Advances in Sciences and Arts

Journal homepage: https://asa.must.ac.mw/

RESEARCH ARTICLE
Vol. 2 Issue 1

Category
Science

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Citation
Mwambyale *et al*. (2025). Antioxidant properties and safety of Paederia bojeriana (A.Rich. ex DC.) Drake used by traditional herbal practitioners to manage asthma in Malawi. *Advances in Sciences and Arts*. 2(1). https://doi.org/10.37872/2025.0201.04

Supporting info
Please refer to the journal's official website on https://asa.must.ac.mw/

Received
13th Nov 2024

Accepted
1st Feb, 2025

Published
14th Mar, 2025

DOI
https://doi.org/10.37872/2025.0201.04

Antioxidant properties and safety of *Paederia bojeriana (A.Rich. ex DC.) Drake* used by traditional herbal practitioners to manage asthma in Malawi

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Abstract This study assessed the phytochemical composition, antioxidant activity and safety of *Paederia bojeriana (A.Rich. ex DC.) Drake* claimed to manage asthma by Malawian traditional herbal practitioners. Crude extracts were analysed for total phenolic content (TPC), total flavonoid content (TFC), ferric reducing antioxidant power (FRAP) and 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) using standard assays. The TPC and TFC ranged from -1.67 ± 0.01 mg GAE/g to -22.60 ± 0.20 mg GAE/g DW and 13.49 ± 0.01 mg QE/g to 64.37 ± 0.06 mg QE/g DW, respectively. FRAP values ranged from 6.00 ± 0.08 mg TEAC/g DW to 9.42 ± 1.00 mg TEAC/g DW, while the scavenging activity (SA_{50}) of the extracts ranged from 0.13 ± 0.01 µg/mL to 2.65 ± 0.01 µg/mL of extract. The extracts exhibited the Brine shrimp lethality *in vitro* LC_{50} value of 11.09 µg/mL whilst acute oral toxicity *in vivo* LC_{50} category of > 2000 mg/kg - 5000 mg/kg. SA_{50} values of less than 50 ug/mL imply that *Paederia bojeriana (A.Rich. ex DC.) Drake* has strong DPPH antioxidant activity. Based on the levels of phenolic compounds and antioxidant activity, *Paederia bojeriana (A.Rich. ex DC.) Drake* seems to scave and antioxidants in the management of asthma.

Keywords: *Phytochemicals; asthma; phenols; toxicity; antioxidant activity; medicinal plants*

Background

Asthma is a chronic non-communicable lung disease characterized by recurrent attacks of breathlessness and wheezing, which vary in severity and frequency from person to person (WHO, 2013). Asthma involves an imbalance between T helper (Th)1- and Th2-related factors (Keyhanmanesh et al., 2015; Sandeep et al., 2010), including interleukin (IL)-12, interferon-γ (IFN-γ), IL-4, IL-5, IL-13, IL-6 and tumor necrosis factor-α (TNF-α). The cytokines, IL-4 and IL-13 play important roles in asthma (Murad & Hasanin, 2014). IL-4 attracts eosinophils into the interspaces of pulmonary tissues (Seo et al., 2016). IL-13 activates B cells and induces asthma-related changes such as the excessive production of mucus, goblet cell hyperplasia, epithelial cell shedding, basement membrane thickening, and lymphocyte infiltration (Lee et al., 2009; Seo et al., 2016). There is no conventional drug used to cure asthma. The current available drugs are meant to alleviate symptoms temporarily and these include corticosteroids, leukotriene antagonists, bronchodilators, and recently developed anti-IgE antibodies (Global Asthma Network, 2018). Moreover, remedies such as corticosteroids have well known adverse effects including growth inhibition in children (Houglum, 2000), hypertension, peptic ulcers, myopathy and immunosuppressive effects (Lloyd & Hawrylowicz, 2009) necessitating the search for new anti-asthma agents from natural sources including plants, which are cheaper, more effective and safer in terms of toxicity (Focho et al., 2009; Mponda & Cheng, 2010). Key body organs that respond to toxicity levels include the kidney and liver (Weerakoon et al., 2020). The nephrotoxicity involves renal free radical production and accumulation, consumption of antioxidant defense mechanisms, glomerular congestion, and acute tubular necrosis (Elfarra et al., 1994; Mingeot-Leclercq & Tulkens, 1999) leading to diminished creatinine clearance and renal dysfunction. The pathological mechanisms also involve significant increase in macrophage infiltration into the renal cortex and medulla, augmentation of oxidative stress, apoptosis and necrosis (Geleilete et al., 2002). Liver hepatocytes are damaged by oxidative stress (Li et al., 2015). Furthermore, Kupffer cells, hepatic stellate cells, and endothelial cells may be more exposed to or susceptible to oxidative stress-related substances. Kupffer cells produce a multitude of cytokines in response to oxidative stress, including TNF-α, which can increase inflammation and apoptosis (Li et al., 2015; Muriel & Gordillo, 2016). Centrilobular cell necrosis, inflammatory cell infiltration, particularly neutrophils, and persistent cell death with morphological hallmarks of necrosis are pathological responses to drug-induced toxicity in severe liver injury (Li et al., 2015). Inflammation and reactive oxygen substances play an important role in acute kidney and liver injury pathophysiology.

Paederia bojeriana (A.Rich. ex DC.) Drake is one of the plants used for medicinal and nutritional purposes. The plant belongs to the family *Rubiaceae* (Backlund et al., 2007). However, other plants belonging to the same genus *Paederia* such as *Paederia foetida* and *Paederia scandens* have been linked to wound healing, treatment of allergies, abdominal ulcers, infertility, diabetes, dysentery and inflammation due to their high levels of phenols, flavonoids and antioxidant activity (Chu et al., 2008; Ma et al., 2009; Tan et al., 2019). In Malawi, *Paederia bojeriana (A.Rich. ex DC.) Drake* leaves and roots have been reported by the local traditional herbal practitioners in the management of asthma (Mponda & Cheng, 2010) although literature on the chemical composition and bioactivities of *Paederia bojeriana (A.Rich. ex DC.) Drake* is limited. It was because of this lack of knowledge that this study was designed to assess the phytochemical composition, antioxidant activity and safety of *Paederia bojeriana (A.Rich. ex DC.) Drake* claimed to manage asthma by Malawian traditional herbal practitioners.

1. Materials and methods

Chemicals

Chemicals such as ascorbic acid ((R)-3,4-dihydroxy-5-((S)-1,2-dihydroxyethyl)furan-2(5H)-one), Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), gallic acid (3,4,5-trihydroxybenzoic acid), quercetin dehydrate (3,5,7-trihydroxy-2-(3,4-dihydroxyphenyl)-4H-chromen-4-one dihydrate) and caffeine (1,3,7-trimethyl-1H-purine-2,6(3H,7H)-dione) that were used as standards were of analytical reagent (AR) grade. A solution of 98 % v/v sulphuric acid, anhydrous sodium sulphate, ammonium molybdate, citric acid monohydrate, glacial acetic acid, sodium acetate trihydrate, iron (III) chloride hexahydrate, aluminium (III) chloride, di-Sodium orthophosphate (Na_2HPO_4), citric acid monohydrate, anhydrous sodium carbonate, 32 % v/v hydrochloric acid, sodium acetate trihydrate, sodium hydroxide pellets, bromocresol green, and chloroform were purchased from Saarchem (RSA); Folin-Ciocalteu phenol reagent, 2,4,6-tris-2-pyridyl-s-triazine (TPTZ) were purchased from Sigma (USA) while 2,2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sicco Research Laboratories (India). Trolox was purchased from Calbiochem (Germany); Quercetin dihydrate was from EMD Millipore (USA), while caffeine was purchased from BDH Chemicals (UK).

Collection and identification of plant material

The Kamuzu University of Health Sciences, Formerly University of Malawi´s College of Medicine Research and Ethics Committee (CoMREC) approved the study with a clearance certificate number P.11/18/2523. Fresh *Paederia bojeriana (A.Rich. ex DC.) Drake* plant was sustainably harvested during the dry cold season of 2020 from Chikwawa district, Malawi and authenticated by the botanist of the National Herbarium and Botanical Gardens of Malawi.

Preparation of the plant extract

Using standard procedures, the roots were cleaned with distilled water and air-dried under the shade at room temperature. The dried roots were processed into powder using a blender (Kenwood) and stored in air-tight containers in a cool, dry place, away from direct sunlight. The powder was macerated with 99.9% Ethanol for 48 hours, filtered using MN 615, 125mm filter paper. The filtrate was evaporated using a rotary evaporator (Buchi, Switzerland) to obtain the crude extract.

Phytochemical analysis

Crude extracts were analysed for total phenolic content (TPC), total flavonoid content (TFC), total alkaloid content (TAC), ferric reducing antioxidant power (FRAP) and 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) using standard assays.

Total phenolic content

Total phenolic content (TPC) was determined using Folin- Ciocalteu (FC) assay as previously described (Blainski et al., 2013). Standards of gallic acid ranging from 0-100 mg/L and blank (80% v/v methanol) were prepared. Aliquots of both standards, 1 mg/mL extracts, and blank (1 mL), were transferred into 15 mL falcon tubes using Eppendorf micropipette followed by addition of 10-fold diluted FC reagent (5 mL) and 1 M sodium carbonate (4 mL) using a sample dispenser. The preparation of the samples and reagents was done within 3-8 minutes followed by vortexing for 1 minute and left to stand for 2 hours to allow colour development. The samples were then transferred into 10 mm cuvettes and their absorbance read at 765 nm using a UV/Vis spectrophotometer. The TPC analysis was done in triplicate, and the results were expressed as milligram of gallic acid equivalents per gram of dry weight (mg GAE g^{-1} DW).

Total flavonoid content

Total flavonoid content (TFC) was determined by aluminum chloride method using quercetin as a standard (Da Silva et al., 2015) with absorbance at 517 nm. The TFC analysis was done in triplicate,

and the results were expressed as milligram of quercetin equivalent per gram of dry weight (mg QE g^{-1} DW).

Antioxidant assays

DPPH Free radical scavenging capacity

The free radical scavenging capacity of *Paederia bojeriana (A.Rich. ex DC.) Drake* was determined by percentage inhibition using the following formula: *DPPH Scavenged (%) = ((AB–AA)/AB) ×100* where, AB is absorbance of blank at t=0 min; AA is absorbance of the antioxidant at t=30 min (Kedare & Singh, 2011).

Ferric reducing antioxidant power (FRAP) The FRAP method (ferric reducing antioxidant power) was used to assess how best *Paederia bojeriana (A.Rich. ex DC.) Drake* can chelate iron (from Fe^{3+} to Fe^{2+}). Absorbance was read at 750 nm and Trolox was used as a standard (Rubio et al., 2016). FRAP results were expressed in µM Trolox/100mL of *Paederia bojeriana (A.Rich. ex DC.) Drake.*

Brine shrimp lethality assay

The Brine shrimp lethality assay was used to test toxicity of the herbal extracts. The assay provides broad-spectrum preliminary toxicity screening. It is also works as an effective predictor of bioactivity and more importantly, commonly used to test cytotoxicity of medicinal plants (Pisutthanan et al., 2004; Sarah et al., 2017). A stock solution of the herbal extracts was prepared whereby 4 mg of crude extract was diluted in 4 mL of 0.5% DMSO, which gives a concentration of 1 mg/mL (1000 µg/mL) and serial dilution was done by taking 1 mL from the stock solution and adding 9 mL of 0.5% DMSO. In order to obtain the lower concentrations, 1 mL was taken from the previous concentration and 9 mL of 0.5% DMSO was added; the process was repeated until the desirable concentrations were reached.

The process started by hatching the shrimp eggs and incubating the eggs under a fluorescent light (60-watt bulb) for 48 hours in saline solution prepared from table salt (38 g in 1-liter of distilled water) in a 2-liter rectangular container. The eggs hatched into larvae (nauplii). Ten larvae were placed into each of the test tubes (40 larvae per herbal extract) using pipettes; herbal extracts were added to the test tubes in concentrations of 1, 10, 100 and 1000 µg/mL as per standard protocol (Sarah et al., 2017) and were checked after 24 hours to check the amount of live nauplii, and the figures recorded. The mortality of the nauplii was calculated in percentages (Moshi et al., 2010; Olowa & Nuneza, 2013; Pisutthanan et al., 2004; Sarah et al., 2017). For each of the concentrations, positive and negative controls were prepared in triplicates.

The results were then classified using standard Clarkson toxicity criteria and Meyer's toxicity index as previously described (Daniel & Ekam, 2016). Meyer's toxicity index classifies extracts of LC_{50} <1000 µg/mL as toxic whilst extracts of LC_{50} > 1000 µg/mL as non-toxic. Clarkson toxicity criteria further groups the extracts into detailed classification such as LC_{50} > 1000 µg/mL as non - toxic, 500 – 1000 µg/mL as low toxicity, 100 – 500 µg/mL as moderate toxicity and 0 – 100 µg/mL as high toxicity (Famewo et al., 2017; R. Hamidi et al., 2014).

Animals and acute oral toxicity test

Twenty-four female albino, nulliparous and non-pregnant Wistar rats, 8 to 12 weeks old, weighing 160-300 g were housed in a temperature-controlled room under 12-hr-light/-dark period in polypropylene cages (3 rats per cage). They were provided with food and water *ad libitum* (Moraal et al., 2012). Rats were randomly selected, grouped and marked for individual identification and caged per dose. Herbal extracts were administered in a constant volume over two fixed dose levels of 300 and 2000 mg/kg body weight (Awounfack et al., 2016a; Omotoso et al., 2017; *Test No. 436: Acute Inhalation Toxicity – Acute Toxic Class Method*, 2009) and three animals were used for each dose. The rats were fasted overnight; however, water was *ad libitum*. After the fast, the rats were weighed, and the extract was administered in a single dose by gavage using an intubation cannula after which

food was withheld for a further 3-4 hours (Omotoso et al., 2017; *Test No. 436: Acute Inhalation Toxicity – Acute Toxic Class Method*, 2009)

After dosing, the rats were observed once at the first 30 minutes then at the 4th hour and daily over the span of 14 days.

Observations were made for signs of toxicity, which included changes in behavior, cardiorespiratory, autonomic, neuromuscular, skin, fur, eyes and mucosal membranes. All observations were recorded for each rat. Any animals found in severe distress, pain and morbid condition, were euthanized (Awounfack et al., 2016b; El Kabbaoui et al., 2017; *Test No. 436: Acute Inhalation Toxicity – Acute Toxic Class Method*, 2009). After the span of 14 days, the rats were euthanized. Gross necroscopy and microscopic examination of the liver and kidney were done, and pathological changes recorded for each animal.

Histological tissue preparation, microscopy and image analysis

Using standard procedures, kidney and liver samples for hematoxylin and eosin staining and light microscopy were fixed in 10% buffered formalin for 48 hours (Karaer et al., 2005), then placed in an automatic tissue processor (Shandon Citadel 1000, Labotek, South Africa) in which samples were incubated in a series of 70%, 95%, 95%, 95%, 100%, 100%, 100% ethanol, then in chloroform and finally in paraffin wax. Samples were then embedded in paraffin wax. All histological glass slides that were used in this study were gelatin-coated (Jan & Lashuel, 2013) in order to stick the tissue sections to the slides during the staining and washing process. Histological sections (5µm) were cut using a Leica RM 2245 Biocut microtome (Leica, Nussloch, Germany) and then dewaxed overnight in an oven at 60^0C, then immersed in histoclear for 5 minutes and then repeated in fresh histoclear for 5 minutes, rehydrated through a graded series of ethanol (100%, 100%, 95%, 80%, 70% and 60%) for 30 seconds each, then washed in running water for 5 minutes (Karaer et al., 2005). Histo-morphometric parameters evaluated are shown in Table 1, which were examined and measured using the Zeiss Axioscope A1. Images were acquired using the Zeiss Axiocam 105 color camera with Zeiss software (Carl Zeiss, Jena, Germany).

A total of 20 images per animal were assessed to generate the results. The observed fields were randomly selected and assessed by three independent histologists. Light microscopic examinations were done on the liver and kidney tissues in order to identify structural changes caused by the herbal plant extracts. The effect of the herbal extracts was measured using a semiquantitative histological scoring system adapted from previous studies (A. Ahmed et al., 2012) (Table 1).

Liver	***Kidney***
1. Hepatocellular necrosis or degeneration	1. Tubule interstitial leukocytic infiltration or nephritis in cortex and medulla
2. Sinusoidal congestion	2. Widening of the urinary space
3. Portal triad and lobular inflammation	3. Tubular epithelial degeneration (necrosis with dark acidophilic cytoplasm; acellular sections of tubules; loss of tubular epithelial cells into tubular lumen)
4. Cytoplasmic vacuolization	4. Congestion of glomeruli
	5. Podocyte hyperplasia
	6. Diffuse cortical and medullary regions

Histological grading: 0 = normal histological tissue; 1 = mild (1-10%); 2 = moderate (11-25%); 3 = severe (26-50%); 4 = very severe (51-100%).

Statistical analysis

Data were analyzed using a one-way ANOVA followed by a Tukey-Kramer *post hoc*. Data were expressed as means ± standard deviations and differences were considered statistically significant when $p<0.05$.

Results

Qualitative phytochemical screening

The preliminary qualitative phytochemical screening of both extracts dissolved in 0.5% DMSO and 99% ethanol showed the presence of phenols, tannins, saponins, glycosides and phytosterols.

Quantitative phytochemical analysis

The quantitative phytochemical screening showed moderate amounts of flavonoids, negligible amount of phenols and high scavenging activity as described in Table 2.

Table 2: Total phenol, flavonoid content and antioxidant capacity of *Paederia bojeriana (A.Rich. ex DC.) Drake* extracts dissolved in 0.5% DMSO and 99% ethanol.

Assay	**Extract**	**Parameter**	**p-value**	**Calibration curve and R^2 values**
DPPH Free Radical Scavenging Activity	0.5% DMSO	80.68±0.04 Inhibition (%)	0.80	
	99% (v/v) Ethanol	82.85±0.07 Inhibition (%)		
FRAP	0.5% DMSO	6.00±0.08 mg/TAEC/g	0.23	y = 0.0031x+0.1248
	99% (v/v) Ethanol	9.42±1.00 mg/TAEC/g		R^2 = 0.9959
Flavonoid	0.5% DMSO	13.49±0.01 QE/mg	0.05	y = 0.0002x + 0.098
	99% (v/v) Ethanol	64.37±0.06 QE/mg		R^2 = 0.9926
Phenol	0.5% DMSO	-1.67±0.01 mg GAE/g	0.33	y = 0.0009x – 0.103
	99% (v/v) Ethanol	-22.60±0.20 mg GAE/g		R^2 = 0.9997

Paederia bojeriana (A.Rich. ex DC.) Drake extracts have good DPPH scavenging potential; moderate FRAP activity; moderate flavonoids and little phenols.

Brine shrimp assay

The preliminary study to determine the toxicity potential of *Paederia bojeriana (A.Rich. ex DC.) Drake* extracts was done using the Brine shrimp assay. The results shown in Figure 1, demonstrate that the LC_{50} value of ethanolic root extracts of *Paederia bojeriana (A.Rich. ex DC.) Drake* is 11.09 µg/mL which is low and toxic according to Clarkson toxicity criteria (0 – 100 µg/mL) and Meyer's toxicity index (LC_{50} < 1000 µg/mL) (Kamadyaapa et al., 2018). The extracts show significant level of toxicity *in vitro*.

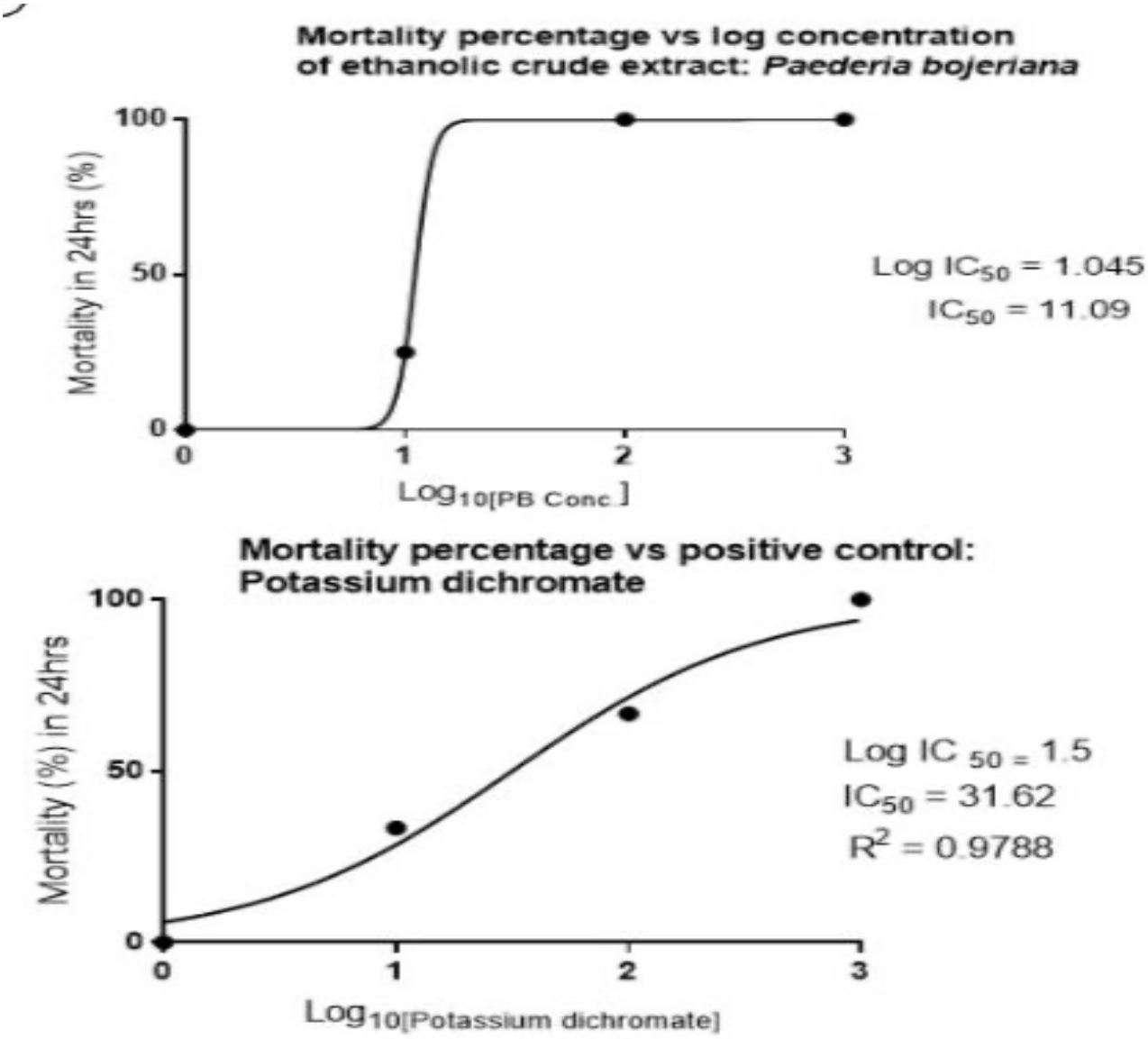

Figure 1: Brine shrimp mortality percentage (24hrs) for *Paederia bojeriana (A.Rich. ex DC.) Drake* and positive control. The LC_{50} value of potassium dichromate is 31.62 µg/mL while that of the ethanolic root extract is 11.09 µg/mL, which is low and toxic.

Acute oral toxicity test

General observations

The rats showed no abnormal behavioral changes during the course of the study with no death occurring in all the 4 groups inclusive of the control group (Table 3).

Table 3: General observations on rats treated with *Paederia bojeriana (A.Rich. ex DC.) Drake*

Observations	30 min	4hrs	24hrs	48hrs	1 week	2 weeks
Eyes	Normal	Normal	Normal	Normal	Normal	Normal
Skin and Fur	Normal	Normal	Normal	Normal	Normal	Normal
Vomiting	N.O	N.O	N.O	N.O	N.O	N.O
Diarrhea	N.O	N.O	N.O	N.O	N.O	N.O
Salivation	Normal	Normal	Normal	Normal	Normal	Normal
Convulsion	N.O	N.O	N.O	N.O	N.O	N.O
Lethargy	N.O	N.O	N.O	N.O	N.O	N.O
Coma	N.O	N.O	N.O	N.O	N.O	N.O

N.O = not observed

Effects of crude extracts on biochemical parameters

The effects of the *Paederia bojeriana (A.Rich. ex DC.) Drake* crude extracts on the biochemical parameters (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and creatinine) in the acute oral toxicity test in Wistar rats are summarized in Figure 2. There is no significant difference ($p \geq 0.05$) in all the biochemical parameters between control and treated groups indicating that the administration of the crude *Paederia bojeriana (A.Rich. ex DC.) Drake* extracts does not affect the biochemical parameters of the rats.

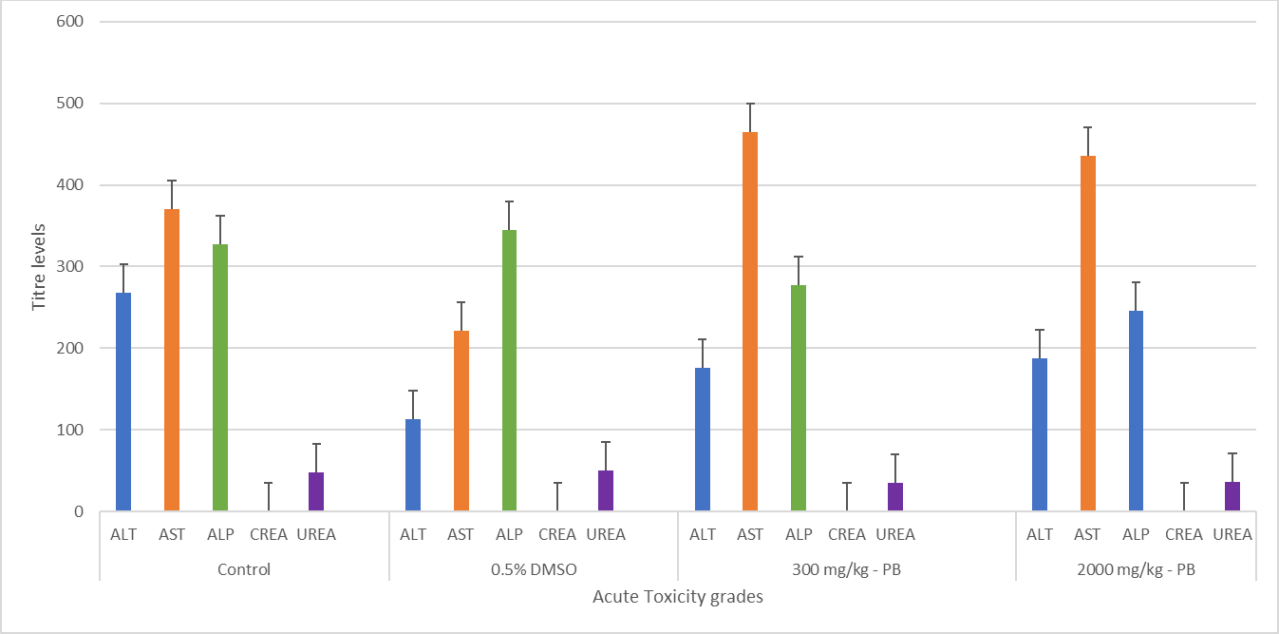

Figure 2: Acute oral toxicity biochemical parameters in the rats treated with 300 mg/kg and 2000 mg/kg dosages of the crude extracts of *Paederia bojeriana (A.Rich. ex DC.) Drake*. AST: aspartate aminotransferase, ALT: alanine aminotransferase, ALP: alkaline phosphatase, CREA: creatinine. The parameters generally showed a lowered ALT, and ALP, elevated AST and unchanged amounts for creatinine and urea in rats treated with 300 mg/kg of crude extract when compared with the control. The parameters showed a lowered ALT, and ALP, elevated AST and unchanged amounts for creatinine and urea in rats treated with 2000 mg/kg of crude extract compared with the control.

Histological architecture of the liver

The following liver histological parameters were assessed: cytoplasmic vacuolization, hepatocellular necrosis and degeneration, portal triad and lobular inflammation, sinusoidal dilation and congestion. There are significant differences ($p \leq 0.05$) between the control groups and *Paederia bojeriana (A.Rich. ex DC.) Drake* 300 mg/kg and 2000 mg/kg treated groups in three histological parameters (cytoplasmic vacuolization, hepatocellular necrosis and degeneration, portal triad and lobular inflammation) except sinusoidal dilation and congestion (Figure 3 and Figure 4); indicating that the administration of the crude extracts generally affected the histological architecture of the liver. There are no significant differences ($p \geq 0.05$) in the liver architecture between the control group and 0.5% DMSO treated rats.

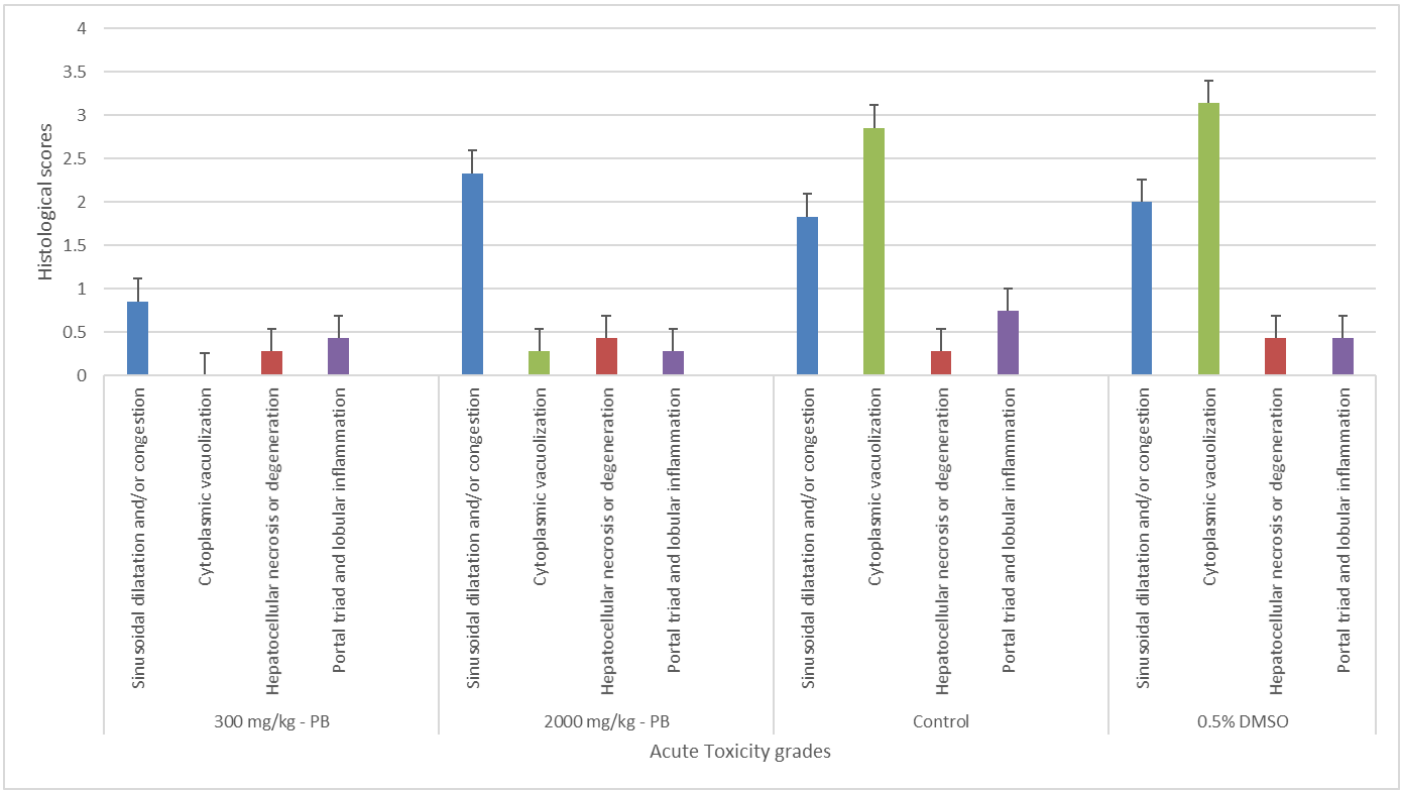

Figure 3: Histological grading for rat liver. There are significant differences ($p \leq 0.05$) between the control groups and *Paederia bojeriana (A.Rich. ex DC.) Drake* 300 mg/kg and 2000 mg/kg treated groups in cytoplasmic vacuolization, hepatocellular necrosis and degeneration, portal triad and lobular inflammation) except sinusoidal dilation and congestion.

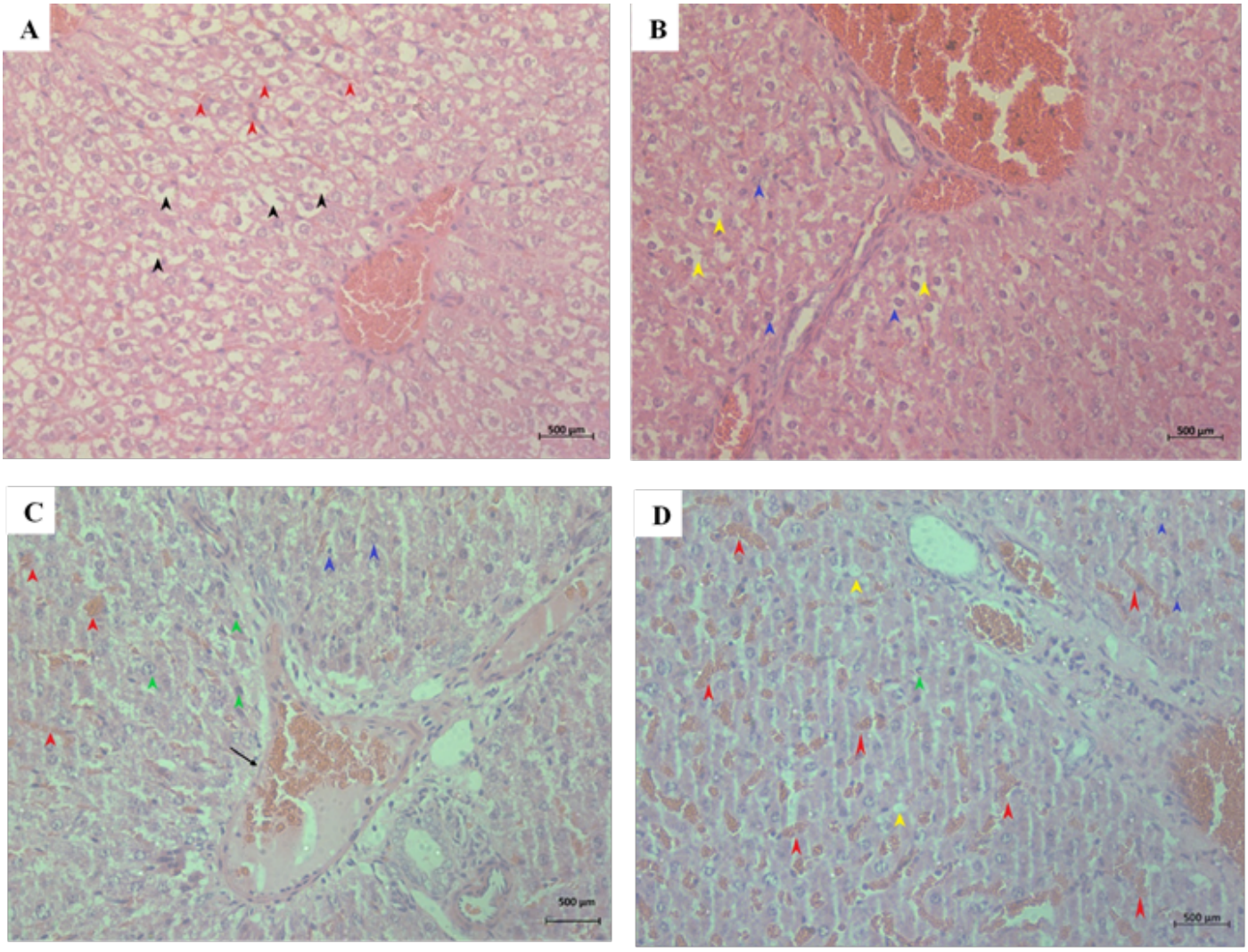

Figure 4: Micrograph showing histological parameters: cytoplasmic vacuolization, hepatocellular necrosis and degeneration, portal triad and lobular inflammation, sinusoidal dilation and congestion in the treated and untreated rat liver; Hematoxylin and Eosin (H & E). (A) Control (Untreated) (x10), (B) 0.5% DMSO(x10), (C) 300 mg/kg *P.bojeriana* (x10), (D) 2000 mg/kg *P.bojeriana* (x10). Red arrowheads - sinusoidal congestion; Blue arrowheads - hepatocellular necrosis; Green arrowheads - lobular inflammation; Yellow and Black arrowheads - cytoplasmic vacuolization. All images are representative of the histological architecture from all the rats in each of the treatment regime.

Histological architecture of the kidney

The following parameters were assessed: leukocyte infiltration, urinary space widening, tubular epithelial degeneration, glomerular congestion, podocyte hyperplasia, diffuse cortical and medullary regions in the rats treated with 300 mg/kg, 2000 mg/kg of the crude extract and control groups. There are no significant differences ($p \geq 0.05$) between the control and 0.5% DMSO treated, 300 mg/kg, 2000 mg/kg of the crude extract in all histological parameters (Figure 5 and Figure 6).

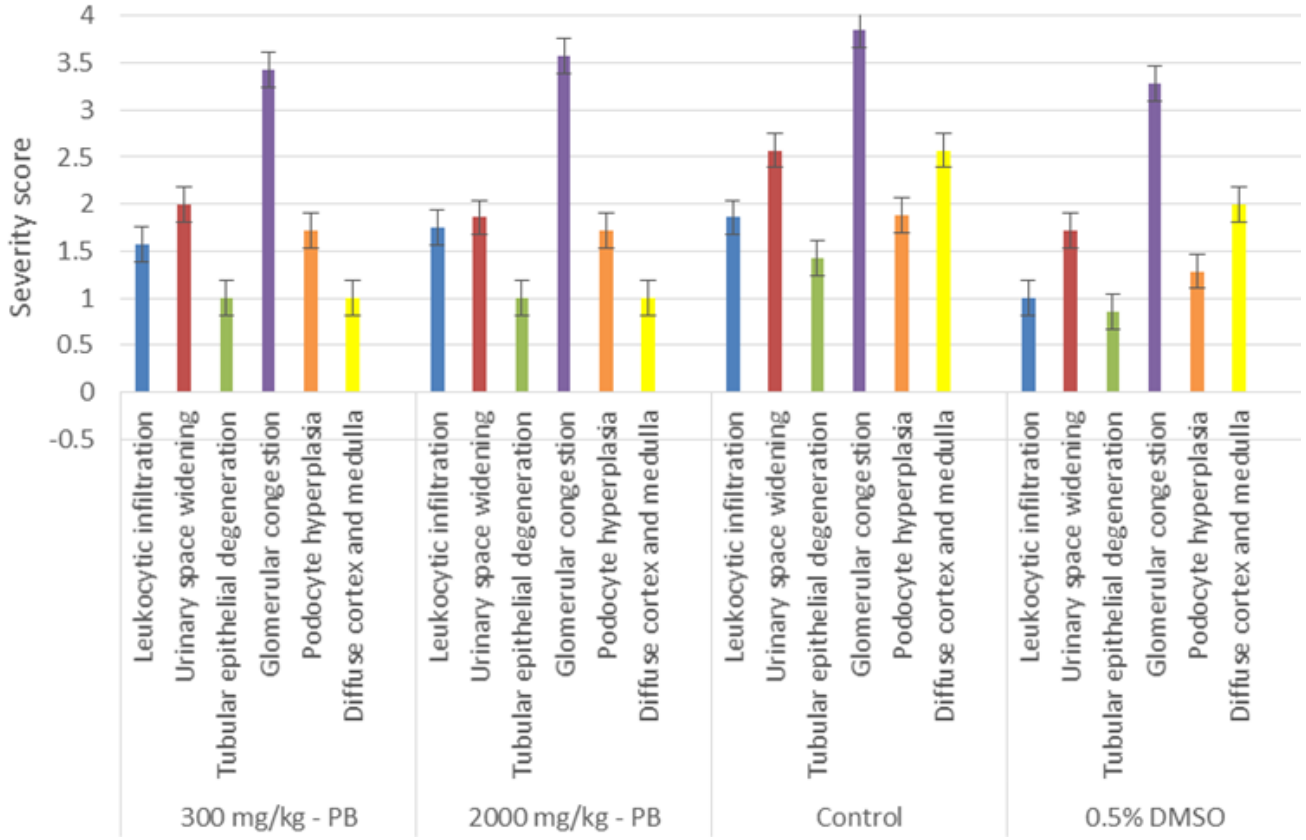

Figure 5: Main histological characteristics of the rat kidney. There are no significant differences between the control and 0.5% DMSO treated; No significant difference is noted between the 300 mg/kg and 2000 mg/kg crude extract treated in all histological parameters ($p \geq 0.05$). Generally, the kidney tissues show mild leukocytic infiltration, moderate urinary space widening, mild tubular epithelial degeneration, severe glomerular congestion, mild podocyte hyperplasia and mild diffuse cortical and medullary regions in the rats treated with the 300 mg/kg of crude extract whilst in the rats treated with 2000 mg/kg of the crude extract, there is moderate leukocytic infiltration, moderate urinary space widening, mild tubular epithelial degeneration, severe glomerular congestion, mild podocyte hyperplasia and mild diffuse cortical and medullary regions.

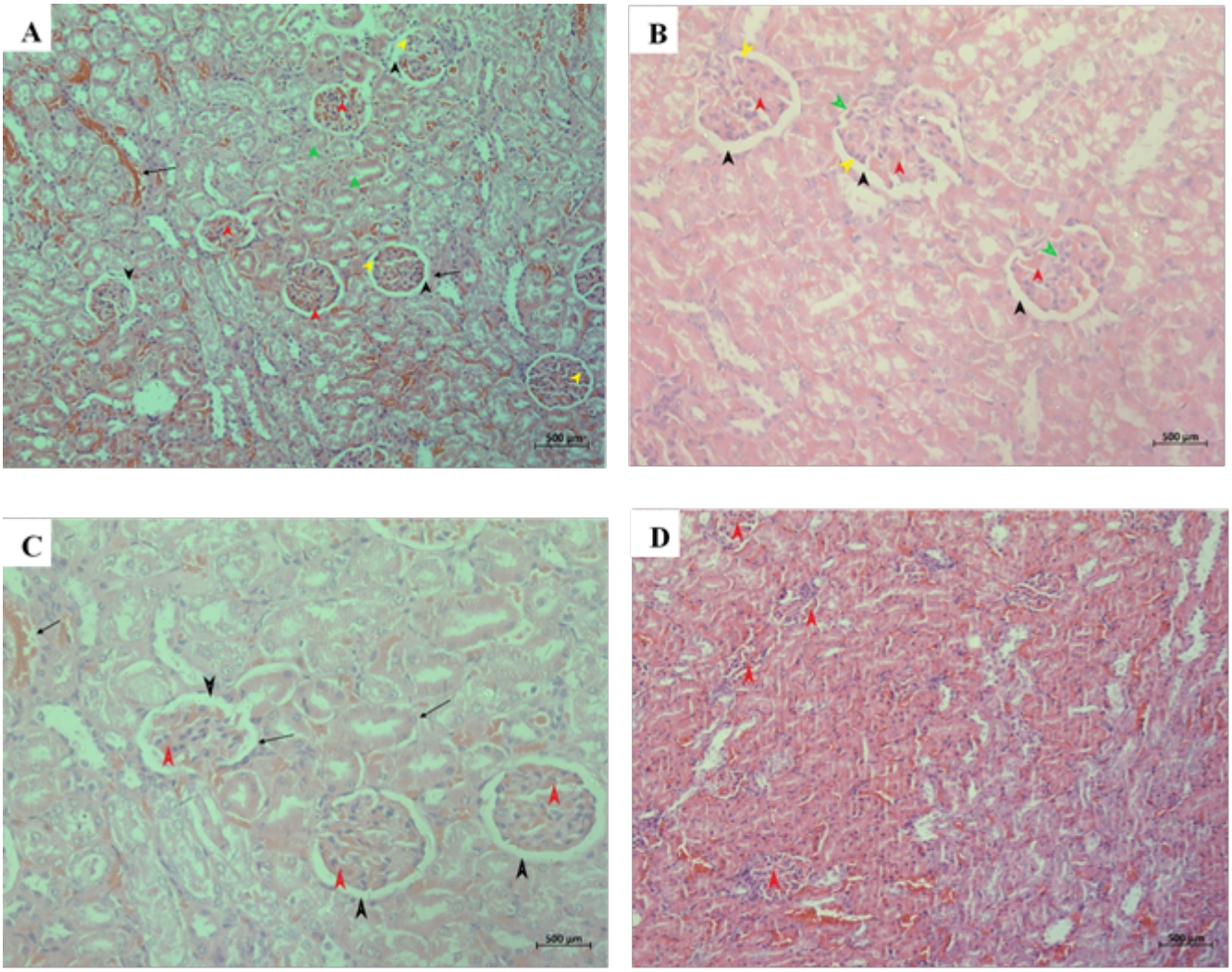

Figure 6: Micrograph showing toxicity histological parameters: leukocytic infiltration, urinary space widening, tubular epithelial degeneration, glomerular congestion, podocyte hyperplasia and diffuse cortical and medullary regions in the treated and untreated rat kidney; Hematoxylin and Eosin (H & E). (A) Control (x10), (B) 0.5% DMSO(x10), (C) 300 mg/kg *P.bojeriana* (x10), (D) 2000 mg/kg *P.bojeriana (*x10). Red arrowheads - glomerular congestion; Yellow arrowheads – podocyte hyperplasia; Green arrowheads – leukocyte inflammation; Black arrowheads – urinary space widening; Black arrows – blood vessels and collecting ducts. All images are representative of the histological architecture from all the rats in each of the treatment regime.

Discussion

In this study, the preliminary phytochemical screening of *Paederia bojeriana (A.Rich. ex DC.) Drake* shows the presence of phenols, tannins, saponins, flavonoids, glycosides and phytosterols. The quantitative phytochemical analysis of the *Paederia bojeriana (A.Rich. ex DC.) Drake* extracts further show trace amount of phenols, moderate amount of flavonoids and high scavenging activity, which might be due to the solubility of the compounds in the extraction solvents. The soluble phenolics are easily extracted by a solvent compared to bound phenolics which are covalently bound within the plant structure (Su et al., 2014). In most studies, it has been established that DMSO provides a good environment for phenolic activity (Eroglu & Girgin, 2021; Sricharoen et al., 2015). This explains the high antioxidant activity of the crude extract implying the interpretation that *Paederia bojeriana (A.Rich. ex DC.) Drake* might have high free radical scavenging capacity, which reduces reactive oxygen species (ROS). This may reduce inflammatory activities suggesting its potential use

in the management of asthma (Edwards, n.d.). Similarly, previous studies have demonstrated the role of flavonoids as phenolic substances with the capacity of suppressing reactive oxygen species (ROS) formation either by inhibition of enzymes or chelating trace elements involved in free radical production, scavenging ROS, and up-regulating antioxidant defenses (Gordon & Graham, 2006). In other words, the free radical scavenging activity of these compounds is due to their free phenolic groups which can donate electrons to the radicals (Mandal et al., 2009). For flavonoids, the O-dihydroxyl structure in the B ring, the 2,3- double bond in conjunction with the 4-oxo function in the C ring, and the 3- and 5-hydroxyl groups with hydrogen bonding to the keto group may be responsible for the antioxidant activity (Yenesew et al., 2009).

This means that inflammatory mediators, such as reactive oxygen species (ROS), histamine and products of arachidonic acid metabolism may induce airway remodeling such as the occurrence of goblet cell hyperplasia, enlarged submucosal mucus glands and vascular congestion, thickening of airway walls, infiltration of inflammatory cells, increases in smooth muscle mass, airway epithelial shedding, and the production of mucus plugs occluding medium and small bronchi (Barrios et al., 2006; Tagaya & Tamaoki, 2007). These structural changes in the airways may lead to airflow obstruction and asthmatic problems (Shifren et al., 2012).

Taken together, the findings from the present study appear to demonstrate the anti-inflammatory potential of *Paederia bojeriana (A.Rich. ex DC.) Drake* against excessive mucus production, goblet cell hyperplasia, epithelial cell shedding, basement membrane thickening, and eosinophil and lymphocyte infiltration suggesting its potential use in the management of asthma (Lee et al., 2009; Seo et al., 2016).

Notably, there are no phytochemical studies found for *Paederia bojeriana (A.Rich. ex DC.) Drake*; however, results from the current study agree with those of Osman et al (Osman et al., 2009) on the methanolic extract of leaves of *Paederia foetida* (same genus), which contain high levels of antioxidant activity (ABTS scavenging activity of dry leaves 67.74% and fresh leaves 75.38%) with high amount of phenols and flavonoids. This is consistent with findings from a study done by Upadhyaya (Upadhyaya, 2013) on the ethanolic extracts of the leaves of *Paederia foetida* which show 74.7% – 88.2% DPPH scavenging activity with high levels of phenols and flavonoids. Moreover, an *in vitro* and *in vivo* study shows that treatment with *Paederia foetida* reduces paw edema, arthritic index induced by Complete Fruend's Adjuvant (CFA) in the knee joints and suppresses prostaglandin E_2 and COX – 2 expression (Kumar et al., 2015). Additionally, a poly herbal formulation containing *Paederia foetida* shows significant reduction in carrageenan induced paw edema and potent analgesic activity (Uddin et al., 2013). Similarly, extracts of *Paederia scandens* have shown to suppress pro-inflammatory mediators (TNF-α and IL-1β) in monosodium urate crystals-induced gouty arthritis rats in synovial tissue (Ma et al., 2009). Iridoid glycosides of *Paederia scandens* have also shown to suppress NF-κBp65, MCP-1, α-SMA, TNF-α and TGF-β1 activity and expression in renal tissue in uric acid nephropathy induced rats (Hou et al., 2014) suggesting that the *Paederia* genus in general seems to have impressive anti - inflammatory and immunomodulatory effects, which correspond with their traditional use in the management of asthma.

Paederia bojeriana (A.Rich. ex DC.) Drake appears to be toxic, which complements previous studies by Ahmed et al (A. M. A. Ahmed et al., 2014) and Morshed et al (Morshed et al., 2012) using Brine Shrimp assay whereby the methanolic leaf extract and whole parts of the plant of *Paederia foetida* showed LC_{50} of 65.31 µg/mL and 1.5625 µg/mL suggesting cytotoxicity of the leaves and whole plant respectively. The crude plant extracts in the current study generally show high toxicity at the cellular level, which could manifest adverse effects when used as traditional medicines

suggesting the interpretation that this needs to be explored further in human cells.

The overall results of the acute oral toxicity test show that the herbal extracts of *Paederia bojeriana (A.Rich. ex DC.) Drake* are of category 5 (>2000 mg/kg – 5000 mg/kg) (*Test No. 436: Acute Inhalation Toxicity – Acute Toxic Class Method*, 2009) with the LC_{50} cut-off mg/kg b.w = ∞ mg/kg with rats not demonstrating abnormal behavioral changes during the course of the study with no death occurring in all the 4 groups inclusive of the control group. The rats have not shown signs of toxic effects. This means that although the extracts exhibit toxicity *in vitro*, they appear safe *in vivo* up to 2000 mg/kg with mild to moderate hepatic and renal toxicity suggesting further studies to explore the sub-chronic, chronic and anti-asthmatic effects of *Paederia bojeriana (A.Rich. ex DC.) Drake* extracts.

Conclusion

Based on the levels of phenolic compounds and antioxidant activity, *Paederia bojeriana (A.Rich. ex DC.) Drake* seems to scavenge free radicals and reduce reactive oxygen species, which may reduce inflammatory activities suggesting its potential use as a medicinal agent and source of natural bioactive compounds and antioxidants in the management of asthma. The extracts exhibit toxicity *in vitro* but appear safe *in vivo* up to 2000 mg/kg with mild to moderate hepatic and renal toxicity suggesting further studies to explore the sub-chronic, chronic and anti-asthmatic effects of *Paederia bojeriana (A.Rich. ex DC.) Drake* extracts.

Acknowledgments

Authors would like to thank the African Centre of Excellence in Public Health and Herbal Medicine (ACEPHEM) for funding this study and the Blantyre to Blantyre Laboratory Facility, Kamuzu University of Health Sciences for providing laboratory equipment for the assays. We are also thankful to the staff of the Malawi National Herbarium and Botanical Gardens of Malawi, Mzuzu office for their technical assistance.

Conflict of interest statement

We declare that we have no conflict of interest.

Author contributions

TM came up with the research concept and was awarded an MPhil research study. MM supervised and provided guidance including processing of the manuscript while IC and AM were involved during data analysis and drafting the manuscript together with the whole team.

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