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Full Length Research Article

ANTI-INFLAMMATORY EFFECT OF THE AQUEOUS EXTRACTS OF LEAVES AND STEM OF *NELSONIA CANESCENS* ON INDUCED OEDEMA IN WISTAR RATS

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ABSTRACT
Inflammation is a critical physiological response to tissue injury or infection, often associated with pain, swelling, and redness. Despite the availability of synthetic anti-inflammatory drugs, their long-term use is frequently accompanied by adverse effects, necessitating the exploration of safer, plant-based alternatives. *Nelsonia canescens*, a herb with traditional medicinal applications in inflammatory conditions, has drawn scientific interest for its therapeutic potential. This study evaluated the phytochemical composition, anti-inflammatory activity, and hematological and histological effects of *Nelsonia canescens* leaf and stem extracts. The extracts were prepared using a cold maceration method, and their phytochemical profiles were analyzed, confirming the presence of significant phytochemicals, including alkaloids, flavonoids, terpenoids, tannins, and saponins, which are known for their therapeutic properties. Anti-inflammatory activity was assessed in adult Wistar rats through a carrageenan-induced paw edema model, where various doses of the aqueous extracts (100, 200, and 400 mg/kg) were administered and compared to a standard anti-inflammatory drug, diclofenac sodium (10 mg/kg). The results showed a significant, dose-dependent reduction in paw edema, with the aqueous leaf extract exhibiting higher activity compared to the stem extract, particularly at higher doses. Furthermore, hematological assessments revealed no significant alterations in white blood cell counts, hemoglobin concentrations, or platelet counts, suggesting that the extracts do not adversely affect blood parameters. These findings shows the potential of *Nelsonia canescens* leaf extract as a natural anti-inflammatory agent, providing a scientific basis for its traditional use in treating inflammatory conditions, while emphasizing its safety profile and efficacy in reducing inflammation.

Keywords: Inflammation, *Nelsonia canescens*, Phytochemical, edema, doses, *haematological* and extracts

INTRODUCTION

Inflammation is a natural defense mechanism triggered by tissue damage, involving processes such as granuloma formation, leukocyte infiltration, and oedema (Hannoodee and Nasuruddin, 2024). Common signs include pain, redness, heat, and swelling, often resulting from increased vascular permeability and accumulation of inflammatory mediators like histamine, serotonin, and bradykinin (Hannoodee and Nasuruddin, 2024; Stone *et al.*, 2024). Acute inflammation is an early, rapid response preceding adaptive immunity and can be studied using carrageenan-induced paw oedema in rats, a widely accepted model that presents biphasic inflammation. The early phase involves histamine and serotonin release, while the late phase is mediated by prostaglandins, neutrophil infiltration, nitric oxide, and cytokines such as IL-1β and TNF-α (Ayertey *et al.*, 2021). Chronic inflammation may result from prolonged mediator activity. Inflammatory stimuli like lipopolysaccharide (LPS) also trigger macrophage activation, further promoting the release of pro-inflammatory agents (Branco *et al.*, 2018; Ishida *et al.*, 2023). Conventional anti-inflammatory drugs such as NSAIDs, steroids, and immunosuppressants are effective but associated with adverse effects like gastrointestinal bleeding and renal toxicity (Amri *et al.*, 2018; Ayertey *et al.*, 2021). As such, research increasingly focuses on plant-based alternatives with fewer side effects. Ethnopharmacology has helped identify many natural compounds with anti-inflammatory potential (Upadhyay and Thakur, 2024). *Nelsonia canescens*, traditionally used in Borno State, Nigeria, for treating inflammatory disorders, offers a promising option for scientific validation. This study evaluated the anti-inflammatory effects of its aqueous leaf and stem extracts in carrageenan-induced oedema in Wistar rats.

MATERIALS AND METHODS

Plant Collection and Extraction

Leaves and stems of *Nelsonia canescens* were collected from Ribadu Cantonment, Kaduna, Nigeria, and authenticated at the Department of Biological Science, Nigerian Defence Academy (voucher number: NDA/BIOH/202447). The plant materials were air-dried, pulverized, and extracted by cold maceration: 300 g of each powdered sample was soaked in 1 L of sterile distilled water with intermittent agitation for 48 hours. The extracts were filtered using muslin cloth and Whatman No.1 filter paper, concentrated at 40°C using a water bath, and stored for use (Judith *et al.*, 2021).

Phytochemical Screening

Qualitative tests for alkaloids, steroids, flavonoids, saponins, phenols, tannins, glycosides, and terpenoids were conducted using standard procedures (Kumar *et al.*, 2009; Ugochukwu *et al.*, 2013; Patil and Nasreen, 2015; Amabye and Tadesse, 2016). Quantitative phytochemical constituents were further analyzed using GC-MS at the Multi-user Laboratory, Ahmadu Bello University, Zaria.

Experimental Animals and Design

Thirty-six adult Wistar rats (120–180 g) were housed under standard conditions and randomly assigned to six groups. Treatments included vehicle (control), aqueous extracts at doses of 100, 200, and 400 mg/kg, and a standard anti-inflammatory drug (diclofenac sodium 10 mg/kg). All treatments were administered orally one hour before carrageenan-induced paw edema, which

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was induced by sub-plantar injection of 0.1 ml of 1% carrageenan in 1% CMC. Paw thickness was measured at 0, 1, 2, 3, and 4 hours using a Vernier caliper. The percentage inhibition of edema was calculated using the method described by (Mohaddesi *et al.*, 2015).

Hematological Analysis
Blood samples collected post-treatment were analyzed for white blood cells (WBC), lymphocytes (LYM), granulocytes (GRAN), red blood cells (RBC), hemoglobin (HGB), and platelets (PLT) using a Diatron Abacus Junior hematology analyzer, following the method of (Olaniyan *et al.*, 2016).

Statistical Analysis
All experiments were performed in triplicate. Data were analyzed using SPSS version 27 and expressed as mean ± SEM. One-way ANOVA was used to compare groups, followed by Tukey's post hoc test. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Extract Yield and Phytochemical Content
The percentage yield of the aqueous extract of *Nelsonia canescens* was higher in the leaves (16.3%) than in the stem (12.3%) as presented in Table 1. Phytochemical screening (Table 2) revealed the presence of alkaloids, flavonoids, saponins, phenols, tannins, and glycosides in the leaf extract, while the stem extract lacked saponins and terpenoids. These constituents are known to contribute to anti-inflammatory activities.

Table 1: *Nelsonia canescens* Leaves and Stem Extracts Characteristics

Plant Material	Initial Weight of Sample (g)	Weight of Extract (g)	Percentage Yield (%)	Colour/Texture
Aqueous Leaf Extract	300.00	43.00	16.3	Greenish-brown, semi-solid
Aqueous Stem Extract	300.00	27.00	12.3	Dark-brown, semi-solid

Table 2: Qualitative Phytochemical Constituents of *Nelsonia canescens* Extract

Phytochemical Compounds	Extracts	
	Leaf	Stem
Alkaloids	+	+
Terpenoids	–	–
Flavonoids	+	+
Saponins	+	–
Phenols	+	+
Tannins	+	+
Glycosides	+	+

Key: Absent (**-**), Present (**+**).

GC-MS analysis of *Nelsonia canescens* extracts
The GC-MS analysis of the aqueous leaf extract of *Nelsonia canescens* revealed 19 distinct phytoconstituents (Table 3). Major compounds identified include thymol, 2-methoxy-4-vinylphenol, umbelliferone, phytol, and methyl palmitate, each detected at specific retention times and peak areas reflecting their relative abundance in the sample. In contrast, the aqueous stem extract yielded 13 compounds (Table 4), with dominant constituents such as methyl palmitate, methyl oleate, methyl linoleate, and palmitic acid. The spectral data for both extracts presented clear peaks associated with various classes of natural products, including fatty acid esters, phenolic compounds, and aromatic derivatives. These constituents were identified based on their unique retention times, molecular weights, and spectral intensities recorded in the GC-MS output.

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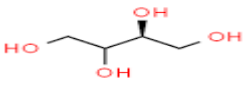
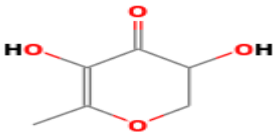
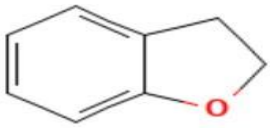
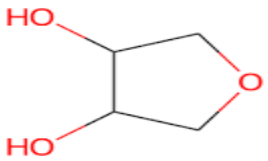
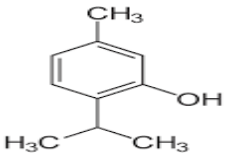
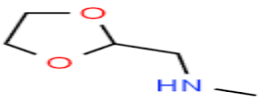
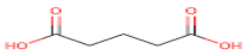
Table 3: GC-MS Spectral Analysis of *Nelsonia canescens* Leaf Extract

S/N	RT	Area %	Formula	Mol. Weight (g/mol)	Bioactive Compound	Compound Structure
1	7.6424	0.1369	C4H10O4	122.12	Butane-1,2,3,4-tetrol	
2	10.5068	2.4627	C6H8O4	144.1	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	
3	12.8305	5.3641	C9H8O3	164.16	Benzofuran, 2,3-dihydro-	
4	11.763	0.6378	C4H8O3	104.10	3,4-Furandiol, tetrahydro-, trans-	
5	12.3681	2.7026	CH3CHO	104.1	2-Butene ozonide	
6	14.6363	3.1391	C10H14O	150.2	Thymol	
7	24.5863	0.5882	C5H11NO2	117.15	2-Methylaminomethyl-1,3-dioxolane	
8	25.1922	0.5165	C5H8O4	32.12	Pentanedioic acid] (Folic acid derivative)	
9	29.1762	5.5824	C17H34O	270.45	Pentadecanoic acid, 14-methyl-, methyl ester	

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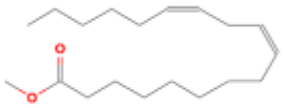
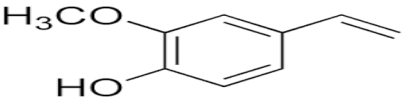
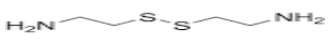
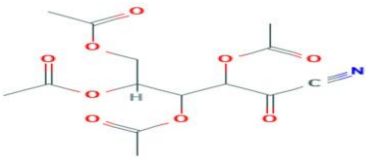
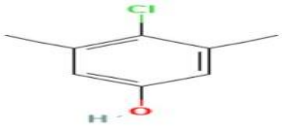
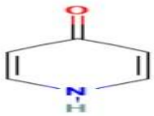
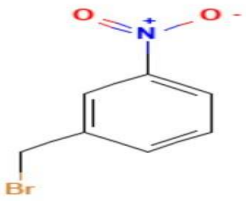
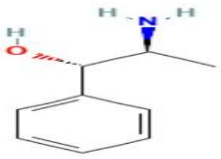
10	32.5134	0.2372	C19H36O	296.48	9-Octadecenoic acid (Z)-, methyl ester	
11	15.0447	1.301	C9H10O2	150.17	2-Methoxy-4-vinylphenol	
12	26.3672	0.5913	C4H12N2S2	152.28	Cystamine	
13	8.9647	0.0917	C4H8O2	88.11	Butanal, 3-hydroxy-	
14	13.4361	12.6928	C14H17NO9	343.29	Tetraacetyl-d-xylonic nitrile	
15	17.2161	0.0896	C8H9ClO	156.61	Chloroxylenol	
16	18.6787	0.0396	C5H5NO	95.10	4-Pyridinol	
17	19.007	0.6391	C7H6BrNO2	216.03	Benzene, 1-(bromomethyl)-3-nitro-	
18	22.7912	0.038	C9H13NO	151.21	Norpseudoephedrine	

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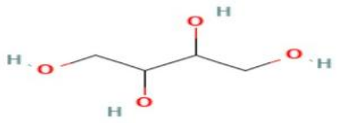
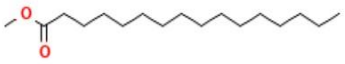
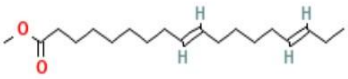
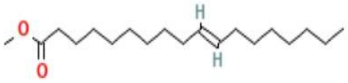
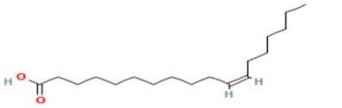
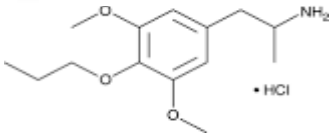
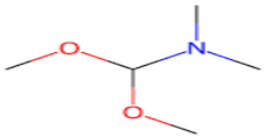
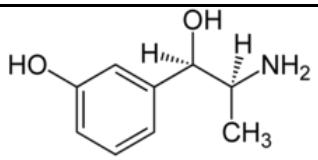
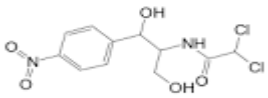
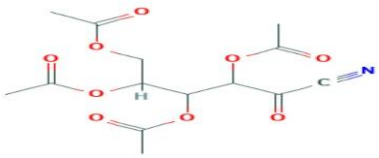
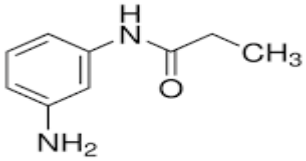
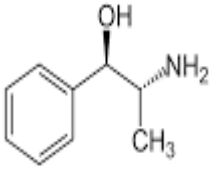
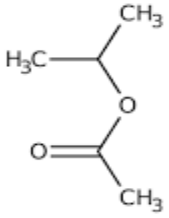
19	27.121	1.7597	C4H10O6	122.12	1,2,3,4-Butanetetrol, [S-(R*,R*)]-	

Table 4: GC-MS Spectral Analysis of *Nelsonia canescens* Stem Extract

S/N	RT	Area %	Formula	Mol. Weight (g/mol)	Compound Name	Compound Structure
1	29.1742	1.6167	C18H36O2	270.45	Hexadecanoic acid, methyl ester	
2	32.5789	4.5123	C19H32O	292.45	9,15-Octadecadienoic acid, methyl ester, (Z,Z)-	
3	32.649	5.6526	C19H36O2	296.48	10-Octadecenoic acid, methyl ester	
4	33.3358	11.1159	C18H34O2	282.46	cis-Vaccenic acid cis-11-octadecenoic acid	
5	36.6703	0.1276	C14H23NO3	221.29	3-Propoxyamphetamine	
6	36.808	0.1667	C4H9NO2	103.1	N-Methoxymethyl-N-methylformamide	
7	37.9365	0.221	C4H8O3S	136.17	Thiophene-3-ol, tetrahydro-, 1,1-dioxide	

8	38.1799	0.2251	C9H13NO2	167.20	Metaraminol (3,β-dihydroxyampheta mine)	
9	38.2035	0.5051	C2H3Cl2NO	127.95	Acetamide, 2,2-dichloro-	
10	38.2671	0.2726	C14H17NO9	343.28	Tetraacetyl-d-xylonic nitrile	
11	38.7332	4.7684	C11H15NO3S	241.3	Propanamide, 3-(3,4-dimethylphenylsulf onyl)-	
12	38.8211	1.0102	C9H13NO	151.2	Norpseudoephedri ne	
13	39.0969	2.1116	C18H33N5OS	367.56	N'-Isopropylureidoace tic acid	

Anti-inflammatory Activity (Carrageenan Model)

The anti-inflammatory activity was evaluated by measuring paw edema inhibition in rats (Table 4). The aqueous leaf extract (ALE) significantly reduced paw swelling at all doses (100, 200, and 400 mg/kg), with the highest effect observed at 400 mg/kg. The aqueous stem extract (ASE) showed significant inhibition only at 400 mg/kg, while the combined extracts (ALE+ASE) exhibited a synergistic anti-inflammatory effect, particularly at 200 and 400 mg/kg. The standard drug diclofenac (10 mg/kg) showed a similar degree of edema suppression.

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Table 5: Anti-inflammatory effect of aqueous leaf and stem extracts of *Nelsonia canescens*

Groups	0 Min	1st Hour	2nd Hour	3rd Hour	4th Hour
Negative control	0.20±0.00	0.20±0.00*	0.20±0.00*	0.20±0.00*	0.20±0.00*
Positive control	0.25±0.03	0.68±0.03	0.73±0.05	0.78±0.03	0.85±0.03
ALE (100 mg/kg)	0.25±0.03	0. 43±0.03*	0.45±0.06*	0.43±0.06*	0.53±0.06*
ALE (200 mg/kg)	0.23±0.03	0.40±0.04*	0.40±0.05*	0.50±0.04*	0.48±0.03*
ALE (400 mg/kg)	0.23±0.03	0.33±0.05*	0.33±0.04*	0.40±0.04*	0.43±0.03*
ASE (100 mg/kg)	0.28±0.03	0.63±0.05	0.65±0.03	0.70±0.00	0.70±0.04
ASE (200 mg/kg)	0.25±0.03	0.65±0.03	0.65±0.03	0.73±0.03	0.73±0.03
ASE (400 mg/kg)	0.25±0.03	0.50±0.04*	0.55±0.03	0.60±0.04	0.53±0.03*
ALE + ASE (100 mg/kg)	0.28±0.03	0.55±0.05	0.60±0.03	0.65±0.05	0.55±0.03*
ALE + ASE (200 mg/kg)	0.20±0.00	0.48±0.05	0.58±0.02	0.63±0.05	0.55±0.06*
ALE + ASE (400 mg/kg)	0.20±0.00	0.43±0.03*	0.45±0.00*	0.53±0.03*	0.55±0.03*
Diclofenac (10 mg/kg)	0.23±0.03	0.58±0.05	0.55±0.03*	0.55±0.03*	0.53±0.03*

Values are presented as mean ± SEM and superscript (*) indicate statistical significant difference (p<0.05) compared to positive control within the column at 95% confidence interval. ALE- aqueous leaf extract, ASE- aqueous stem extract

Hematological Profile of Experimental Animals

Analysis of hematological indices (Table 6) indicated that rats treated with ALE (particularly at 200 and 400 mg/kg) showed a reduction in white blood cell (WBC) count compared to the positive control group. Additionally, there was a slight increase in red blood cell (RBC) count and hemoglobin (Hb) concentration, suggesting a protective or stimulatory effect on erythropoiesis. Platelet counts remained relatively stable across treatment groups.

Table 6: Hematological Parameters of Experimental Animals

Groups	**WBC (103/µL)**	**LYM (%)**	**GRAN (%)**	**RBC (106/µL)**	**HGB (g/dL)**	**PLT (103/µL)**
Negative control	5.4±0.07*	32.4±0.41*	39.6±0.42*	1.7±0.08*	10.5±0.70*	206.0±0.28*
Positive control	29.2±0.25	52.7±0.41	23.1±0.45	0.3±0.52	7.0±3.61	6.0±0.08
ALE (100 mg/kg)	7.3±0.12	47±0.41	30.2±0.47*	0.1±0.16	0.8±0.94*	685.0±0.89*
ALE (200 mg/kg)	60.0±0.76*	59.9±0.55*	32.1±0.38*	5.9±0.51*	32.9±0.66*	909.0±0.97*
ALE (400 mg/kg)	72.8±0.32*	0±0*	40.0±0.00*	9.4±0.08*	50.5±0.79*	44.0±1.51*
ASE (100 mg/kg)	44.3±0.12*	68.2±0.54*	17.2±0.45*	4.0±0.07*	29.8±1.25*	89.0±0.06*
ASE (200 mg/kg)	53.9±0.38*	60±0.42*	22.5±0.41	8.2±0.04*	36.8±2.11*	56.0±0.47*
ASE (400 mg/kg)	58.4±0.79*	54.2±0.53	26.7±0.45	11.4±0.12*	45.0±1.91*	25.0±0.44*
ALE + ASE (100 mg/kg)	20.6±0.24*	75.1±0.44*	13.2±0.45*	0.0±0.30	2.2±1.72*	165.0±0.67*
ALE + ASE (200 mg/kg)	38.5±0.27*	15.6±0.34*	47.6±0.45*	1.6±0.28	12.8±0.63*	407.0±0.47*
ALE + ASE (400 mg/kg)	40.4±0.23*	25.9±0.38*	47±0.52*	3.8±0.18*	25.9±0.68*	69.0±0.31*
Diclofenac (10 mg/kg)	50.5±0.23*	49.6±0.38	34.4±0.38*	0.1±0.25	41.5±2.00*	223.0±0.48*

Values are presented as mean ± SEM and superscript (*) indicate statistical significant difference (p<0.05) compared to positive control within the column at 95% confidence interval. ALE- aqueous leaf extract, ASE- aqueous stem extract, WBC- White Blood Cell Count, LYM- Lymphocytes, GRAN- Granulocytes, RBC- Red Blood Cell Count, HGB- Hemoglobin, and PLT- Platelet Count.

DISCUSSION

One of the major challenges in modern medicine is the discovery of new, safe, and effective anti-inflammatory agents (Djova *et al.*, 2019). Natural products derived from medicinal plants offer promising alternatives due to their bioactive compounds with minimal side effects (Baali *et al.*, 2020). This study investigated the phytochemical composition, anti-inflammatory efficacy, and heamatogical effects of *Nelsonia canescens* leaves and stem extracts. Phytochemicals are substances derived from plants. Plants use primary or secondary metabolism to produce substances known as phytochemicals (from the Greek phyto, meaning "plant"). In the plant host, they typically exhibit biological activity and contribute to the growth of the plant or its defense against predators, diseases, and competition (Zaynab *et al.*, 2018). The qualitative phytochemical screening of *Nelsonia canescens* leaves extracts revealed the presence of Alkaloids, Flavonoids, Saponins, Phenols, Tannins and Glycosides, while Alkaloids, Flavnoids, Phenols, Tannins and Glycosides are present in the aqueous stem extract of *Nelsonia canescens* as shown in table 4.2. These compounds may be the cause of the plants potential anti-inflammatory properties (Owoyele *et al.*, 2005). The Gas Chromatography-Mass Spectrometry (GC-MS) analysis of the leaves and stem of *Nelsonia canescens* showed presence of wide range of phenolic compounds. The GC-MS Analysis of *Nelsonia canescens* aqueous leaves extract identified 2-Methyl-2,3-epoxy-2,3-dihydro-1,4-naphthoquinoneN-Methoxy-N-methylacetamideN-Acetylmannosamine as the most abundant compound, this compound has shown anti-inflammatory properties by inhibiting COX enzymes and reducing inflammation in various studies (Daniel *et al.*, 2022).

The Aqueous leaves and stem extract of *Nelsonia canescens* showed anti-inflammatory activity on an acute inflammatory process like in carrageenan-induced paw oedema in rats. The anti-inflammatory effect of the leaves and stem extracts of *Nelsonia canenscens* can be attributed to one or more of the phytochemicals observed in the extract. Several studies on medicinal plants revealed the presence of different phytochemicals such as Alkaloids, flavonoids phenols among others. These chemical constituents have revealed various medicinal properties such as antinociceptive, antipytretic and anti-inflammatory among others (Zhang and Ghosh, 2001; Choi *et al.*, 2019; Carrasco-Marín *et al.*, 2024). Anti-inflammatory effect of Aqueous leaves and stem extracts of *Nelsonia canescens* and its synergy at ratio 50:50 were determined to ascertain the most effective of the three different extracts used. According to table 4.4 *Nelsonia canescens* aqueous leaves extract showed greater anti-inflammatory activity while the aqueous stem extract revealed the less anti-inflammatory activity.
The hematological analysis further supported the anti-inflammatory efficacy of *Nelsonia canescens*. The treated groups, particularly those receiving ALE at 200 and 400 mg/kg, exhibited a reduction in white blood cell (WBC) counts, suggesting a dampening of excessive immune activation. Elevated WBC counts in the untreated inflammatory group indicate heightened immune response, which can contribute to tissue damage. Increased red blood cell (RBC) counts and hemoglobin (HGB) levels in treated groups suggest potential erythropoietic stimulation, possibly due to the presence of bioactive compounds that promote hematopoiesis. The stabilization of platelet (PLT) levels in treated groups further highlights the plant's role in reducing inflammatory-induced hematological disruptions. Diclofenac, a non-steroidal anti-inflammatory drug (NSAID), was used as the standard reference drug. While it significantly reduced edema and inflammatory cell infiltration, its effects on hematological parameters suggest possible immunosuppressive tendencies. *Nelsonia canescens*, on the other hand, demonstrated a balanced anti-inflammatory effect by reducing edema while maintaining hematological stability, suggesting its potential as a safer alternative to synthetic NSAIDs. The anti-inflammatory effect of *Nelsonia canescens* is likely due to the synergistic action of multiple bioactive compounds acting through various pathways: inhibition of COX enzymes and pro-inflammatory cytokines (umbelliferone, thymol, eugenol), reduction of oxidative stress through antioxidant properties (methyl oleate, cis-vaccenic acid), stabilization of immune response and reduction of inflammatory cell infiltration (methyl palmitate, thymol), and modulation of lipid metabolism and cellular protection (cis-vaccenic acid, methyl stearate). These findings align with previous studies (Liyanagamage *et al.*, 2020; Shahid *et al.*, 2020), further reinforcing the therapeutic potential of *Nelsonia canescens* as an anti-inflammatory agent.

Conclusion

This study shows that *Nelsonia canescens* contains several useful plant compounds with anti-inflammatory effects. These include umbelliferone, thymol, methyl oleate, eugenol, and others, which may help reduce inflammation through different ways. The extracts appear to be safe and may serve as good options for developing new anti-inflammatory medicines. Overall, the findings support the possible use of *Nelsonia canescens* in treating inflammation and related health problems.

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