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Anti-inflammatory and Elemental Evaluation of Aqueous and Ethanolic Extracts of *Adenodolichos paniculatus* Leaves and Stems

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Abstract

Objective: To determine the acute oral toxicity and evaluate the anti-inflammatory activity and elemental profiles of aqueous and ethanolic leaf and stem extracts of *Adenodolichos paniculatus* in rats using the carrageenan-induced paw oedema model.

Methods: Leaves and stems were sequentially extracted with distilled water and 70% ethanol. Elemental analysis was performed by digesting 1 g of each extract with 9 mL aqua regia (HNO_3:HCl, 1:3 v/v) until complete dissolution, followed by filtration and analysis using Atomic Absorption Spectroscopy (BUCK Scientific ACCUSYS 211). Acute toxicity (LD_{50}) was determined according to OECD-425 guidelines. Carrageenan-induced paw oedema was quantified at 30-min intervals for 3 h. Male Wistar rats received the extracts (200 or 400 mg/kg, p.o.) or diclofenac (10 mg/kg).

Results: All extracts were safe (LD_{50} > 2,000 mg/kg) with no mortality or severe toxicity. Elemental analysis showed higher magnesium (8.50–11.60 vs. 6.00–9.40 mg/g) and potassium (18.10 vs. 1.83 mg/g in aqueous extracts) in leaves compared to stems, with trace calcium, zinc, iron, and copper detected. Leaf extracts exhibited significant dose-dependent anti-inflammatory activity ($P < 0.05$). Aqueous leaf extract (400 mg/kg) achieved 41.12% inhibition at 150 minutes, surpassing diclofenac (26.29%), while ethanolic leaf extract (400 mg/kg) reached 33.48%. Stem extracts showed markedly lower activity (12.58–24.72% maximum inhibition).

Conclusions: *A. paniculatus* leaf extracts demonstrate promising anti-inflammatory activity with favorable safety profiles, supporting their traditional medicinal use. The superior efficacy of aqueous leaf extracts over diclofenac warrants further investigation into bioactive constituents and mechanisms of action. Higher magnesium and potassium content in leaves may contribute to the observed anti-inflammatory effects, suggesting potential elemental contributions to bioactivity.

Keywords: *Adenodolichos paniculatus*; anti-inflammatory; carrageenan; medicinal plants; toxicity

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1. Introduction

Inflammation is an indispensable innate defence mechanism, yet when dysregulated, it drives the pathology of chronic disorders such as rheumatoid arthritis, psoriasis and inflammatory bowel disease [1]. Although, non-steroidal anti-inflammatory drugs (NSAIDs) remain first-line therapy, their gastrointestinal and cardiovascular liabilities warrant the exploration of safer alternatives [23,24]. Medicinal plants, long used in folk medicine, constitute a prolific source of anti-inflammatory lead compounds [26]. *Adenodolichos paniculatus* (Fabaceae), locally known as “waken wuta”, is traditionally applied in West Africa for burns, cough, sore throat and general pain relief [12,16].

Phytochemical studies reveal the presence of flavonoids, saponins and triterpenes that could mediate immunomodulatory activity [5,25]. While methanolic leaf extracts have shown analgesic and anti-inflammatory effects [25], the activity of aqueous and ethanolic stem extracts and aqueous leaf extracts has not been investigated. The present study therefore aimed (i) to determine the acute oral toxicity of aqueous and ethanolic leaf and stem extracts of *A. paniculatus*, (ii) to compare their acute anti-inflammatory efficacy in a validated carrageenan-induced rat paw oedema model [29], and (iii) to also carryout the elemental analysis of *A. paniculatus.*

2. Materials And Methods

2.1 Plant material and extraction

Whole plants of *A. paniculatus* were collected in Ilorin, Nigeria and authenticated by a plant taxonomist [12]. Leaves and stems were air-dried (25 °C), pulverised and extracted separately by maceration either in distilled water or 70 % ethanol (1:10 w/v, 48 h). Filtrates were concentrated (rotary evaporator or freeze-dryer) to yield four dried extracts.

Table 1. Extractive yield of *A. paniculatus* leaf and stem extracts

Extract	Yield (%)
Ethanolic leaf	8.5
Aqueous leaf	2.7
Ethanolic stem	6.1
Aqueous stem	3.9

2.2 Animals and ethics

Male Wistar rats (110–150 g) were housed under standard conditions with ad libitum access to feed and water. Protocols were approved by the University of Ilorin Animal Ethics Committee (UREC/FUM/1642) and conformed to OECD-425 guidelines [10].

2.3 Acute toxicity (LD_{50})

OECD-425 limit testing was performed: single oral doses of 2000 mg kg^{-1} followed, when non-lethal, by confirmation at 1000 and 500 mg kg^{-1} (n = 3 per dose/extract) [11]. Animals were observed for 24 h.

2.4 Elemental Analysis

To 1g of each extract, 9 mL of freshly prepared aqua regia (HNO_3 and HCl in ratio of 1:3) was added. Then, the mixture was boiled gently over a water bath until the sample had completely dissolved [31].

During the digestion procedures, the inner walls of the beakers were washed with 2 mL of deionized water to prevent the loss of the sample, the samples were filtered with Whatman no. 1 filter paper. Then, a sufficient amount of deionized water was added to make the final volume up to 100 mL. The analysis was conducted using Atomic Absorption Spectrophotometer (AAS), model; BUCK scientific ACCUSYS 211 Atomic Absorption Spectrophotometer.

2.5 Carrageenan-induced paw oedema

Rats were randomised into twelve groups (n = 5) receiving extracts (200 or 400 mg kg^{-1} p.o.), diclofenac (10 mg kg^{-1}) or vehicle. One hour later, 0.1 mL of 1 % carrageenan was injected sub-plantarly into the left hind paw. Paw diameter was measured every 30 min for 3 h [29]. Oedema inhibition (%) was calculated versus vehicle. Data were analysed by one-way ANOVA with Bonferroni post-hoc correction ($\alpha = 0.05$).

3. Results

No mortality or severe behavioural changes occurred at 2 000 mg kg^{-1}, assigning all extracts to OECD category 5 (LD_{50} > 2 000 mg kg^{-1}) [10].

Carrageenan injection produced a biphasic oedema that peaked at 90 min in controls [28]. Aqueous and ethanolic leaf extracts produced dose-dependent inhibition of paw swelling, with the A. Leaf 400 mg/kg dose reaching a peak inhibition of 41.12% at 150 minutes. This efficacy was superior to the Positive Control (diclofenac 10 mg/kg), which achieved a peak inhibition of 26.29% at the same time point. Elemental analysis revealed higher levels of anti-inflammatory co-factors Mg^{2+} and K^{+} in leaf extracts, which may partly explain their superior activity.Elemental analysis revealed higher levels of anti-inflammatory co-factors Mg^{2+} and K^{+} in leaf extracts, which may partly explain their superior activity.

Table 2. Temporal Profile of Paw Oedema in Rats Following Administration of Aqueous and Ethanolic Leaf and Stem Extracts of *Adenocholitos paniculatus* at 200 mg/kg and 400 mg/kg, Compared to Positive and Negative Controls

Time (Min)	Positive Control	Negative Control	E. Leaf 200 mg/kg	E. Leaf Extract 400 mg/kg	A. Leaf Extract 200 mg/kg	A. Leaf Extract 400 mg/kg	E. Stem 200 mg/kg	E. Stem 400 mg/kg	A. Stem 200 mg/kg	A. Stem 400 mg/kg
0.0	2.50	3.37	2.57	2.49	2.69	2.67	2.98	2.79	2.84	2.59
30.0	3.66	4.38	3.90	4.12	3.13	3.34	4.23	4.19	4.23	4.14
60.0	3.63	4.67	3.47	3.58	3.03	2.93	4.08	4.01	4.27	4.1
90.0	3.38	4.37	3.47	3.58	2.92	2.9	4.33	3.75	3.95	3.72
120.0	3.34	4.24	3.29	3.06	2.79	2.85	4.08	3.98	3.9	3.6
150.0	3.28	4.45	3.11	2.96	2.80	2.62	3.89	3.72	3.82	3.35
180.0	3.07	4.07	3.01	2.8	2.77	2.90	3.83	3.76	3.81	3.22

Table 3. Elemental Composition (mg/L) of Aqueous and Ethanolic Leaf and Stem Extracts of *Adenodolichos paniculatus.*

Extracts	Mg	Ca	Zn	K	Fe	Cu
Ethanolic Stem	6.00	3.23	0.03	1.66	0.05	0.75
Ethanolic Leaf	11.60	0.36	0.01	1.83	0.02	0.04
Aqueous Stem	9.40	3.80	0.04	17.60	0.85	0.73
Aqueous Leaf	8.50	4.07	0.03	18.10	0.45	0.05

Table 4. Time-dependent percentage inhibition of E. leaf, A. leaf, E. stem, and A. stem extracts on Paw Oedema.

Time (Min)	Positive Control	E. Leaf 200 mg/kg	E. Leaf 400 mg/kg	A. Leaf 200 mg/kg	A. Leaf 400 mg/kg	E. Stem 200 mg/kg	E. Stem 400 mg/kg	A. Stem 200 mg/kg	A. Stem 400 mg/kg
0.0	25.82	23.74	26.11	20.18	20.77	11.57	17.21	15.73	23.15
30.0	16.44	10.96	5.94	28.54	23.74	3.42	4.34	3.42	5.48
60.0	22.27	25.70	23.34	35.12	37.26	12.63	14.13	8.57	12.21
90.0	22.65	20.59	18.08	33.18	33.64	0.92	14.19	9.61	14.87
120.0	21.23	22.41	27.83	34.20	32.78	3.77	6.13	8.02	15.09
150.0	26.29	30.11	33.48	37.08	41.12	12.58	16.40	14.16	24.72
180.0	24.57	26.04	31.20	31.94	28.75	5.90	7.62	6.39	20.88

The percentage inhibition is calculated relative to the Negative Control group's change in paw volume. Negative values indicate a pro-inflammatory effect (swelling greater than the control).

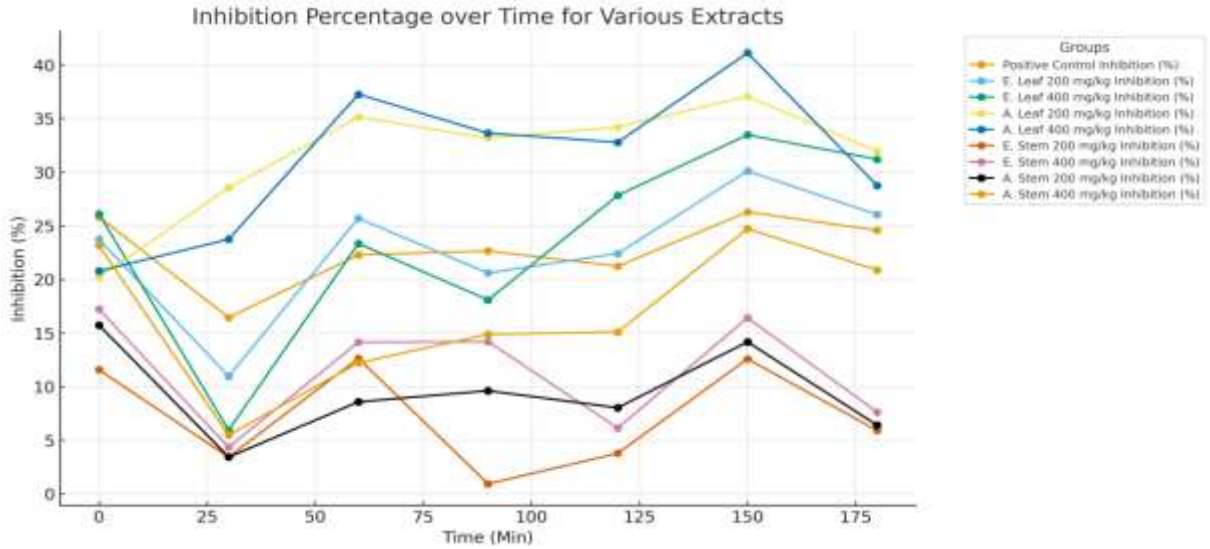
$$\% \text{ Inhibition} = \left\{ \left(NC_t - T_t \right) / NC_t \right\} \times 100$$

Where:
NC_t = Negative Control Paw Volume at time t
T_t = Treatment Group Paw Volume at time t

The complete temporal profile of paw edema percentage inhibition for all treatment groups is detailed in Table 4. As shown,The A. Leaf extracts were identified as the most potent anti-inflammatory agents, primarily targeting the late stage of inflammation. The A. Leaf 400 mg/kg dose achieved a peak inhibition of 41.12% at 150 minutes, demonstrating effective and sustained anti-inflammatory action maintained at >33% inhibition post-90 minutes.The high positive inhibition rates suggest a strong capacity to suppress the prostaglandin-mediated phase of the inflammatory response, significantly outperforming the Positive Control in overall efficacy.

Conversely, the stem extracts (E. stem and A. stem) demonstrated substantially lower anti-inflammatory activity compared to leaf extracts. A. Stem 400 mg/kg showed minimal inhibition, with the lowest value of 5.48% at 30 minutes, indicating limited therapeutic effect. This suggests the stem extracts are pro-inflammatory or contain irritants that amplify the early phase of swelling.

Figure 1. Anti-inflammatory activity of aqueous and ethanolic extracts of *Adenodolichos paniculatus* leaves and stems in carrageenan-induced rat paw oedema. Paw diameter was measured at 30-minute intervals post-induction.



4. Discussion

The present findings corroborate earlier reports on the anti-inflammatory potential of *Adenodolichos paniculatus* leaves [25] and extend them by demonstrating activity in aqueous matrices, aligning with traditional usage. The significant inhibition of carrageenan-induced paw oedema by aqueous and ethanolic leaf extracts, particularly at 400 mg /kg - indicates that bioactive constituents are both water - and ethanol-soluble. This supports ethnomedicinal use where aqueous decoctions are common.

The absence of comparable activity in stem extracts suggests tissue-specific localization of anti-inflammatory compounds. This is consistent with earlier phytochemical reports indicating higher concentrations of flavonoids, saponins, and triterpenoids in leaves than in stems [5]. Flavonoids are known to inhibit cyclooxygenase (COX), lipoxygenase (LOX), and nitric oxide pathways, thereby modulating both the early (histamine, serotonin) and late (prostaglandin-mediated) phases of inflammation [4,6]. Saponins can suppress pro-inflammatory cytokines such as TNF-α and IL-6 via NF-κB and MAPK signalling inhibition.

The biphasic oedema pattern observed in the carrageenan model, with peak swelling at 90 minutes, reflects prostaglandin-driven inflammation, making it ideal for screening COX inhibitors [28,29]. The time-dependent reduction in paw volume, particularly in the aqueous leaf group, suggests early onset of action, possibly due to the presence of rapidly absorbed hydrophilic components. The ethanolic leaf extract, while slightly slower in onset, produced comparable overall suppression. This aligns with known pharmacodynamics of flavonoid-rich extracts, where lipophilic constituents modulate late-phase mediators.

Elemental analysis showed elevated levels of magnesium and potassium in leaf extracts, both of which play roles in modulating inflammatory processes. Magnesium acts as a calcium antagonist, potentially dampening calcium-dependent inflammatory cascades, while potassium may modulate membrane excitability and cellular immune response. These ions may synergize with phytoconstituents to produce the observed effects.

Importantly, none of the extracts induced mortality or observable toxicity at the limit dose (2000 mg kg^{-1}), classifying them under OECD Category 5 [10,11]. This positions *A. paniculatus* leaf extracts as candidates for further chronic toxicity evaluation and pharmacokinetic profiling.

Comparing our findings with NSAIDs, the inhibition produced by the A. Leaf 400 mg/kg (41.12%) surpassed that of diclofenac 10 mg/kg (26.29%), suggesting superior efficacy with better safety margins. However, NSAIDs act primarily through COX-1 and COX-2 inhibition, whereas plant-based extracts may offer broader immunomodulation with multi-targeted mechanisms [23,24].

Nonetheless, some limitations exist. The acute nature of the model does not reflect chronic inflammatory conditions such as arthritis or colitis. Furthermore, no histopathological assessment or cytokine quantification was performed. The exact phytoconstituents responsible for the activity were not isolated, and synergistic or antagonistic interactions between compounds remain unexplored.

5. Conclusion

This study demonstrates that *A. paniculatus* leaf extracts possess significant anti-inflammatory activity with favorable safety profiles (LD_{50} > 2,000 mg/kg). The aqueous leaf extract at 400 mg/kg exhibited superior efficacy (41.12% inhibition) compared to standard diclofenac (26.29%), validating traditional medicinal applications. The dose-dependent response and plant part-specific activity (leaves > stems) suggest concentrated bioactive constituents in foliar tissues.

Elemental profiling revealed higher magnesium and potassium concentrations in leaves compared to stems, which may contribute to the observed anti-inflammatory mechanisms through modulation of cellular signaling pathways. The correlation between mineral content and bioactivity warrants further investigation. These findings support the ethnomedicinal use of *A. paniculatus* and justify advanced phytochemical characterization to isolate active compounds, elucidate molecular mechanisms, and explore formulation development for therapeutic applications. Future studies should investigate chronic toxicity, additional inflammatory models, and clinical translation potential.

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