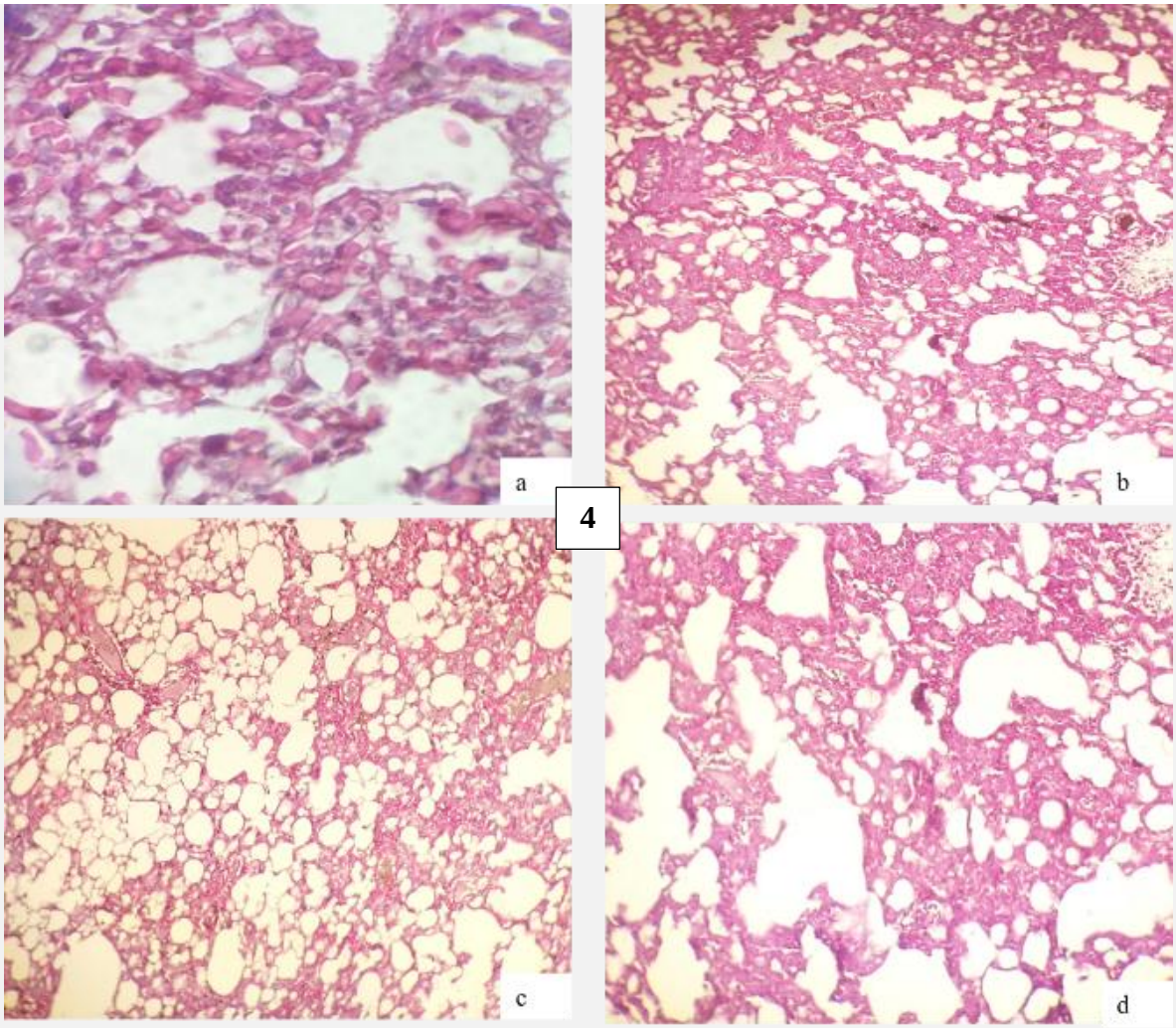

Figure 4 - Histological sections of the organs of the test chickens (Gx400). **a:** batch control witness ; **b:** batch exposed to the ethanolic extract of *Mallotus oppositifolius*; **c**: batch exposed to the aqueous extract *of Mallotus oppositifolius*; **d**: batch exposed ethanolic extract of *Kalanchoe crenata.* **1**: heart organ; **2**: kidney organ; **3**: liver organ; **4**: lung organ

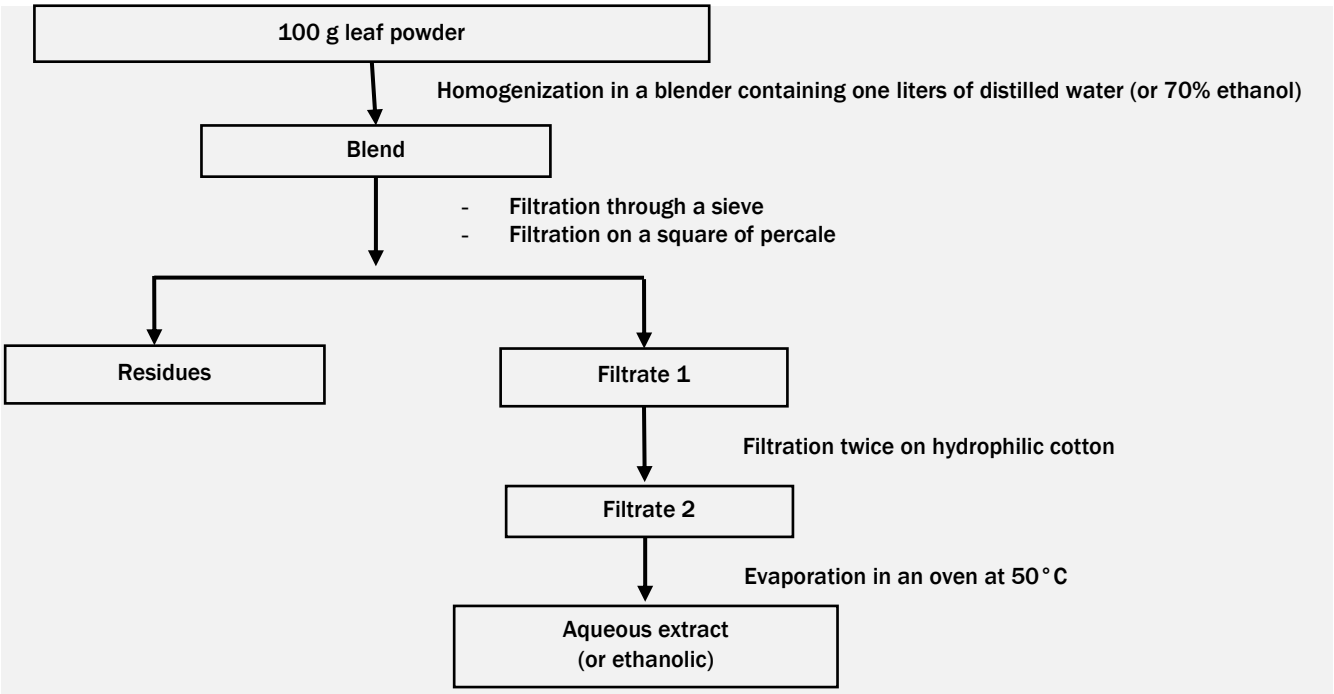

Figure 3 - Protocol for obtaining aqueous and ethanolic extracts from the plants in the study

CONCLUSION

This study shows that the aqueous and ethanolic extracts of *Mallotus oppositifolius* and the ethanolic extract of *Kalanchoe crenata*, having antibacterial properties on different serotypes of multi-resistant avian *Salmonella* strains, possess molecules belonging to large phytochemical groups such as polyphenols, catechin tannins, anthraquinones, saponosides and sterols. In addition, these extracts do not present any acute toxicity for a dose less than or equal to 2000 mg/kg. No signs of behavioral, tissue or biochemical toxicity were observed. These extracts can be considered as promising candidates for the development of alternative antibacterials, in the fight against the *Samonella* pathogen in poultry farming.

DECLARATIONS

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Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Author contribution

ASSANDI Kouamé Rivière spearheaded the design and execution of the experiments, conducted results analysis, and contributed to the writing. TOURE Alassane spearheaded the execution histological analyses. BONNY Aya Carole was involved in the design, writing, results analysis, and refining the manuscript's structure. KAROU Tago Germain played a key role in establishing execution sites and coordinating with participating author.

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Ethical approval

This experimental study was carried out with the agreement of the National Ethics Committee for Life and Health Sciences (CNESVS) of the Pasteur Institute of Côte d'Ivoire (N/ Ref: 037-23/ MSHPCMU / CNECVS – km).

Consent to participate

All authors agree to the execution of this experimentation.

Consent for publication

All authors agree to the publication of this manuscript.

Competing interests

The authors declare that they have no competing interests.

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Not applicable.

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