

**Anti-Inflammatory, Analgesic and Antioxidant Activities of the Methanol Extract and Solvent Fractions of *Psychotria vogeliana* Benth. (Rubiaceae) in Wistar Rats and Mice**

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Abstract

Inflammation and pain remain leading causes of morbidity globally. Non-steroidal anti-inflammatory drugs and opioids, though widely used, are constrained by gastrointestinal, cardiovascular and dependence-related adverse effects. *Psychotria vogeliana* Benth. (Rubiaceae) is employed in Nigerian ethnomedicine for pain, bruises and inflammatory swellings. This traditional use, coupled with evidence of anti-inflammatory phytoconstituents within the genus, prompted its systematic pharmacological evaluation. This study therefore evaluated the analgesic, anti-inflammatory and antioxidant activities of the methanol extract and solvent fractions of the aerial parts of *P. vogeliana*. The methanol extract (ME) was obtained by cold maceration and sequentially partitioned into n-hexane (HF), ethyl acetate (EF) and methanol (MF) fractions. Acute oral toxicity was determined by Lorke's method. Analgesic activity was assessed using the hot-water tail-immersion and acetic acid-induced writhing models in mice. Anti-inflammatory activity was evaluated via egg albumin-induced paw oedema, formaldehyde-induced arthritis and cotton pellet-induced granuloma models in rats. Antioxidant potential of the most active fraction (EF) was quantified by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and ferrous ion chelating assays. ME contained tannins, flavonoids, steroids, terpenoids, saponins, carbohydrates, resins, proteins and phenolics; alkaloids were detected only in EF. The median lethal dose (LD₅₀) exceeded 5000 mg/kg. ME (100–400 mg/kg) produced dose-related but statistically non-significant increases in tail-flick latency, whereas acetic acid-induced writhing was reduced significantly and dose-dependently at 200 and 400 mg/kg (35.6% and 47.6% inhibition, respectively; $P < 0.05$). ME did not significantly suppress acute egg albumin-induced oedema. In formaldehyde-induced chronic arthritis, ME and EF at 400 mg/kg significantly reduced paw thickness at discrete time points ($P < 0.05$), with EF producing the greatest cumulative inhibition (9.5%) among the fractions. HF significantly and dose-dependently reduced granuloma dry weight ($P < 0.05$), although EF and ME produced numerically larger, non-significant reductions at 400 mg/kg. EF demonstrated potent antioxidant activity, with DPPH scavenging $> 88\%$ and Fe^{2+} chelation $> 80\%$ across 15.63–500 $\mu\text{g/mL}$, surpassing ascorbic acid at all concentrations. The extract and particularly its ethyl acetate fraction exhibit peripherally mediated analgesic, appreciable chronic anti-inflammatory and marked antioxidant activities, plausibly attributable to flavonoids, tannins and terpenoids constituents, thereby providing pharmacological credence for the ethnomedicinal use of *P. vogeliana*.

Keywords: *Psychotria vogeliana*; Rubiaceae; anti-inflammatory; analgesic; antioxidant; DPPH; arthritis.

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08033501106

<https://doi.org/10.61594/tnpr.v7i4.2026.184>

Page No.: 375-387

Volume: Volume 7 Issue 4, 2026

Trends in Natural Products Research

Copy Right: NAPREG

Received 13/9/26, Revised 20/9/29, Accepted 25/9/26, Published Online 30/09/26

Introduction

Inflammation is the coordinated vascular and cellular response by which tissue defends itself against injury, infection and other noxious stimuli, and it underlies the pathogenesis of conditions ranging from arthritis to cardiovascular and neurodegenerative disease (Medzhitov, 2010). Pain, now formally defined by the International Association for the Study of Pain as an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage, frequently accompanies inflammatory states and is itself a major cause of disability (Raja *et al.*, 2020). Non-steroidal anti-inflammatory drugs remain the mainstay of pharmacological management of both conditions, but their long-term use is constrained by gastrointestinal ulceration, nephrotoxicity and an increased risk of cardiovascular events, while opioid analgesics carry a well-documented liability for tolerance and dependence (Ahsan and Sahu, 2025). These limitations sustain the search for plant-derived agents that combine efficacy with an improved safety margin, and a growing body of pharmacological evidence continues to demonstrate that polyphenols, flavonoids and terpenoids of plant origin modulate the same inflammatory pathways, including cyclo-oxygenase activity, nuclear factor- κ B signalling and pro-inflammatory cytokine release, that are targeted by conventional drugs (Nakadate *et al.*, 2025).

Nigerian medicinal flora has proven to be a rich reservoir of such leads. For instance, anti-inflammatory lipophilic fractions for topical application and membrane-stabilising stigmastane steroids have been previously isolated and characterised from the leaves of *Alchornea floribunda* and *Alchornea cordifolia* (Okoye *et al.*, 2010, 2011).

Psychotria (Rubiaceae) is among the largest angiosperm genera and is a long-recognised reservoir of structurally diverse alkaloids, flavonoids and terpenoids with antinociceptive, antioxidant and central nervous system activity (Adeyemi *et al.*, 2004; Formagio *et al.*, 2014; Calixto *et al.*, 2016). *Psychotria vogeliana* is a scrambling shrub of West and Central African deciduous forest and savanna woodland whose leaves, roots and bark are applied in folk medicine to bruises, boils, arthritic swelling and pain, and whose aerial parts have separately been documented as a component of antimalarial plant recipes in southern Benin (Seinde *et al.*, 2016). A pharmacognostic and phytochemical survey of the leaf confirmed a rich content of alkaloids, tannins, flavonoids and cardiac glycosides together with antimicrobial activity (Mba *et al.*, 2021), and a subsequent *in vitro* study reported that the methanol leaf extract inhibited albumin denaturation and phospholipase A2 activity with potency comparable to

diclofenac, implicating phospholipase A2 inhibition as a plausible mechanism of the plant's traditional anti-inflammatory use (Chukwuma *et al.*, 2024).

Taken together, the ethnomedicinal application of *P. vogeliana* to painful and inflammatory conditions in some parts of Nigeria, and this emerging *in vitro* mechanistic evidence provided the rationale for the present study, which was undertaken to establish, in whole-animal models, whether these effects extend to analgesia, chronic anti-inflammatory activity and free-radical scavenging, an *in vivo* profile that had not previously been reported for this species. The present study was designed to provide pharmacological validation for the ethnomedicinal application of *Psychotria vogeliana*.

Materials and Methods

Experimental animals

Adult Wistar rats (150–200 g) and Swiss albino mice (17–25 g) of either sex were obtained from the Laboratory Animal Facility, Department of Pharmacology and Toxicology, University of Nigeria, Nsukka. Animals were housed in well-ventilated steel cages at ambient temperature with free access to water and standard livestock pellets and were acclimatised for one week before use. All procedures conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Pub. No. 85-23, revised 1985) and were approved by the Research Ethics Committee, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka (approval number FPSRA/UNN/021/0105).

Plant material

Fresh aerial parts of *P. vogeliana* were collected in July 2019 from Anambra State, Nigeria, and authenticated by Mr A. Ozioko, taxonomist at the Bioresources Development and Conservation Programme (BDGP) Centre, Nsukka, Enugu State. A voucher specimen (PCG/UNN/0083) was deposited at the Pharmacognosy Herbarium, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka. The plant material was cleaned, cut into smaller pieces, shade-dried and pulverised using a laboratory mill (Christy and Norris Ltd, England).

Extraction and fractionation

The powdered plant material (1 kg) was cold-macerated in methanol for 72 h with intermittent agitation. The extract was filtered (Whatman No. 1) and concentrated under reduced pressure at 40 °C to yield the methanol extract (ME), which was stored at

4 °C. A portion of ME was sequentially partitioned between n-hexane, ethylacetate and methanol, in order of increasing polarity, using a separating funnel, and each fraction was concentrated to dryness under reduced pressure to obtain the n-hexane fraction (HF), ethylacetate fraction (EF) and methanol fraction (MF).

Phytochemical screening

ME and the HF, EF and MF fractions were screened qualitatively for alkaloids, glycosides, flavonoids, resins, reducing sugars, carbohydrates, steroids and terpenoids, saponins, tannins, fats and oils, proteins and phenolics using standard colour and precipitation reactions (Harborne, 2001; Trease and Evans, 2002).

Acute toxicity study

The median lethal dose (LD₅₀) of ME was estimated in mice by the method of Lorke (1983) in two stages. In stage one, nine mice (n = 3/group) received 10, 100 and 1000 mg/kg ME (p.o.) and were observed for 24 h. As no deaths occurred, a fresh batch of four mice (n = 1/group) received 1600, 2900, 3600 and 5000 mg/kg ME in stage two and were similarly observed.

Analgesic studies

Effect of the methanol extract on hot-water tail-immersion nociception

The lower two-thirds of the mouse tail was immersed in a water bath maintained at 55 °C and the latency to tail withdrawal was recorded (cut-off 15–20 s) before and at 30, 60, 90, 120 and 180 min after oral administration of distilled water (2 mL/kg, negative control), tramadol (10 mg/kg, positive control) or ME (100, 200 or 400 mg/kg), following the method of D'Amour and Smith (1941).

Effects of the methanol extract on acetic acid-induced writhing.

Five groups of mice (n=6/group) received distilled water (10 mL/kg), ASA (10 mg/kg, p.o.) or ME (100, 200 or 400 mg/kg, p.o.) respectively. One hour later, 0.6% v/v acetic acid (10 mL/kg, i.p.) was administered and the number of writhes was counted over 30 min, beginning 5 min after acetic acid injection (Koster *et al.*, 1959; Kumar *et al.*, 2009). Percentage inhibition was calculated relative to the control group.

Anti-inflammatory studies

Effects of the methanol extract on egg albumin-induced paw oedema

Five groups of rats (n = 6/group) received distilled water (10 mL/kg, negative control), ME (100, 200 or 400 mg/kg) or ASA (100 mg/kg, positive control) orally respectively, and one hour later, oedema was induced by sub-plantar injection of 0.1 mL fresh egg albumin into the right hind paw (Akah and Nwambie, 1994). Paw volume was measured by water displacement at baseline and at 0.5, 1, 2, 3, 4, 5 and 6 h post-injection. Oedema was calculated as the increase in paw volume relative to the pre-injection (0 h) baseline for each group, and percentage inhibition was calculated as $[(Ec - Et)/Ec] \times 100$, where Ec and Et are the mean oedema of the control and treated groups, respectively, at each time point.

Effects of the methanol extract and fractions on Formaldehyde-induced arthritis

Arthritis was induced in rats by sub-plantar injection of 0.1 mL of 2% formaldehyde. One hour after the first treatment, the groups received daily orally, the vehicle, ASA (100 mg/kg), ME (100, 200 or 400 mg/kg) or, in a separate cohort, HF, EF or MF (200 or 400 mg/kg), following the method of Shivaji *et al.* (2000). Paw thickness was measured with a vernier calliper at baseline, 0.5, 1, 2, 3 and 4 h on day 1 and once daily thereafter through day 7. The cumulative inflammatory response for each group was expressed as the area under the paw thickness–time curve (AUC), calculated by the trapezoidal rule, and percentage inhibition relative to the control AUC.

Effects of the methanol extract and fractions on cotton pellet-induced granuloma

Under ketamine–xylazine anaesthesia, two sterile cotton pellets (20 mg each) were implanted subcutaneously in the axillary region of each rat. From day 2 the animals received distilled water, ASA (300 mg/kg), ME (100, 200 or 400 mg/kg) or, in a separate cohort, HF, EF or MF (200 or 400 mg/kg each) once daily for seven days (Winter and Porter, 1957). On day 8, the animals were sacrificed, and the pellets were excised, weighed wet and then dried to constant weight at 60 °C. Granuloma formation was expressed as the wet and dry weight of tissue accreted on the pellet, and percentage inhibition relative to control.

Determination of antioxidant potential

The fraction with the strongest chronic anti-inflammatory activity (EF) was evaluated for free-radical scavenging using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay (Gyamfi *et al.*, 1999). Two-fold serial dilutions of EF or L-ascorbic acid (15.63–500 µg/mL in 80% methanol) were mixed with 0.076 mM

of DPPH and incubated in the dark for 25 min; absorbance was read at 517 nm. Ferrous ion (Fe^{2+}) chelating activity was determined by the ferrozine method (Mishra *et al.*, 2013). Graded concentrations of EF or ascorbic acid were incubated with FeCl_2 (2 mM) and ferrozine (5 mM), and absorbance of the resulting complex was read at 562 nm. Percentage inhibition was calculated relative to a reagent blank in both assays.

Statistical analysis

Data are expressed as mean $\pm$ standard error of the mean (SEM) and were analysed by one-way analysis of variance (ANOVA) followed by Dunnett's post-hoc test using GraphPad Prism (GraphPad Software, San Diego, CA). $P < 0.05$ was considered statistically significant.

Results

Extraction yield

Cold maceration of the powdered aerial part of *P. vogeliana* with methanol yielded ME (1.64% w/w). Fractionation of ME resulted in HF, EF and MF yields of 14.7%, 18.2% and 13.8% (w/w of ME), respectively.

Phytochemical constituents

ME tested positive for saponins, carbohydrates, glycosides, proteins, tannins, flavonoids, steroids, terpenoids, resins, fats and oils, and phenolics.

Alkaloids were detected only in EF. Steroids and terpenoids were abundant in ME and in all three fractions (Table 1).

Acute toxicity

No mortality or overt signs of toxicity were observed in mice given ME at doses up to 5000 mg/kg over 24 h, placing the oral LD_{50} of ME above 5000 mg/kg.

Analgesic activity

In the hot-water tail-immersion test, ME elicited a non-dose-dependent and non-significant ($p > 0.05$) increase in tail-flick latency at all doses when compared with the negative control (Table 2).

In the acetic acid-induced writhing test, ME produced a dose-related reduction in the mean number of writhes. The reductions at 200 mg/kg (35.6%) and 400 mg/kg (47.6%), as well as ASA were statistically significant compared with control ($p < 0.05$), (Table 3).

Anti-inflammatory activity Effects of the methanol extract on egg albumin-induced paw oedema

ME did not produce a consistent, dose-dependent reduction in egg albumin-induced paw oedema. At the highest dose (400 mg/kg), paw volume was transiently higher than control at 0.5 h before falling below control from 2 h onward, whereas the 100 and 200 mg/kg doses showed little activity throughout the observation period (Table 4).

Table 1: Phytochemical constituents of the methanol extract and solvent fractions of *P. vogeliana*

Phytoconstituent	ME	HF	EF	MF
Tannins	+++	+	+++	+++
Saponins	++	–	++	–
Flavonoids	++	+	+++	++
Steroids	+++	+++	+++	+++
Alkaloids	–	–	+	–
Terpenoids	+++	+++	+++	+++
Carbohydrates	+++	++	+++	++
Resins	++	++	+	++
Proteins	+++	–	+	+
Phenolics	+++	+	+++	+++

ME = methanol extract; HF = n-hexane fraction; EF = ethylacetate fraction; MF = methanol fraction. +++ abundant, ++ moderate, + slight, – absent.

Table 2: Effect of the methanol extract of *P. vogeliana* on tail-flick latency in the hot-water immersion test

		Latency (sec)					
Group	Dose (mg/kg)	0 min	30 min	60 min	90 min	120 min	180 min
Control	2 mL/kg	2.49±0.18	2.48±0.22	2.13±0.19	1.91±0.19	2.16±0.18	2.35±0.25
Tramadol	10	3.25±0.54	2.92±0.62	2.87±0.40	2.53±0.34	3.16±1.26	2.45±0.41
ME	100	3.09±0.48	8.11±4.02	4.17±1.39	2.97±0.65	2.59±0.38	3.32±0.42
ME	200	2.79±0.27	2.74±0.43	2.55±0.21	3.36±0.48	2.22±0.22	3.47±0.55
ME	400	2.31±0.44	4.09±1.40	3.14±0.84	3.47±1.02	2.31±0.16	3.04±0.37

Values are mean ± SEM tail-withdrawal latency (s); n = 6. None of the treatment means differed significantly from control (one-way ANOVA, Dunnett's post-hoc test, $P > 0.05$).

Table 3: Effect of the methanol extract of *P. vogeliana* on acetic acid-induced writhing in mice

Group	Dose (mg/kg)	Number of writhes	Inhibition (%)
Control	10 mL/kg	80.17±10.61	–
ASA	10	64.67±21.61*	19.3
ME	100	70.00±22.47	12.7
ME	200	51.67±12.90*	35.6
ME	400	42.00±13.97*	47.6

Values are mean ± SEM; n = 6. ASA = acetylsalicylic acid. * P < 0.05.

Table 4: Effect of the methanol extract of *P. vogeliana* on egg albumin-induced paw oedema in rats

		Paw volume (ml)							
Group	Dose (mg/kg)	0 h	0.5 h	1 h	2 h	3 h	4 h	5 h	6 h
Control	10 mL/kg	0.47±0.08	1.18±.18	1.15±.14	0.97±.12	0.92±.13	0.78±.09	0.87±.07	0.80±.07
ASA	100	0.57±.03	1.08±.09 (28.2)	1.10±.07 (22.1)	0.90±.06 (34.0)	0.77±.08 (55.6)	0.90±.04 (NI)	0.88±.06 (22.5)	0.92±.07 (NI)
ME	100	0.47±.04	1.20±.08 (NI)	1.10±.04 (7.4)	1.08±.05 (NI)	1.03±.04 (NI)	0.90±.06 (NI)	1.00±.09 (NI)	0.93±.06 (NI)
ME	200	0.47±.08	1.25±.05 (NI)	1.07±.03 (11.8)	1.05±.04 (NI)	0.97±.06 (NI)	0.92±.05 (NI)	1.00±.09 (NI)	0.97±.08 (NI)
ME	400	0.73±.04	1.27±.07 (24.0)	1.13±.08 (41.2)	1.10±.07 (26.0)	0.92±.05 (57.8)	0.92±.08 (38.7)	1.03±.08 (25.0)	0.87±.06 (57.6)

Values are mean ± SEM paw volume (mL); n = 6. Figures in parentheses are percentage inhibition of oedema relative to control; Figures in parentheses are percentage inhibition of oedema (increase in paw volume above the 0 h baseline) relative to control, NI = no inhibition

Effects of the methanol extract and fractions on Formaldehyde-induced arthritis

ME (400 mg/kg) produced a significant reduction in paw thickness at 1 h ($P < 0.05$) relative to control, and both the 200 and 400 mg/kg doses significantly reduced paw thickness on day 5 ($P < 0.05$) (Figure 1A). Among the fractions, EF (400 mg/kg) was the most consistently active, producing a significant reduction in paw thickness on day 5 ($P < 0.05$) and the greatest cumulative suppression of the oedematous response. HF (400 mg/kg) also showed a modest

cumulative inhibition (Figure 1B). Cumulative response over the seven-day observation period, expressed as the area under the paw thickness–time curve (AUC), is summarised for all groups in Table 5. ME (200 and 400 mg/kg) gave the largest cumulative reductions (38.2% and 39.0%, respectively), followed by EF (400 mg/kg, 9.5%) and HF (400 mg/kg, 2.9%); the remaining fractions showed negligible or no net inhibition over the full observation window despite transient significant effects at individual time points.

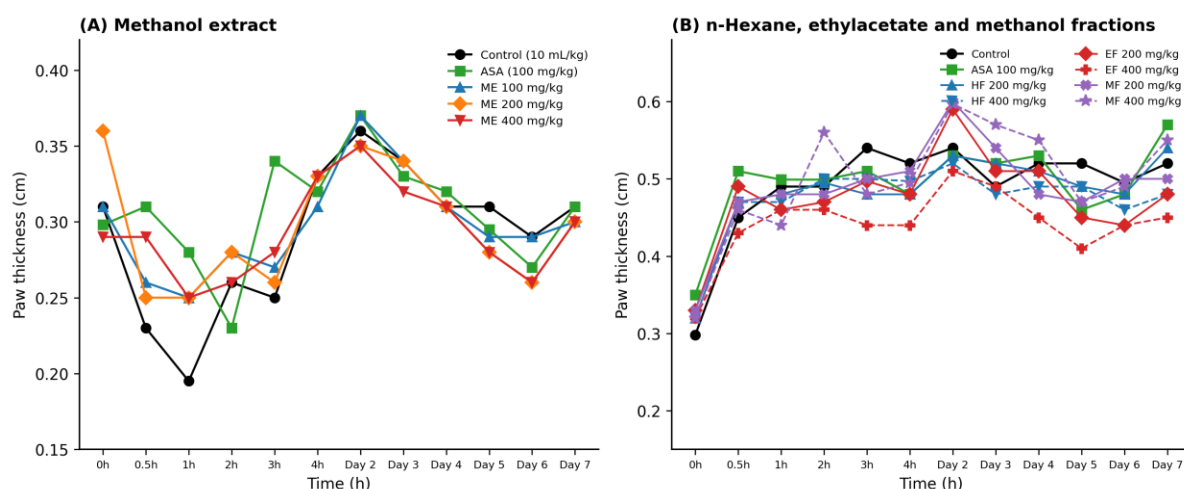


Figure 1. Time course of paw thickness in formaldehyde-induced arthritis in rats treated with (A) the methanol extract (ME) of *P. vogeliana* or (B) its n-hexane (HF), ethylacetate (EF) and methanol (MF) fractions. Values are mean $\pm$ SEM (error bars omitted for clarity; $n = 5$); ASA = acetylsalicylic acid (positive control).

Table 5: Area under the paw thickness–time curve (AUC) and percentage inhibition of formaldehyde-induced arthritis by the methanol extract and fractions of *P. vogeliana*

Group	Dose (mg/kg)	AUC (0–168 h)	Inhibition (%)
Control (ME cohort)	10 mL/kg	–	–
ME	100	53.28±0.97	1.7
ME	200	52.37±1.27	38.2
ME	400	51.63±1.09	39.0
Control (fraction cohort)	10 mL/kg	84.69±2.59	–
ASA	100	86.45±2.45	NI
HF	200	84.81±2.88	NI
HF	400	82.21±2.50	2.9
EF	200	83.36±1.29	1.6
EF	400	76.69±3.70	9.5
MF	200	87.21±2.23	NI
MF	400	87.57±3.56	NI

Values are mean ± SEM; n = 5. ME and the fractions (HF, EF, MF) were evaluated in two separate cohorts, each with its own concurrent control; the two control AUCs are therefore not directly comparable (see Discussion). NI = no net inhibition over the observation period. Inhibition (%) calculated relative to the respective cohort control AUC by the trapezoidal method (Shivaji *et al.*, 2000).

Effects of the methanol extract and fractions on cotton pellet-induced granuloma

Seven-day treatment with the extract and its fractions produced dose-related reductions in both the wet and dry weight of the cotton pellet granuloma relative to control (Figure 2). Statistical significance was reached only for HF, which reduced granuloma weight at both doses in a dose-dependent manner. The highest percentage reductions in dry weight ($p > 0.05$) were recorded for EF (400 mg/kg, 76.9%) and ME (400 mg/kg, 59.2%), followed by HF (400 mg/kg, 40.6%) and ME (100 mg/kg, 48.0%); ME inhibited granuloma dry weight at all three doses tested (38.3 to 59.2%) in a non-dose-response manner, whereas MF showed only modest activity (Figure 2).

Antioxidant activity of the ethylacetate fraction

EF produced concentration-dependent DPPH radical scavenging ranging from 88.9% to 99.1%, exceeding the scavenging activity of the L-ascorbic acid (62.6–64.2%) (Figure 3). A precise IC_{50} could not be determined by non-linear regression because scavenging had already plateaued within the concentration range tested.

In the ferrous ion chelating assay, EF similarly showed concentration-dependent activity ranging from 80.4% to 96.2%, exceeding ascorbic acid at every concentration above 15.63 µg/mL (Figure 3). As with the DPPH assay, the near-maximal response at low concentrations precluded precise IC_{50} determination within the tested range.

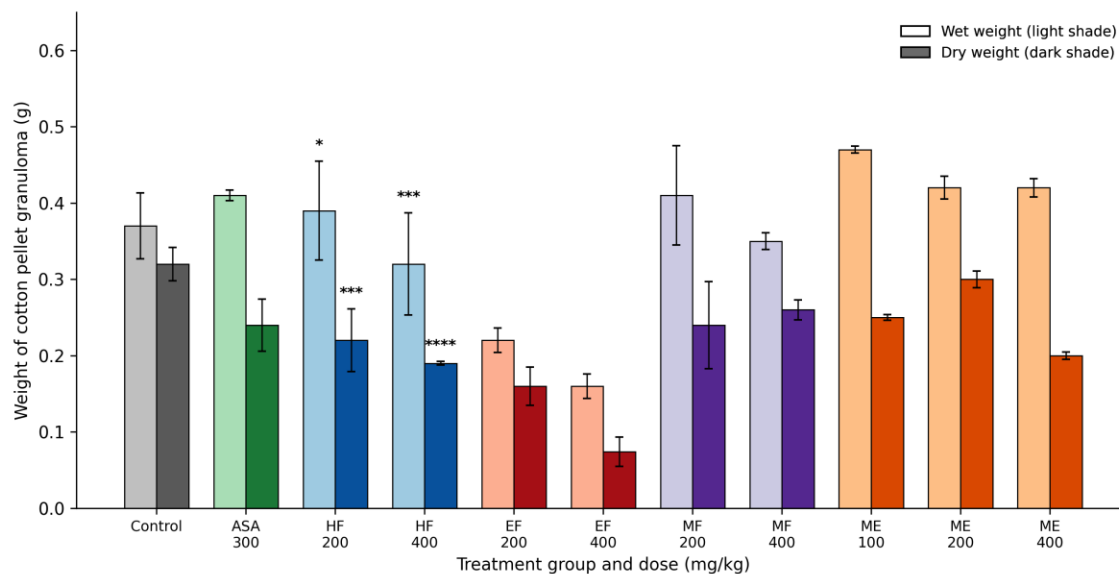


Figure 2. Wet and dry weight of cotton pellet granulomas in rats treated for seven days with the methanol extract (ME) or fractions of *P. vogeliana*. Values are mean $\pm$ SEM; n = 5. *p<0.05, ***p<0.001, ****p<0.0001 versus control (one-way ANOVA, Dunnett's post-hoc test).

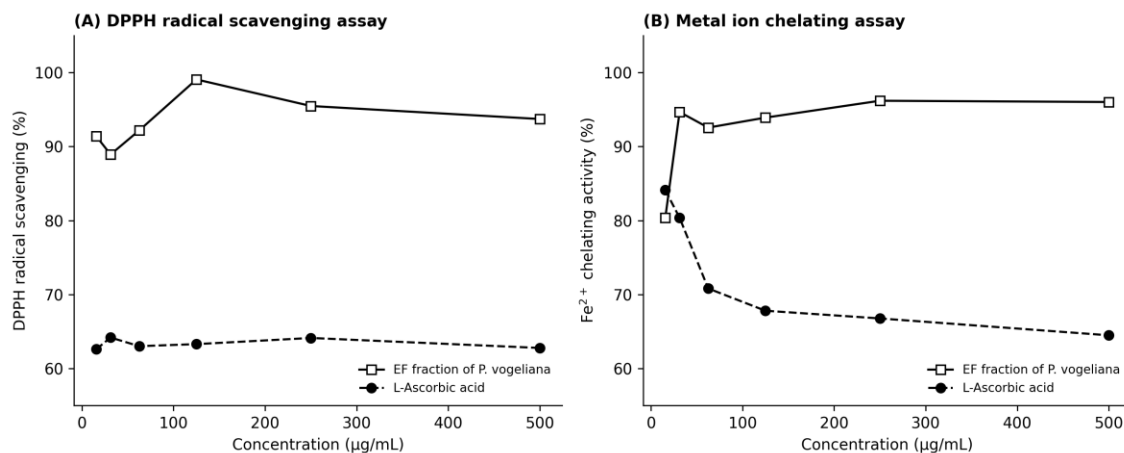


Figure 3. (A) DPPH radical scavenging and (B) ferrous ion chelating activity of the ethylacetate fraction (EF) of *P. vogeliana* compared with L-ascorbic acid. Values are mean of triplicate determinations.

Discussion

This study provides *in vivo* evidence that *P. vogeliana* possesses analgesic, chronic anti-inflammatory and marked antioxidant activities, and the differential

pattern of activity offers valuable mechanistic insights. ME and its fractions were essentially inactive in the histamine- and serotonin-mediated acute phase of egg albumin-induced oedema. Since the egg albumin model is most sensitive to inhibitors of early mediator release (histamine, serotonin, bradykinin),

and the formaldehyde and cotton pellet models respectively probe fibroblastic proliferation and granulomatous tissue deposition (Olajide *et al.*, 2000; Ndebia *et al.*, 2007), this pattern suggests that the extract and its fractions act preferentially on the later proliferative phase of inflammation rather than on early vasoactive mediator release. This interpretation is strengthened by a recent independent report that the methanol leaf extract of *P. vogeliana* inhibits phospholipase A2 and albumin denaturation *in vitro* with potency comparable to diclofenac (Chukwuma *et al.*, 2024). Phospholipase A2 liberates arachidonic acid for downstream prostaglandin synthesis (Dennis *et al.*, 2011), a pathway more consequential for the sustained oedema of chronic models than for the immediate histaminergic response captured in the first hour of the egg albumin assay. The present *in vivo* findings are therefore mechanistically consistent with, and extend, that *in vitro* observation.

The displayed pharmacological profile can be rationalised on phytochemical grounds. ME and its fractions were rich in tannins, flavonoids, steroids and terpenoids, classes with well-established anti-inflammatory and analgesic properties (Nakadate *et al.*, 2025). Steroidal constituents are known to inhibit prostaglandin biosynthesis via phospholipase A2 and cyclo-oxygenase (Uma *et al.*, 2010), a mechanism previously invoked to account for the anti-inflammatory and membrane-stabilising activities of stigmastane steroids isolated from the related Euphorbiaceous species *Alchornea floribunda* (Okoye *et al.*, 2010). Flavonoids and tannins exert their effects principally through free-radical scavenging and suppression of cyclo-oxygenase-2 and inducible nitric oxide synthase, thereby interrupting the nuclear factor- κ B-driven pathways implicated in the maintenance phase of inflammation (Nakadate *et al.*, 2025). Saponins and alkaloids, present in EF, have also been reported to attenuate nociceptive signalling and cytokine release in other plant extracts (Marzouk *et al.*, 2009). The concentration of these constituents was consistently highest in EF, which correspondingly gave both the strongest chronic anti-inflammatory activity and, by a wide margin, the strongest antioxidant activity, an internally consistent pattern across assays.

The pattern of analgesic activity observed for ME was model-dependent and mechanistically informative. In the tail-immersion test, which reflects centrally mediated, supraspinal antinociception, ME produced no statistically significant change in latency at any dose. In contrast, ME produced a dose-dependent and statistically significant reduction in acetic acid-induced writhing at 200 and 400 mg/kg (35.6% and

47.6% inhibition, respectively; $p < 0.05$), a magnitude comparable to that of the reference analgesic ASA (19.3% inhibition, $p < 0.05$). Because the acetic acid-writhing response is driven principally by peripheral release of prostaglandins and other inflammatory mediators that sensitise visceral nociceptors, rather than by centrally mediated mechanisms (Deraedt *et al.*, 1980; Koster *et al.*, 1959), this dissociation suggests that the analgesic activity of ME is mediated predominantly through peripheral, inflammation-linked pathways rather than central antinociception. This interpretation is consistent with, and mechanistically unified by, the phospholipase A2-inhibitory activity separately reported for the methanol leaf extract of *P. vogeliana* (Chukwuma *et al.*, 2024) and with the chronic anti-inflammatory activity of ME demonstrated in the present study. Any agent that limits peripheral prostaglandin generation would be expected to attenuate acetic acid-induced writhing without necessarily altering centrally mediated thermal nociceptive thresholds. The absence of effect on tail-immersion latency therefore suggests mechanistic selectivity for peripheral pathways rather than a general absence of antinociceptive activity.

The oral LD₅₀ of ME exceeding 5000 mg/kg places the extract in the practically non-toxic category by the classification of Lorke (1983), consistent with the LD₅₀ greater than 5000 mg/kg subsequently reported for the leaf extract (Chukwuma *et al.*, 2024) and lends preliminary support to the safety of traditional oral use of the plant.

The DPPH and metal-chelating assays showed near-maximal scavenging already at the lowest concentration tested (15.63 μ g/mL). This plateau precludes precise IC₅₀ determination within the tested range and indicates that EF's radical-scavenging capacity, consistent with its high tannins and flavonoids content, is realised at concentrations below those assayed here.

These findings align with, and are complemented by, a growing independent literature on this species. Pharmacognostic and phytochemical characterisation of the leaf has confirmed a similarly rich content of tannins, flavonoids and alkaloids (Mba *et al.*, 2021), and a separate report describes amelioration of biochemical markers of complete Freund's adjuvant-induced rheumatoid arthritis by the ethylacetate fraction of the leaf (Ezeani *et al.*, 2024), broadly consistent with the chronic anti-inflammatory activity of EF. Taken together with the present results, the overall weight of evidence supports *P. vogeliana* as a source of anti-inflammatory and antioxidant leads meriting further investigation, including bioassay-

guided isolation of the active compounds and evaluation in validated models of chronic inflammatory disease.

Conclusion

The methanol extract and ethylacetate fraction of *Psychotria vogeliana* show a low order of acute toxicity, dose-related analgesic activity that was statistically significant against peripheral (acetic acid-writhing) but not central (thermal) nociception, and significant chronic anti-inflammatory and antioxidant activity, most plausibly mediated by their tannin, flavonoid, steroid and terpenoid content. These findings provide pharmacological support for the traditional use of *P. vogeliana* in the management of pain and inflammatory conditions, particularly those of a chronic or proliferative nature.

Declarations

Ethical approval

All animal procedures were approved by the Research Ethics Committee, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka (approval number FPSRA/UNN/021/0105), and conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Pub. No. 85-23, revised 1985).

Author contributions (CRediT)

Angela O. Okpor and Chinonyelum E. Agbo: Investigation, Methodology, Formal analysis, Data curation, Writing – original draft. Collins A. Onyeto: Supervision and Validation. Chukwuemeka S. Nworu: Conceptualization, Resources, Supervision, Project administration, Review & Editing of the manuscript. All authors read and approved the final manuscript.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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CITATION Okpor AO, Agbo CE, Onyeto CA, Nworu CS (2026) Anti-Inflammatory, Analgesic and Antioxidant Activities of the Methanol Extract and Solvent Fractions of *Psychotria vogeliana* Benth. (Rubiaceae) in Wistar Rats and Mice
Trend Nat Prod Res Vol 7(4) 375-387. <https://doi.org/10.61594/tnpr.v7i4.2026.184>