

Terpenoid Profiling, Biological Activity, Biodegradability and Toxicological Evaluation of *Euphorbia kamerunica* Pax and *Euphorbia poisonii* Pax Extracts

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ABSTRACT

Conventional synthetic antiriot agents are effective, toxic and environmentally persistent, giving rise to the need to search for renewable and potentially biodegradable alternatives whose biological activity and toxicological profiles require systematic evaluation that can be derived from natural sources. Plant-based phytochemicals, such as alkaloids, phenolics and terpenoids from our flora have been reported as useful alternatives to synthetic incapacitating agents. In this study, the terpenoidal contents of the aerial parts of *Euphorbia kamerunica* Pax and stems of *Euphorbia poisonii* Pax were investigated for their composition, antiriot potential, acetylcholinesterase (AChE) inhibitory activity, biodegradability and toxicological profiles. Statistically significant differences were determined using one-way analysis of variance (ANOVA), followed by Tukey-Kramer post-hoc comparison at $p < 0.05$. Profiling and quantification of crude chloroform extracts of *Euphorbia kamerunica* (Ek) and *Euphorbia poisonii* (Ep) using gas chromatography-mass spectrometry revealed a significant mixture of sesquiterpenoids (55.25%) and triterpenoids (43.58%) in Ek and significant presence of sesquiterpenoids (96 %) in Ep. Evaluation of the antiriot potential of each extract at varying concentrations (2-10%) using the spray-induced discomfort and incapacitation model revealed that activity for both extracts was dose-dependent and compared favourably well with that of commercial pepper spray (3%) at 10%. Forty-five male mice were used for the study and randomly assigned into nine groups of five animals each. Both extracts also inhibited acetylcholinesterase enzyme (AChE), with the inhibition increasing progressively from 0% to 64% and 0% to 86.90% for Ek and Ep, respectively, at 10% in both extracts. Biodegradability assessment of both extracts showed a progressive increase in CO₂ evolution, microbial population, soil pH and a decrease in the amount of the extract, showing a level of decomposition in the soil under the experimental conditions. The toxicological profiles of both extracts showed relatively low values of adverse effects at lower concentrations and the higher concentrations caused changes in some haematological, biochemical and histopathological parameters. In conclusion, *E. kamerunica* and *E. poisonii* extract demonstrated antiriot potential as alternatives to synthetic antiriot agents with favorable biodegradability in soil. However, further safety evaluation of these extracts should be done to ascertain whether their eco-friendly nature compatible to the environment.

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INTRODUCTION

In many countries, the use of synthetic chemicals is a common practice in maintaining law and order in the country. These chemicals, also known as tear gases/riot control agents/crowd control agents/incapacitating agents are meant to temporarily incapacitate people by causing irritation to the mucous membranes of their eyes, nose, mouth and lungs (Rothenberg *et al.*, 2016). These agents, however, have short durations of effectiveness and are effective at dispersing the crowd, but contain toxic gases that may have harmful health and environmental impacts, such as; ortho-chlorobenzylidene malononitrile (CS), dibenzoxazepine (CR) and chloroacetophenone (CN) (Abbas, 2020; Rani *et al.*, 2021; Ma *et al.*, 2024). For instance, soil and aquatic residues of CS

gases were found, weeks after application (Gheorghe *et al.*, 2023). These issues later inspired the use of plant extracts and their metabolites, particularly the plants that have burning/pepperish/irritant properties (Mtewa *et al.*, 2021; El Saadony *et al.*, 2025). One noteworthy example is Capsaicin (alkaloid) extracted from the oily extract of chili peppers (*Capsicum annum*, family: Solanaceae), which is commonly used to make pepper spray, a contemporary natural antiriot agent used by the police as a crowd control means (Fischer *et al.*, 2020; Keel and Walters, 2023). Over the last few years, scientists have been using phytochemicals derived from plant sources, with a potent effect against rioting. Some of them include Piperine (alkaloid) from *Piper nigrum*; Cinnamaldehyde (phenolic) from *Cinnamomum* spp.;

Eugenol (phenolic) from *Ocimum gratissimum* and Allicin (thiosulfate) from *Allium sativum*; Nerolidol (sesquiterpenoid) from *Zingiber officinale* and Kaempferol (flavonol) from *Euphorbia perkinensis* (Senapati, et al., 2025). All of them are known to have irritant, toxic and incapacitating properties that in some instances are comparable or superior to the effects of synthetic agents (Awuchi, 2020). The risk of persistent ecological contamination is usually avoided, due to the low toxicity and fast degradation in the environment of such biodegradable sustainable plant-based agents that do not tend to persist (Souto et al., 2021; Gheorghe et al., 2023). This shift toward sustainable chemistry aligns with the UN SDGs, particularly SDG 3, SDG 9 and SDG 13 and hence, continued search and exploitation of the secondary metabolites of the diverse flora and fauna of Nigeria and beyond, for anti-riot compounds, become pertinent in order to come up with safer, environmentally safe plant based anti-riot compounds. *Euphorbia* species (Family: Euphorbiaceae) are well distributed in the tropics and sub-tropics, very abundant in Nigeria and have been reported for their extensive chemical and biological diversity (Benjamaa et al., 2022). *Euphorbia kamerunica* Pax and *Euphorbia poissonii* Pax possess many terpenoidal constituents and biologically active latex, which can be responsible for their irritant and deterrent properties (Kgosiemang et al., 2025). The terpenoid profile, biological properties, environmental biodegradability and toxicological properties of these two species, however, have not been comparatively studied in detail. The aim of this study was thus to compare the terpenoid composition, biological activities related to anti-riot potential and biodegradability and acute toxicity of extracts from *E. kamerunica* and *E. poissonii*. Specifically, the study aimed to identify the terpenoidal content of *E. kamerunica* and *E. poissonii*, to assess the antiriot potential related biological activity of the extracts using the appropriate experimental models, to assess the biodegradability and environmental dissipation of the extracts under the experimental conditions and to determine the acute toxicological effects of the extracts in experimental animals. The study was hypothesized that terpenoid composition and biological activity of the two *Euphorbia* species would be significantly different, and that their plant extracts would show measurable biodegradability and different toxicological profiles.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

The stems of *Euphorbia poissonii* were collected from a farmland in Ekiti Local Government Area, Kwara State, Nigeria, identified and authenticated at the Herbarium Unit, Department of Biological Sciences, Ahmadu Bello University (ABU), Zaria, Kaduna State (Voucher No. ABU0298).. The aerial parts of *Euphorbia kamerunica* were collected from Shiroro Local Government Area, Niger State, Nigeria, identified and authenticated at the National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja (Voucher No. NIPRD/H/22278. Both plant materials were washed with clean water to remove surface impurities, air-dried at room temperature for several weeks, and subsequently pulverized into fine powder using a mortar and pestle.

Extraction Procedure

Each air-dried pulverized plant sample was separately cold macerated at 27 ± 2 °C with 3.5 L of chloroform for a week, with occasional agitation. Each resulting mixture was filtered and the respective filtrates concentrated *in-vacuo* and finally

dried over a water bath at 30°C to afford crude extracts, coded Ek (*E. kamerunica*) and Ep (*E. poissonii*). Both extracts were weighed and stored at 4°C until required for further analysis.

Preliminary Qualitative Screening of Extracts

Qualitative tests were performed on extract Ek and Ep using standard methods to detect the presence/absence of terpenes/terpenoids and their various classes using the methods described by Awe et al. (2023), Fadipe et al. (2020), and Tanko et al. (2020).

Liebermann-Burchard's Test

Extract Ek and Ep (0.2 g each) in separate tubes was dissolved in 1 cm³ of acetic anhydride, followed by the careful addition of 1 cm³ of concentrated tetraoxosulphate (VI) acid (H₂SO₄) to each resulting mixture. Each mixture was gently shaken and subsequently heated on a water bath at 50°C for 5 minutes. The development of a reddish-brown colouration indicates the general presence of terpenoids.

Salkowski's Test

Each extract (0.5 g) in separate tubes was dissolved in 5 cm³ of distilled water and filtered. A few drops of concentrated tetraoxosulphate (VI) acid (H₂SO₄) was then carefully added to each filtrate. The appearance of a reddish-brown colouration at the interface was taken as positive for the general presence of terpenoids.

Test for Monoterpenoids

To each extract (0.2 g) in separate tubes, 1 cm³ of sodium nitroprusside reagent was added and gently shaken. The development of a pink colouration indicates the presence of monoterpenoids.

Test for Sesquiterpenoids

Extract Ek and Ep (0.2 g each) in separate tubes, 5 cm³ of vanillin-H₂SO₄ acid was added and the resulting mixture gently shaken. The development of a reddish-brown colouration indicates the presence of sesquiterpenoids.

Test for Diterpenoids

Each extract (0.5 g) in separate tubes, was dissolved in 5 cm³ of distilled water and filtered. 3 drops of copper acetate solution was then added to each filtrate. The development of an emerald green colouration indicates the presence of diterpenoids.

Test for Sesterpenoids

To each extract (0.2 g), 5cm³ portion of ammonium nitrate in 5% methanol was added and mixed gently. Formation of a reddish-brown colouration indicates the presence of sesterpenoids.

Test for Triterpenoids

Each extract (0.2 g) was separately heated with 2 drops of a saturated solution of antimony trichloride in chloroform. The development of a blue-green colouration, which subsequently changed to red, indicates the presence of triterpenoids.

Test for Tetraterpenoids

Concentrated tetraoxosulphate (VI) acid (H₂SO₄) was carefully added to 0.2 g of extract Ek and Ep in separate test tubes. The violet color precipitate formed shows the presence of tetraterpenoids.

Gas Chromatography-mass Spectrometry profiling of Extracts

The extract Ek and Ep were analysed by GC-MS on Agilent HP-5 MS capillary column (30 m × 0.25 mm × 0.25 µm), with carrier gas helium (99.999%) at 1.61 mL/min. Temperature in the oven was set from 60°C to 280°C in 2 ramping sequences. The extracts were dissolved in hexane (5 mg/mL), filtered and injected (1.0 µL) into the autosampler in split entry mode (25:1). Compounds were identified based on the NIST mass spectral library; the relative percentage peak areas were reported (Gao *et al.*, 2024).

Evaluation of the Antiriot Potential of Extracts

The extracts Ek and Ep were serially concentrated at 2%, 4%, 8% and 10% (w/v) by dissolving separately 2 g, 4 g, 8 g and 10 g of each extract in absolute ethanol and then making up to 100 cm³ of solution. Glycerol (10 cm³) was added to each mixture as a dispersant. The antiriot potential of each extract was determined by the method described by Awe *et al.* (2023) with a few modifications. Six weeks mice (male) weighing between 150-173 g were obtained from the animal house facility, Department of Biochemistry, Federal University of Technology, Minna. The animals were kept under standard laboratory conditions (12-hour light/dark cycle, temperature 22–25°C, relative humidity 50–60%) with free access to feeds and water. The animals were kept for three weeks in a pharmacology laboratory conditions to get acclimatized before experiment. The mice were randomly distributed among a total of nine (9) groups with 5 individual mouse in each group, the mice in the first four groups were sprayed with concentration of extract Ek (2%, 4%, 8% and 10% (w/v)), the mice in the second four groups were sprayed with concentration of extract Ep (2%, 4%, 8% and 10% (w/v)) and the last group was sprayed with pepper spray as a Positive control. The mice sprayed were observed for skin reactions, behavioral changes and signs of discomfort at 20 second intervals. The time required for each mouse to return to normal physiological state was also noted. A commercial pepper spray with Oleoresin capsicum (3% mixture of Oleoresin capsicum, ethanol and carbon (IV) oxide) was used as positive control.

Acetylcholinesterase Inhibition Studies of Extracts

The acetylcholinesterase (AChE) inhibitory activity of extract Ek and Ep was assessed using the method of Organisation for Economic Co-operation and Development (OECD) as described by Lin *et al.* (2021), with slight modifications. Specifically, 140 µL of phosphate buffer was dispensed into each well of a 96-well microplate, followed by 10 µL of each extract (2%, 4%, 8%, and 10% w/v) and 10 µL of AChE solution (0.5 U/mL). After incubation at 25°C for 10 minutes, 5,5'- dithiobis (2-nitrobenzoic acid), (10 mM) and acetylthiocholine iodide (14 mM) were added to initiate the enzymatic reaction. The absorbance was measured at 60 seconds interval for 10 minutes and to determine the percentage AChE inhibition (I) of each extract, Equation 1 was used in which the untreated group (control) served as the 100% reference.

$$I = \frac{Ac - As}{Ac} \times 100 \quad 1$$

Where; I = percentage AChE inhibition, Ac = absorbance of the enzyme in the control, As = absorbance of the enzyme presence in treated group (inhibitor).

Biodegradability Studies of Extracts

The biodegradability of the extract Ek and Ep was evaluated by soil burial/incubation method according to OCED, 2025

for a period of 28 days using 2%, 4%, 8% and 10% (w/w) of the extract concentration. The microbial population count, soil pH, CO₂ evolution, residual extract weight were observed at 28 days intervals. The percentage degradation was determined by equation 2:

$$\% \text{ Degradation} = 1 - \frac{C_t}{C_0} \times 100 \quad 2$$

Where; D = percentage degradability, C₀ = initial concentration/amount of the substance and C_t = concentration/amount remaining after time t.

Toxicological Studies of Extracts

Lethal Dose (LD₅₀)

The acute dermal toxicity of Ek and Ep extracts was tested in mice according to OECD Test Guideline 402 (OECD, 2017). The extracts were made in concentrations of 2%, 4%, 8% and 10% (w/v) and applied to the skin directly by spraying. Animals were followed for mortality and clinical signs of toxicity (behavioural, locomotor and respiratory) during the observation period of twenty-four (24) hours. The effect on the body weight and treatment-related effects were monitored thereafter. Acute dermal toxicity was determined from the observations of mortality and behavioural reactions at the various treatment levels.

Biochemical Analysis

The biochemical parameters of blood samples of mice sprayed with different concentrations of extract Ek and Ep was evaluated. The parameters, included; alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total protein, albumin, creatinine, urea, and uric acid. The concentrations of these biochemical indices were spectrophotometrically determined within a wavelength range of 100–1100 nm, as described by Andrei *et al.* (2023).

Haematological Analysis

The haematological parameters of mice sprayed with varying concentrations of extract Ek and Ep were evaluated using an automated haematological analyser (Bamps *et al.*, 2021). The parameters assessed included; haemoglobin concentration (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet count (PLT), red blood cell count (RBCs), and total white blood cell count (TWBC).

Histopathological Studies

Histopathological examination of sections of lung and skin tissues obtained from mice sprayed with varying concentrations of extract Ek and Ep were immediately fixed in neutral buffered formalin solution upon excision. Tissue processing and embedment were carried out using a mixture of chloroform and ethanol (2: 3) at 60°C. Tissue sections of 3 µm thickness were obtained, deparaffinized, and stained with haematoxylin and eosin. Histological changes in the architectural pattern of each tissue was examined under a light microscope at ×40 magnification, as described by Divekar *et al.* (2022).

Statistical Analysis

The data were subjected to statistical analyses with the use of Statistical Package For the Social Sciences (SPSS) version 20.0 for Windows. Data are presented as means ± SEM, and differences between treatment groups were determined by one way analysis of variance (ANOVA) followed by Tukey-Kramer post hoc multiple-comparison test (p < 0.05).

RESULTS AND DISCUSSION

The qualitative terpenoidal contents of crude chloroform extracts of *E. kamerunica* and *E. poisonii* is presented in Table 1.

Table 1: Classes of Terpenoids in *E.kamerunica* (Ek) and *E.poisonii* (Ep) Crude Chloroform Extract

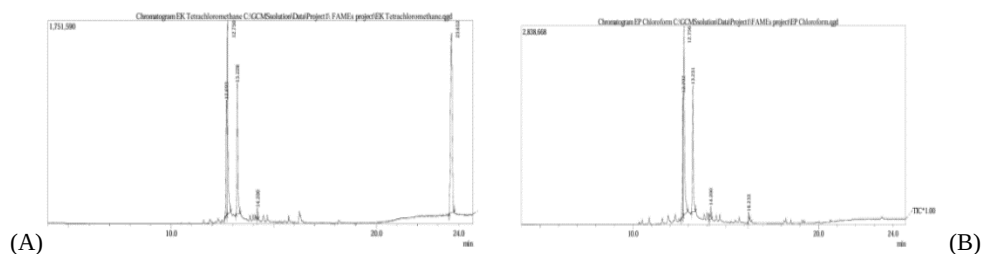
(1) Class of Terpenoid	(2) Test	(3) Observation	Inference	
			(5) Ek	(6) Ep
(7) Terpene/Terpenoids (8) $(-C_5H_8)_n$	(9) Liebermann-Burchard's (10) (11) Salkowski's	(12) Reddish-brown colour observed (13) Reddish-brown colour observed at the interphase	(14) + (15) (16) +	(17) + (18) (19) +
(20) Monoterpenoids $(-C_5H_8)_2$	(21) Sodium nitroprusside	(22) No Pink colouration observed	(23) -	(24) -
(25) Sesquiterpenoids $(-C_5H_8)_3$	(26) Vanillin-sulphuric acid	(27) Reddish-brown colour observed	(28) +	(29) +
(30) Diterpenoids $(-C_5H_8)_4$	(31) Copper acetate	(32) No Emerald green solution observed	(33) - (34)	(35) - (36)
(37) Sesterpenoids $(-C_5H_8)_5$	(38) Ammonium nitrate in 5% MeOH	(39) No reddish-brown colour observed	(40) -	(41) -
(42) Triterpenoids $(-C_5H_8)_6$	(43) $SbCl_3$ in $CHCl_3$	(44) Blue to red colouration not observed/observed	(45) +	(46) -
(47) Tetraterpenoids $(-C_5H_8)_7$	(48) Conc. H_2SO_4	(49) No violet colour observed	(50) -	(51) -

Keys: + Present; - Absent

The crude chloroform extract of *Euphorbia poisonii* (Ep) qualitatively showed the presence of terpenoids (mainly sesquiterpenoidal) and *Euphorbia kamerunica* (Ek) showed the presence of terpenoids (mainly sesquiterpenoidal and triterpenoidal). The terpenoids detected in both extracts are similar to the results reported in several species of the *Euphorbiaceae* family (Kgosiemang et al., 2025). In the general, the quality and bioactivity of a plant depends mainly on the kinds and variety of secondary metabolites that are present (Crowley and Dando, 2024).

Profiling and Quantification of Terpenoidal Compounds in Extracts

Gas chromatography coupled with mass spectrophotometry carried out on crude chloroform extracts of *Euphorbia kamerunica* (Ek) *Euphorbia poisonii* (Ep) confirmed the various classes of terpenoids and their quantities as presented in Figures 1A and 1B, respectively, while a summary of the detected compounds are presented in Table 2.



Figures 1: 1A & 1B: GC-MS Chromatograms of Chloroform Extracts of *E.kamerunica* (Ek) and *E. poisonii* (Ep), Respectively

Table 2: Summary of major terpenoids identified in chloroform extract of *E. kamerunica* (Ek) and *E. poisonii* (Ep) by GC-MS

(52) Peak no	(53) Retention time (mins)	(54) Area (%)	(55) Name of Compound	(56) Molecular Formula	(57) Molecular Weight (g/mol)	(58) Class of Compound
(59) Ek	(60)	(61)	(62)	(63)	(64)	(65)
(66) 1	(67) 12.69	(68) 13.38	(69) Cinnamyl tiglate	(70) $C_{14}H_{16}O_2$	(71) 216	(72) ester
(73) 2	(74) 12.75	(75) 25.71	(76) Ar-Tumerone	(77) $C_{15}H_{20}O$	(78) 216	(79) Sesquiterpenoid
(80) 3	(81) 13.23	(82) 16.43	(83) Curlone	(84) $C_{15}H_{22}O$	(85) 218	(86) sesquiterpenoid
(87) 4	(88) 14.20	(89) 0.90	(90) Indene	(91) $C_{15}H_{16}$	(92) 206	(93) Sesquiterpene
(94) 5	(95) 23.65	(96) 43.58	(97) Lanosterol	(98) $C_{30}H_{50}O$	(99) 426	(100) Triterpenoid
(101) Ep	(102)	(103)	(104)	(105)	(106)	(107)
(108) 1	(109) 12.70	(110) 24.35	(111) Ar-Tumerone	(112) $C_{15}H_{20}O$	(113) 216	(114) Sesquiterpenoid
(115) 2	(116) 12.75	(117) 45.47	(118) Tumerone	(119) $C_{15}H_{22}O$	(120) 218	(121) Sesquiterpenoid

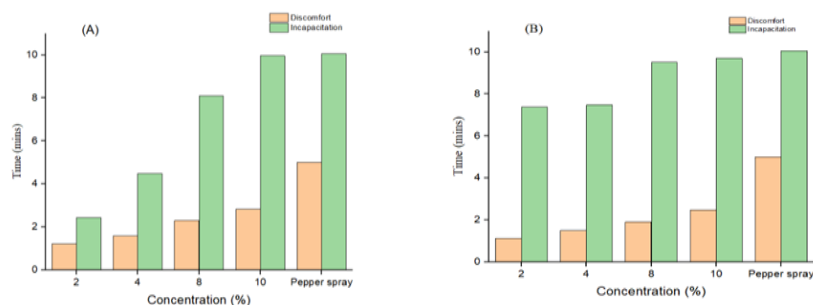
(52) Peak no	(53) Retention time (mins)	(54) Area (%)	(55) Name of Compound	(56) Molecular Formula	(57) Molecular Weight (g/mol)	(58) Class of Compound
122) 3	123) 13.23	124) 26.39	125) Curlone	126) C ₁₅ H ₂₂ O	127) 218	128) Sesquiterpenoid
129) 4	130) 14.20	131) 2.48	132) Indene	133) C ₁₅ H ₂₆	134) 206	135) Sesquiterpene
136) 5	137) 23.23	138) 1.30	139) Palmitic acid	140) C ₁₆ H ₃₂ O ₂	141) 256	142) Fatty acid

The GC-MS analysis of extract Ek and Ep showed the presence of five (5) compounds in each extract with the major component in both extract being sesquiterpenoids of which more than 96% of the Ep was made up of it. The detected sesquiterpenoids are bioactive compounds identified in the plant *Curcuma longa* (Turmeric) which has been reported to have several varieties of biological activities (De Oliveira Barbosa and Neto, 2023; Orellana-Paucar, 2024). The significant presence of Lanosterol (43.58%), a triterpenoid and other compounds in Ek may be acting individually or

synergistically and contributing to the overall biological activity of the extract (Rojas-Jiménez et al., 2024). The presence of a large number of terpenoidal compounds in both extracts was confirmed by GC-MS as earlier detected qualitatively.

Anti-Riot Potential of Extracts

The antiriot potential of extract *E. kamerunica* (Ek) and *E. poisonii* (Ep) at different concentrations are presented in Figures 2A and 2B below.



Figures 2: 2A and 2B: Antiriot Potential of Extract *E. kamerunica*(Ek) and *E. Poisonii* (Ep) at Different Concentrations in Comparison with 3% Pepper Spray

Ek and Ep both showed statistically significant concentration dependent increase in discomfort (onset) time and incapacitation duration. For Ek, discomfort and incapacitating times ranged from 1.20 ± 0.03 and 2.43 ± 0.07 minutes at 2%, progressively increasing to 2.82 ± 0.02 and 9.96 ± 0.03 minutes at 10%, respectively. Likewise, at lower concentrations (2% and 4%) the discomfort (onset) times recorded by Ep ranged from 1.10 – 1.13 minutes, but at 10% increased significantly to 2.48 minutes, which was not statistically different from the commercial pepper spray control (2.40 minutes) at the same concentration. Except for the control group, all extract-treated groups were significantly different ($p < 0.05$). These effects are mostly related to the presence of sesquiterpenoids, which are confirmed as the main constituents by the GC-MS analysis shown in figure 1 and 2, and due to their lipophilic nature they are able to

interact with the sensory receptor very fast and to penetrate the membrane to explain their irritant potency. The extracts exhibited incapacitating effects at 10% concentration similar to the commercial pepper spray, which had a longer onset time due to formulation issues including solvent evaporation and aerosol dispersion (Brown et al., 2021). The results are experimental support for the possible use of *E. kamerunica* and *E. poisonii* as a potential alternative to synthetic antiriot agents, giving meaningful data to help develop sustainable and plant-based non-lethal formulation of deterrents (Kgosiemang et al., 2025).

Acetylcholinesterase Activity of Extracts

The inhibitory activity of acetylcholinesterase enzyme by *E.kamerunica* (Ek) and *E.poisonii*(Ep) extracts are shown in Figure 3.

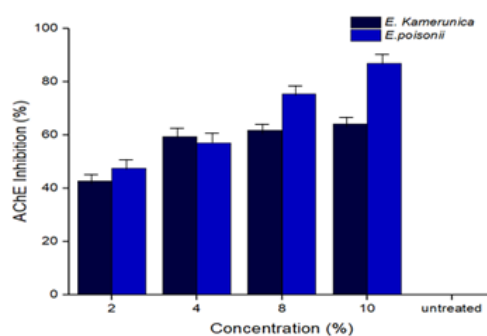


Figure 3: Acetylcholinesterase (AChE) Activity of Extract Ek and Ep at Different Concentrations in Comparison with Untreated Group

The acetylcholinesterase (AChE) inhibitory activity of the chloroform crude extracts of *E. kamerunica* and *E. poisonii* were significantly inhibited in a clear concentration-dependent manner. AChE is an important enzyme in the nervous system and its inhibition causes acetylcholine to build up at cholinergic synapses, causing continuous stimulation of the nerve, contraction of muscle, excessive secretions, bronchoconstriction and sensory discomfort. The control value 0 % represents absence of inhibitory activity in untreated group of mice, this suggest that the concentration of AChE enzyme in the health mice in this study was 100% , an indication of the absence of acetylcholine buildup at the cholinergic synapses that could cause continuous stimulation of the nerve, contraction of muscle, excessive secretions, bronchoconstriction and sensory discomfort, hence supporting systemic balance in the mice. However, the AChE inhibition increased steadily as the concentration increased 0 % to 64.00 % for Ek and 86.90 % for Ep at 10 %

concentration. The highest inhibition obtained at 10% for Ep, indicated higher inhibitory potency. The observed AChE inhibition is attributed mainly to the sesquiterpenoids and this is only a hypothesis as these extracts are crude and unfractionated and further research will require the isolation of the different compounds to determine their specific effect; however, it does indicate that this class of compounds plays a key role in the inhibition. (Sultana *et al.*, 2022; Sun *et al.*, 2024). The same trend was observed in extracts applied in neuroactive and insecticidal uses, with the enzyme suppression being more marked as the concentration of extracts increased (Yiannis *et al.*, 2025). This indicates the increasing effectiveness of extracts as potential antiriot agents.

Biodegradability Capability of Extracts

The biodegradability activity of *E.kamerunica* (Ek) and *E.poisonii* (Ep) extracts are shown in Figure 4.

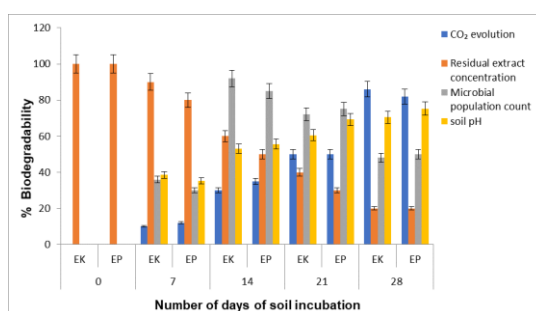


Figure 4: Degree of Biodegradability of *E. kamerunica* and *Euphorbia poisonii* Extracts

The biodegradability assessment shown in Figure 4 illustrated the changes that occurred in *Euphorbia kamerunica* (Ek) and *Euphorbia poisonii* (Ep) extracts over the 28-day soil incubation period. The residual extract weight has been reduced gradually from the initial weight down to its minimum value (about 20% remaining) on day 28 indicating about 80% of the extract materials had been removed. Contrary, the CO₂ evolution has increased gradually with incubation and reached 86% for Ek and 82% for Ep by day 28, indicating in turn enhanced microbial respiration and extract derived organic carbon mineralization. The inverse correlation between the weight of the extract and the amount of CO₂ evolved is also complementary proof for the biological transformation and not for disappearance (Ferreira *et al.*, 2026). The microbial population trend for the other groups was the opposite with significant increases in early incubation, then reaching a peak around day 14, followed by a decline until day 28. This response showed that the easily degradable components of the extracts in the beginning stimulated the growth of the microbes and then as the time passed more and more substrate required to be consumed, resulting in a reduction in microbial growth. It has been observed that the evolution of CO₂ continued even after reducing the microbial population, which further corroborates the fact that there is a lack of direct proportionality in the microbial activity/biome during the later stages of degradation. The difference in Ek and Ep could also be due to their different phytochemical contents, with a higher concentration of sesquiterpenoids in both extracts and a significant amount of triterpenoids in Ek (such as lanosterol). The soil pH slightly decreased at the beginning of incubation and gradually rose in the later phase of incubation, which may be attributed to the formation of acidic decomposition intermediates at the initial

stage of incubation and their conversion and buffering during later incubation stage (Li *et al.*, 2023). Although the biological transformation is significant, with CO₂ evolution increasing, and the initial microbial stimulation decreasing (Li *et al.*, 2024), the amount of extract left after 28 days (around 20%) indicates that the mineralization is not yet finished. The extracts were greatly degraded and eventually lost during the experimental soil incubation under the experimental soil-incubation conditions.

Lethal Dose (LD₅₀)

Lethal Dose (LD₅₀) was used to determine the toxicological profiles and safe exposure limits of both extract Ek and Ep. The LD₅₀ value is the dose of a test substance that is predicted to kill 50% of the animals in an experimental group after exposure to the substance (Sultana *et al.*, 2022). The LD₅₀ of both extracts was 8%; hence both extracts were moderately toxic at this concentration with 50% of the experimental mice exhibiting lethal effects. These LD₅₀ values set a safety limit, so that doses applied in antiriot formulations do not exceed lethal levels. The LD₅₀ for *Euphorbia hirta* was found to be 10% by Praveen *et al.* (2024), a similar value as observed for both extracts in this study. The comparative results corroborate the toxicity profile of both extracts is similar to that of other *Euphorbia* species. The moderate toxicity further corroborates the appropriateness of both extracts for use as potential anti-riot agents with biodegradable tendency.

Haematological Parameters

The effect of different concentrations of *E.kamerunica* (Ek) and *E.poisonii* (Ep) extracts on hematological and biochemical parameters of mice are presented in Tables 3 and 4, respectively.

Table 3: Haematological Parameters of Extract *E. kamerunica* (Ek) and *E. poisonii* (Ep) at Different Concentrations in Comparison with Pepper Spray

Concentration (%)	2		4		8		10		3	
Parameters	Ek	Ep	Ek	Ep	Ek	Ep	Ek	Ep	Pepper spray	Normal range (ICSH, 2023)
Hb (g/dL)	10.45±1.22 ^g	11.75±0.02 ⁱ	10.54±1.57 ^f	10.35±0.01 ^d	10.39±0.37 ^e	9.03± 0.03 ^a	10.25±0.37 ^c	9.10±0.13 ^b	11.04±1.15 ^h	11.50-15.50
PCV (%)	41.00 ± 1.00 ^e	39.40±0.10 ^d	41.100 ± 2.00 ^f	32.70±0.07 ^c	42.00 ± 1.00 ^g	27.10±0.01 ^b	42.02 ± 1.00 ^h	26.03±0.03 ^a	41.00± 1.00 ^e	37-48
MCV (fL)	54.50 ± 1.50 ^c	87.50±0.02 ⁱ	55.50 ± 2.50 ^f	81.50±0.06 ^h	55.00 ± 2.00 ^d	48.05±0.04 ^b	55.40 ± 2.00 ^e	46.12±0.00 ^a	55.50 ± 2.50 ^f	80 – 100
MCH (pg/cell)	47.50 ± 1.50 ^f	29.30±0.01 ^d	45.50 ± 1.50 ^e	27.90±0.09 ^c	51.50 ± 1.50 ^h	25.60±0.01 ^b	52.30 ± 1.00 ⁱ	25.20±0.01 ^a	48.50±2.50 ^e	27 – 33
MCHC (g/dL)	41.00 ± 2.00 ^f	33.20±0.00 ^d	46.50 ± 1.50 ^h	32.10±0.20 ^c	46.00 ± 3.00 ^g	29.40±0.10 ^a	47.00 ± 1.60 ^d	31.20±0.20 ^b	40.50±1.50 ^e	32 – 36
PLT (10 ⁹ /L)	141.50±2.50 ^a	153.±0.30 ⁱ	147.50 ± 2.50 ^f	150.5±0.10 ^g	144.00 ± 2.00 ^e	143.50±0.15 ^d	143.00±2.00 ^c	152.7±0.03 ^h	143.00±2.00 ^e	150 – 450
RBCs (10 ¹² /L)	6.615±0.41 ^g	4.89±0.03 ^c	7.025 ± 0.44 ⁱ	4.32±0.07 ^e	6.170 ± 0.19 ^e	4.14±0.00 ^a	6.173±0.17 ^f	5.40±0.01 ^d	6.985±0.41 ^h	4.32–5.72
TWBCs (10 ⁹ /L)	9.410 ± 1.24 ^a	12.56±0.50 ^f	11.000 ± 1.19 ^b	13.07±0.04 ^g	11.01 ± 1.70 ^c	15.10±0.01 ^h	12.515±0.78 ^e	16.30±0.00 ⁱ	11.01 ±1.70 ^d	4.00–11.00

Values are Expressed as Mean ± Standard Error. Values Bearing Different Superscript Letters Within the Same Row Differ Significantly from the Control (0%) at p<0.05 **Keys:** Hb = Hemoglobin Level, PCV= Packed Cell Volume, MCV=Mean Corpuscular Volume, MCH=Mean Corpuscular Hemoglobin, MCHC= Mean Corpuscular Hemoglobin Concentration, PLT=Platelet, RBCs= Red Blood Cells, TWBC= Total White Blood Cells, ICSH= International Council for Standardization in Hematology

Table 4: Biochemical Parameters of Extract *E. kamerunica*(Ek) and *E. poisonii* (Ep) at Different Concentrations in Comparison with Pepper Spray

Concentration (%)	2		4		8		10		3	
Parameters	Ek	Ep	Ek	Ep	Ek	Ep	Ek	Ep	Pepper spray	Normal range (IFCC, 2023)
ALT (U/L)	19.30±1.35 ^a	23.15±0.01 ^d	23.31±0.82 ^f	23.53±0.00 ^g	23.02±1.70 ^c	31.64±0.03 ^h	23.20±1.50 ^e	34.18±0.02 ⁱ	21.21±2.13 ^b	0-45
AST (U/L)	25.58±1.64 ^a	27.46±0.00 ^d	26.80±1.41 ^c	30.65±0.03 ^g	28.61±1.72 ^e	32.42±0.02 ^h	28.81±1.22 ^f	33.40±0.01 ⁱ	26.55±0.87 ^b	0-40
ALP (U/L)	77.36±2.97 ^e	72.09±0.04 ^a	82.43±3.00 ^g	74.57±0.05 ^b	84.20±1.99 ^h	76.57±0.01 ^c	85.50±1.95 ⁱ	80.51±0.03 ^f	76.73±1.68 ^d	20-140
Albumin (g/L)	21.19±2.24 ^f	32.77±0.05 ⁱ	19.18±0.44 ^d	23.53±0.01 ^h	17.15±1.66 ^b	20.86±0.01 ^e	15.14±1.60 ^a	17.20±0.00 ^c	23.39±2.35 ^g	35.00-52.00
Urea (mg/dL)	28.80±3.40 ^d	20.89±0.02 ^a	31.40±1.81 ^h	23.46±0.11 ^b	30.53±2.61 ^f	31.61±0.00 ⁱ	30.73±2.01 ^g	29.12±0.11 ^e	26.31±2.01 ^c	6 -24
Creatinine (mg/dL)	6.83 ± 1.02 ^c	6.48± 0.05 ^a	8.55 ± 0.66 ^g	7.79± 0.02 ^e	8.94 ± 0.19 ^h	7.95± 0.09 ^f	8.98 ± 0.19 ⁱ	7.00±0.00 ^d	6.48 ± 1.01 ^b	6.20-10.50
Uric Acid (mg/dL)	7.27 ± 1.05 ^e	5.59±0.00 ^a	6.41 ± 0.92 ^c	6.64±0.12 ^d	7.28 ± 0.34 ^f	8.23±0.20 ⁱ	7.68 ± 0.14 ^g	8.11±0.03 ^h	6.23 ± 0.75 ^b	3.5 – 7.20

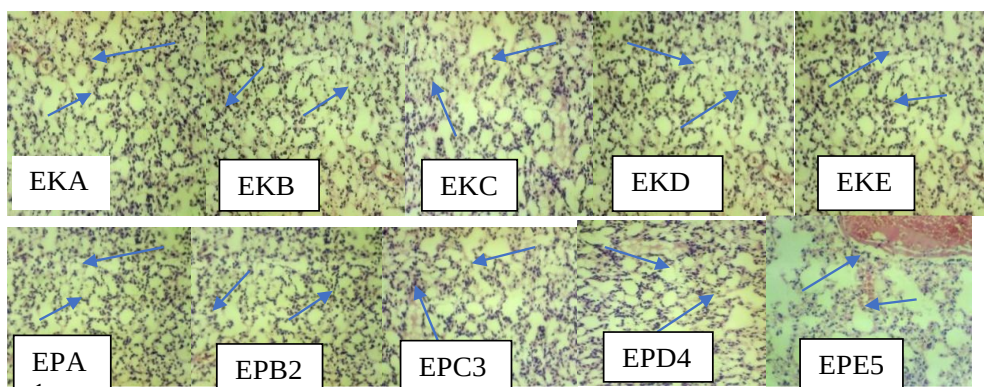
Values are Expressed as Mean ± Standard Error. Values Bearing Different Superscript Letters Within the Same Row Differ Significantly from the Control (0%) at p<0.05 **Keys:** ALT=Alamine Transaminase, AST=Aspartate Transaminase, ALP=Alkaline Phosphatase, IFCC= International Federation of Clinical Chemistry and Laboratory Medicine

Table 3 shows that the haematological evaluation of experimental animals that were subjected to both the chloroform crude extract of *Euphorbia kamerunica* (Ek) and *Euphorbia poissonii* Pax (Ep) revealed contrasting toxicological profile of the two species. The two extracts had different effects on haemoglobin and PCV. Hb ($10.25\text{--}10.54\text{ g/dL}$) and PCV (approximately 41–42%) were relatively stable over the concentrations tested and were within reference limits at all concentrations, except for Hb, which ranged at the lower end of the reference range at each concentration tested compared to the comparator, pepper-spray (11.04 g/dL). Ep, however, had a progressive decrease in both Hb (11.75 g/dL at 2%, 9.10 g/dL at 10%) and PCV (39.40% at 2% to 26.03% at 10%), with only the lower limit of Hb being within the reference interval. The fall in Hb was matched by a corresponding fall in PCV and this would suggest that the erythrocyte disturbance due to Ep was a highly haemolytic and suppressed erythropoiesis rather than purely suppressed erythropoiesis (Obiandu et al., 2023). MCV, MCH and MCHC showed significant differences between the two extracts. However, MCV ($54.50\text{--}55.50\text{ fL}$) was below the reference range ($80\text{--}100\text{ fL}$), and MCH ($45.50\text{--}52.30\text{ pg/cell}$) and MCHC ($41.00\text{--}47.00\text{ g/dL}$) were above their respective reference ranges ($27\text{--}33\text{ pg/cell}$ and $32\text{--}36\text{ g/dL}$). The combination suggests that the main erythrocytic effect of Ek was neither a decrease in red-cell mass but rather a change in cell size and packing of haemoglobin. Ep showed the opposite pattern, with MCV falling from 87.50 fL at 2% (within the reference) to 48.05 fL at 8% and 46.12 fL at 10% (both below $80\text{--}100\text{ fL}$) and MCH falling in parallel, from 29.30 pg/cell at 2% to 25.60 pg/cell at 8% and 25.20 pg/cell at 10% (both marginally below $27\text{--}33\text{ pg/cell}$); MCHC was also found to be below $32\text{--}36\text{ g/dL}$, decreasing from 33.20 g/dL at 2% to 29.40 g/dL at 8% and 31.20 g/dL at 10%. From these observed lower MCV, MCH and MCHC at higher Ep concentrations suggest a tendency to smaller and less haemoglobinised erythrocytes (Patowary et al., 2020; Andrei et al., 2023), which is suggestive of possible interference of the terpenoid constituents with the erythrocyte morphology; while the higher MCH/MCHC, and lower MCV at lower Ep concentrations suggests a different, non-anaemic disturbance of erythrocyte character, suggested by Ek. The difference in RBC count was also quite large for extracts. Despite the low MCV, the cells' haemoglobin content was elevated and reflected in the elevated Ek count ($6.170\text{--}7.025 \times 10^{12}/\text{L}$), meaning more cells with less haematoglobin content. Ep's RBC counts fell progressively from $4.89 \times 10^{12}/\text{L}$ at 2% to a low of $4.14 \times 10^{12}/\text{L}$ at 8% the only concentration falling below the reference range before rising again to $5.40 \times 10^{12}/\text{L}$ at 10%. This partial rebound at the highest concentration in this study does not seem to follow a monotonic dose–response and thus, requires of further investigation. The absolute values of platelets in both extract groups were also near, and mostly below, the lower limit of the reference interval, ranging from $141.50\text{--}147.50 \times 10^6/\text{L}$ in the former, and from $143.50\text{--}153.0 \times 10^6/\text{L}$ in the latter, with no consistent dose-dependent decrease seen in either; it is again noteworthy that the absolute values for this parameter were generally low compared to the reference interval. Both extracts caused a rise in total white blood cells (TWBC) with concentration, but only Ep caused leukocytosis, which exceeded the reference values (4.00--

$11.00 \times 10^9/\text{L}$) at all concentrations ($12.56\text{--}16.30 \times 10^9/\text{L}$); at 2% ($9.41 \times 10^9/\text{L}$) and 4% ($11.00 \times 10^9/\text{L}$), Ek values were within the reference range, and at 8% ($11.01 \times 10^9/\text{L}$) and 10% ($12.515 \times 10^9/\text{L}$) were just at the upper limit of the reference range. This concentration-dependent increase of TWBC is in line with systemic immune activation, likely due to the sesquiterpenoid and triterpenoid content observed by GC–MS after both extracts (Ep in Figure 1A and B) were administered probably indicated only a systemic response (Oloninefa et al., 2023; Miafo et al., 2025). The biochemical assessment of the experimental animals treated with the chloroform crude extracts of *E. kamerunica* (Ek) and *E. poissonii* Pax (Ep) as presented in Table 4 revealed that the main enzymes of the liver (ALT, AST and ALP) were not altered from their respective reference ranges at all the concentrations examined, indicating no hepatocellular damage or biliary obstruction under the experimental conditions. This is in line with the lack of biochemical evidence of liver damage reported at sub-toxic doses of related plant extracts (Izunya et al., 2024; Lu et al., 2024). The concentration of albumin however was not found to be below the reference range ($35.00\text{--}52.00\text{ g/L}$) in both extracts at all the concentrations tested and not in Ep alone. Ek's albumin fell from 21.19 g/L at 2% to 15.14 g/L at 10%, while Ep's fell from 32.77 g/L at 2% to 17.20 g/L at 10%. In all the matched concentrations except 2%, the albumin concentration in the Ek extract was, indeed, lower than the albumin concentration in the Ep extract, and the signal for hepatopathy/inflammation was more evident in the extracts at higher concentrations. Renal indices were variable. Renal impairment was not suggested for either extract in all concentrations because the values of creatinine remained within the reference range of $6.20\text{--}10.50\text{ mg/dL}$. Nevertheless, the concentration of urea was higher than the upper limit for both Ek ($28.80\text{--}31.40\text{ mg/dL}$) and Ep ($20.89\text{--}29.12\text{ mg/dL}$) at all the concentrations tested, not only at high concentrations, and higher than the upper limit for Ep at 8% (31.61 mg/dL) and 10% (29.12 mg/dL) and close to the upper limit for Ep at 4% (23.46 mg/dL). Uric acid levels were consistently higher at 8% and 10% (8.23 mg/dL and 8.11 mg/dL respectively, both above range), showing a more definite dose-dependent trend, with increasing uric acid levels corresponding to an increased risk of joint inflammation or systemic inflammation with doses on the higher end (Abbas, 2020; Habibi et al., 2024). In contrast, the uric acid from Ek was more or less flat across test concentration range (6.41 to 7.68 mg/dL). Both extracts were found to be a safer biochemical profile at the lower concentrations tested, with urea and albumin signals present from the lowest concentration tested through both extracts, but not the same for Ep, where hepatic and renal stress markers (albumin, urea and uric acid) increased with concentration from the lowest concentration tested. This separation, which is largely concentration independent for Ek, and dose dependent for Ep, highlights the need for dose optimization of any formulation of any species developed as a potential antiriot plant.

Histopathology Studies

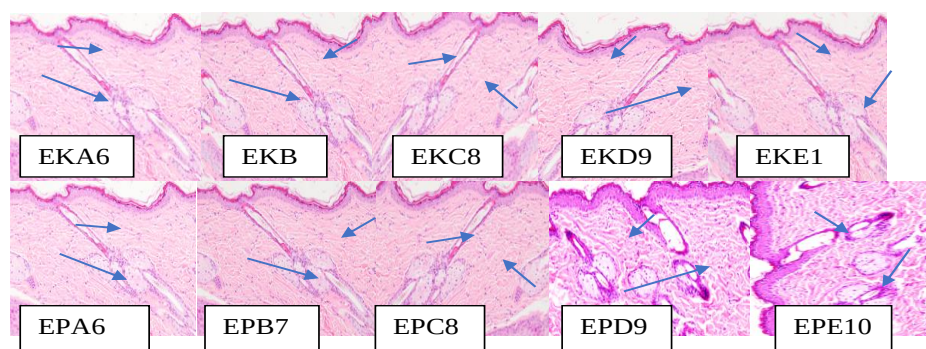
The histopathological examination of sections of lung and skin tissues of mice sprayed with extract Ek and Ep are presented in Plates 1A – E and 2A – E, respectively.



Plates 1: 1A - 1E: A = EKA1 & EPA1 = Normal healthy mice lung tissue sprayed with extract Ek and Ep
 B = EKB2 and EPB2= Lung tissue structure of mice sprayed with 2 % extract Ek and Ep
 C = EKC3 and EPC3= Lung tissue structure of mice sprayed with 4 % extract Ek and Ep
 D = EKD4 and EPD4 = Lung tissue structure of mice sprayed with 8 % extract Ek and Ep
 E = EKE5 and EPE5= Lung tissue structure of mice sprayed with 10 % extract Ek and Ep

The crude extracts from *E. kamerunica* (Ek) and *E. poisonii* (Ep) in chloroform showed different concentration dependent pulmonary profile among two species as shown in Plate I. No histopathological changes were evident in any of the treatment groups, including the highest dosage level tested (10%), suggesting that there was no pulmonary toxicity in the tested dosage range in the administration of extract Ek. Lung tissue by contrast, showed normal structure, with well-defined spaces between alveoli and thin alveolar septa, which is indicative of healthy lung morphology in mice exposed to 2%, 4% and 8% concentration of extract Ep (Rojas-Jiménez, *et al.*, 2024). The alveolar spaces, however, were distorted and tissue injuries were observed with the exposure to 10% concentration of *E. poisonii* extract, which indicated pulmonary response depending on the concentration of the extract at higher doses (Jozwiak *et al.*, 2020). The results of

these analyses have been similar with other *Euphorbia* species such as *Euphorbia humifusa*, *Euphorbia kansui*, and *Euphorbia hirta*, where sub-toxic doses did not cause significant lesions in the respiratory system or disruption of the respiratory tissues (Baba *et al.*, 2025; Noh *et al.*, 2024; Du *et al.*, 2025). Importantly, the severity of the pulmonary injuries was relatively less as compared to the commercial pepper spray (Yiannis *et al.*, 2025). The findings indicate that the magnitude and distribution of tissue changes differed between the extracts and the commercial sprayer under the experimental conditions; however, these observations do not establish comparative safety. Although, histopathological results from this study shows that both extracts are safe for the lungs at lower concentrations and that there is a critical need for dose optimization, especially for the extract Ep when developing plant-based antiriot formulations.



Plates 2: 2A - 2E: A = EKA6 & EPA6 = Normal healthy mice skin sprayed with extract Ek and Ep
 B = EKB7 and EPB7 = Skin structure of mice sprayed with 2 %extract of Ek and Ep
 C = EKC8 and EPC8 = Skin structure of mice sprayed with 4 %extract of Ek and Ep
 D = EKD9 and EPD9 = Skin structure of mice sprayed with 8 % extract of Ek and Ep
 E = EKE10 and EPE10 = Skin structure of mice sprayed with 10 %extract of Ek and Ep

Plates 2A-2E above show the histopathological examination of skin tissues of mice when exposed to the chloroform crude extracts (Ek) of *E. kamerunica* and *Euphorbia poisonii* at different concentrations. In all treatment groups (from 0% to 10%) the architecture of the skin remained preserved. This means that there was no visible dermal damage(s) observed in the tested dosage range. In all extract Ek treated groups, no epidermal disruption, inflammatory cell infiltration, necrosis or oedema was found, indicating that the dermal protective mechanisms of the experimental animals were unaffected at all the administered concentrations. Likewise, the

micrographs of mice exposed to 2% and 4% concentrations of extract Ep showed no significant structural changes in the dermal tissues; this suggests that the protective mechanisms of the skin were able to cope to a significant extent with the irritant effect of the extract which was present at lower concentrations (Zhang *et al.*, 2024). In the micrographs of the mice exposed to 8% and 10% of extract Ep, dermal tissue mild alteration and injuries were observed. These minor histological changes, as indicated by the arrows in the micrographs, suggest tissue alterations manifesting especially, at higher concentrations (Schep *et al.*, 2023). In

addition, studies of biomaterials with scaffolds loaded with extracts from *Euphorbia* species showed no significant cytotoxicity in fibroblast cell lines, and promising in-vivo outcomes in wound healing in the skin, further corroborating the dermal safety profile of *Euphorbia* species at experimental doses (Baba *et al.*, 2025). However, it should be recognized that raw latex from *Euphorbia* spp. are naturally irritant and toxicity of this genus is species-dependent and dose dependent (Kgosiemang *et al.*, 2025).

CONCLUSION

In conclusion, chloroform extract of *E. kamerunica* (Ek) and *E. poisonii* (Ep) revealed significant amount of sesquiterpenoids (55.25% and 96%), respectively, while a significant presence of triterpenoids (43.58 %) was also observed in extract Ek. Both extracts caused a concentration-dependent discomfort and incapacitation, with the incapacitating effect of Ep at 10% being similar to that of commercial pepper spray at 3%. The extracts inhibited acetylcholinesterase enzyme (AChE) in a concentration-dependent fashion with Ep producing 86.90% inhibition than *E. kamerunica* (64%), both at 10%. The biodegradability studies of both extracts revealed a progressive dissipation of both extracts into the environment by a decrease in their weights as the days progressed to 28 days. Also, an increase in the amount of CO₂ generated, as well as; temporal changes in the microbial population and soil pH confirmed the degradability of both extracts, suggesting the mineralization of both extracts under the experimental conditions. The toxicology assessment of both extracts exhibited changes in some haematological, biochemical and histopathological parameters in experimental animals. The results of this study indicate that the bioactive compounds of the aerial parts of *E. kamerunica* and stem of *E. poisonii* could be a source of the observed antiriot potential; hypothetically these effects could occur as a result of the effect of each compound or a synergistic interaction between multiple compounds, since the crude extracts tested in this study were not fractionated to determine the effects of individual compounds. The extracts demonstrated preliminary biological activity and appreciable biodegradation under the experimental conditions, although; at higher concentrations produced adverse effects. It is recommended that further studies should entail the testing of formulations of blends of both extract and further evaluation of these extracts and compound-specific toxicology is required prior to application.

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ETHICAL APPROVAL

Regulated and approved by the Ethical Committee on the use of Laboratory Animals of FUTMINNA, conforming to the National Research Council Guide.

CONFLICT OF INTEREST

The authors declare no potential conflicts of interest with respect to the research authorship, and/or publication of this article.

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