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ANTIOXIDANT-MEDIATED ANTI-INFLAMMATORY EFFECTS OF *VERBENA OFFICINALIS* ROOT EXTRACT IN EXPERIMENTAL MODELS

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ABSTRACT

Arthritis and other inflammatory disorders are closely associated with oxidative stress and excessive production of inflammatory mediators. The present study aimed to evaluate the antioxidant and anti-inflammatory potential of the ethanolic root extract of Verbena officinalis through in vitro and in vivo experimental models. Preliminary phytochemical screening revealed the presence of flavonoids, phenolic compounds, terpenoids, glycosides, alkaloids, and saponins. Antioxidant activity was assessed using DPPH, ABTS, ferric reducing antioxidant power (FRAP), and total antioxidant capacity (TAC) assays. The extract exhibited significant concentration-dependent radical scavenging and reducing activities, indicating strong antioxidant potential. The anti-inflammatory activity was evaluated using the carrageenan-induced paw edema model in Wistar rats. Animals treated with Verbena officinalis extract (200 and 400 mg/kg, p.o.) showed a significant reduction in paw edema compared with the control group. At 4 h, the extract produced 42.97% and 52.34% inhibition of edema at doses of 200 and 400 mg/kg, respectively, while diclofenac sodium (10 mg/kg) produced 57.81% inhibition. The higher dose demonstrated anti-inflammatory activity comparable to the standard drug. The observed pharmacological effects may be attributed to the synergistic action of flavonoids and phenolic compounds, which are known to possess antioxidant and anti-inflammatory properties through free radical scavenging and modulation of inflammatory pathways. These findings provide scientific support for the traditional medicinal use of Verbena officinalis and suggest that its root extract may serve as a promising natural source for the development of plant-based antioxidant and anti-inflammatory therapeutics. Further studies on active compound isolation, molecular mechanisms, toxicity, and clinical efficacy are warranted.

KEYWORDS: Verbena officinalis; antioxidant activity; anti-inflammatory activity; carrageenan-induced paw edema; flavonoids; phenolic compounds; medicinal plants; arthritis.

INTRODUCTION

Arthritis is a chronic musculoskeletal disorder characterized by joint inflammation, pain, stiffness, swelling, and progressive impairment of mobility. It encompasses a heterogeneous group of diseases including osteoarthritis, rheumatoid arthritis, gout, psoriatic arthritis, and juvenile idiopathic arthritis, which collectively represent a major cause of disability worldwide. Among these conditions, rheumatoid arthritis and osteoarthritis are particularly important because of their high prevalence, chronic nature, and significant impact on quality of life. Persistent inflammation and progressive joint destruction can lead to functional impairment, reduced productivity, and increased healthcare burden.

The pathogenesis of arthritis involves a complex interplay of inflammatory mediators, immune dysregulation, oxidative stress, and tissue remodeling. In inflammatory arthritis, activated immune cells release pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6), which contribute to synovial inflammation, cartilage degradation, and bone erosion. Similarly, oxidative stress plays a crucial role in disease progression by driving excessive reactive oxygen species (ROS) production, leading to lipid peroxidation, protein oxidation, DNA damage, and amplification of inflammatory responses. These pathological processes ultimately contribute to pain, joint deformity, and loss of function.

Current therapeutic strategies for arthritis primarily aim to reduce inflammation, relieve pain, and prevent structural joint damage. Commonly used medications include nonsteroidal anti-inflammatory drugs (NSAIDs), corticosteroids, disease-modifying antirheumatic drugs (DMARDs), biologic agents, and targeted synthetic therapies. Although these treatments have significantly improved disease management, their long-term use is often associated with adverse effects such as gastrointestinal complications, hepatotoxicity, nephrotoxicity, cardiovascular risks, immunosuppression, and high treatment costs. Furthermore, some patients fail to achieve adequate therapeutic responses, highlighting the need for safer and more effective alternative approaches.

Natural products and medicinal plants have gained considerable attention as potential sources of novel antiarthritic agents. Numerous plant-derived compounds possess anti-inflammatory, antioxidant, analgesic, and immunomodulatory properties that may be beneficial in the management of arthritic disorders. Phytochemicals such as flavonoids, phenolic acids, terpenoids, iridoids, and tannins have been reported to inhibit inflammatory mediators, suppress oxidative stress, and protect against tissue damage. Consequently, scientific validation of traditionally used medicinal plants has become an important area of pharmacological research.

Verbena officinalis L. (Verbenaceae), commonly known as common vervain, is a medicinal herb widely used in traditional medicine for the treatment of inflammatory disorders, pain, fever, wounds, and various systemic ailments.

Phytochemical investigations have revealed the presence of biologically active constituents including iridoid glycosides, phenylpropanoid glycosides, flavonoids, tannins, terpenoids, and phenolic compounds. Previous studies have demonstrated that extracts of *Verbena officinalis* possess antioxidant, anti-inflammatory, analgesic, antimicrobial, neuroprotective, and immunomodulatory activities. These pharmacological properties suggest that the plant may have therapeutic potential against arthritis, where inflammation and oxidative stress are key pathogenic factors.

Despite its traditional use and reported pharmacological activities, scientific evidence regarding the antiarthritic potential of *Verbena officinalis* root extract remains limited. In particular, comprehensive evaluation using experimental models of arthritis and biochemical markers of oxidative stress is lacking. Therefore, further investigation is necessary to establish its efficacy and provide mechanistic insight into its therapeutic effects.

The present study was undertaken to evaluate the antiarthritic potential of *Verbena officinalis* root extract using experimental models of arthritis. The study aimed to investigate its effects on inflammatory responses, oxidative stress, and arthritis-associated pathological alterations. Furthermore, biochemical parameters and antioxidant markers were assessed to explore possible mechanisms underlying its activity. The findings of this study may provide scientific evidence supporting the traditional use of *Verbena officinalis* and contribute to the development of plant-derived therapeutic agents for the management of arthritis.

MATERIALS AND METHODS

2.1 Plant Material Collection and Authentication

Roots of *Verbena officinalis* L. (Family: Verbenaceae) were collected during the flowering season from India. The plant material was authenticated by a qualified taxonomist, and a voucher specimen was deposited in the herbarium of for future reference. The collected roots were washed thoroughly with distilled water, shade-dried at room temperature (25–30°C) for 10–14 days, and pulverized into coarse powder using a mechanical grinder.

2.2 Preparation of Extract

The powdered roots of *Verbena officinalis* were extracted using a Soxhlet apparatus with ethanol as the extraction solvent. Approximately 100 g of powdered material was extracted for 6–8 h until complete exhaustion. The extract was filtered and concentrated under reduced pressure using a rotary evaporator at 40–50°C. The concentrated extract was dried to obtain a semisolid mass, and the percentage yield was calculated. The extract was stored in airtight containers at 4°C until further use.

Percentage Yield

Yield (%) = (Weight of dried extract / Weight of crude powder) × 100

2.3 Preliminary Phytochemical Screening

The ethanolic extract was subjected to qualitative phytochemical screening for flavonoids, phenolics, tannins, terpenoids, glycosides, and alkaloids using standard procedures. Flavonoids were identified by the Shinoda test, phenolics and tannins by the ferric chloride test, terpenoids by the Salkowski test, glycosides by the Keller–Killiani test, and alkaloids by Dragendorff's test.

2.4 In Vitro Antioxidant Activity

2.4.1 DPPH Radical Scavenging Assay

The antioxidant activity of the extract was evaluated using the DPPH radical scavenging method. Various concentrations of the extract (20–100 µg/mL) were mixed with 0.1 mM DPPH solution and incubated in the dark for 30 min. Absorbance was measured at 517 nm using a UV–Visible spectrophotometer. Ascorbic acid served as the reference standard.[11]

2.4.2 ABTS Radical Scavenging Assay

ABTS radical cation was generated by reacting ABTS solution with potassium persulfate and incubating the mixture in the dark for 12–16 h. The extract (20–100 µg/mL) was mixed with the ABTS solution, and absorbance was recorded at 734 nm after 6 min of incubation.[12]

2.4.3 Ferric Reducing Antioxidant Power (FRAP) Assay

The reducing power of the extract was determined using FRAP reagent consisting of acetate buffer, TPTZ solution, and ferric chloride. Extract samples at different concentrations were incubated with FRAP reagent at 37°C for 30 min, and absorbance was measured at 593 nm.[13]

2.4.4 Total Antioxidant Capacity (TAC)

Total antioxidant capacity was evaluated using the phosphomolybdenum method. The extract was incubated with phosphomolybdenum reagent at 95°C for 90 min, cooled, and absorbance was measured at 695 nm.[14]

Calculation of Radical Scavenging Activity

Percentage inhibition was calculated using the following equation:

$$\% \text{ Inhibition} = [(Ac - As)/Ac] \times 100$$

where Ac represents the absorbance of the control and As represents the absorbance of the sample.

2.5 Experimental Animals

Healthy adult male Wistar rats (180–220 g) were procured from the Institutional Animal House Facility. Animals were maintained under standard laboratory conditions (25 ± 2°C, relative humidity 55–65%, and 12 h light/dark cycle) with free access to standard pellet diet and water ad libitum. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) and conducted in accordance with CPCSEA guidelines.[15]

2.6 Carrageenan-Induced Paw Edema Model

The acute anti-inflammatory activity of the ethanolic root extract of *Verbena officinalis* was evaluated using the carrageenan-induced paw edema model in Wistar rats, a widely accepted experimental model for assessing anti-inflammatory agents.[16] Animals were randomly divided into four groups (n = 6). Group I served as the vehicle control and received 1% carboxymethyl cellulose (CMC), Group II received diclofenac sodium (10 mg/kg, p.o.) as the standard anti-inflammatory drug, while Groups III and IV received *Verbena officinalis* extract at doses of 200 mg/kg and 400 mg/kg body weight, respectively. One hour after oral administration of the respective treatments, acute inflammation was induced by subplantar injection of 0.1 mL of 1% carrageenan suspension into the right hind paw of each animal.[16,17] Paw volume was measured using a digital plethysmometer immediately before carrageenan administration and subsequently at 1, 2, 3, and 4 h after injection. The increase in paw volume was used as an index of edema formation, and the anti-inflammatory effect of the extract was determined by comparing paw volumes in treated groups with those in the control group.[18]

Percentage Inhibition of Edema

$$\% \text{ Inhibition} = [(Vc - Vt)/Vc] \times 100$$

where Vc is the mean paw volume of the control group and Vt is the mean paw volume of the treated group.

2.7 Statistical Analysis

All experimental data were expressed as mean ± SEM (n = 6). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Differences were considered statistically significant at $p < 0.05$.[19]

3. RESULTS

3.1 Extractive Yield

The extraction yield of *Verbena officinalis* root varied according to the solvent employed. Among the tested solvents, the aqueous extract produced the highest yield (12.3 ± 0.5% w/w), followed by the ethanolic extract (9.6 ± 0.4% w/w), whereas petroleum ether yielded the lowest extractive value (2.8 ± 0.2% w/w) (Table 1).

Table 1: Extractive Yield of *Verbena officinalis* Root Extracts.

Solvent	Yield (% w/w)
Petroleum ether	2.8 ± 0.2
Ethanol	9.6 ± 0.4
Aqueous	12.3 ± 0.5

3.2 Preliminary Phytochemical Screening

Qualitative phytochemical analysis of the ethanolic root extract revealed the presence of several bioactive constituents, including flavonoids, phenolics, terpenoids, glycosides, alkaloids, and saponins. Flavonoids and phenolic compounds were found in abundant quantities, whereas alkaloids and saponins were detected in lower amounts (Table 2).

Table 2: Preliminary Phytochemical Screening of *Verbena officinalis* Root Extract.

Phytoconstituent	Result
Flavonoids	+++
Phenolics	+++
Terpenoids	++
Glycosides	++
Alkaloids	+
Saponins	+

3.3 DPPH Radical Scavenging Activity

The ethanolic root extract demonstrated concentration-dependent DPPH radical scavenging activity. The percentage inhibition increased from 28.5 ± 1.2% at 20 µg/mL to 79.4 ± 1.6% at 100 µg/mL. Ascorbic acid exhibited significantly higher activity at all tested concentrations (Table 3). The results indicate notable free-radical scavenging potential of the extract.

Table 3: DPPH Radical Scavenging Activity of *Verbena officinalis* Root Extract.

Concentration (µg/mL)	Extract (% Inhibition)	Ascorbic Acid (% Inhibition)
20	28.5 ± 1.2	45.2 ± 1.0
40	41.3 ± 1.5	62.8 ± 1.3
60	55.7 ± 1.8	75.6 ± 1.4
80	68.9 ± 2.0	86.3 ± 1.2
100	79.4 ± 1.6	92.5 ± 1.1

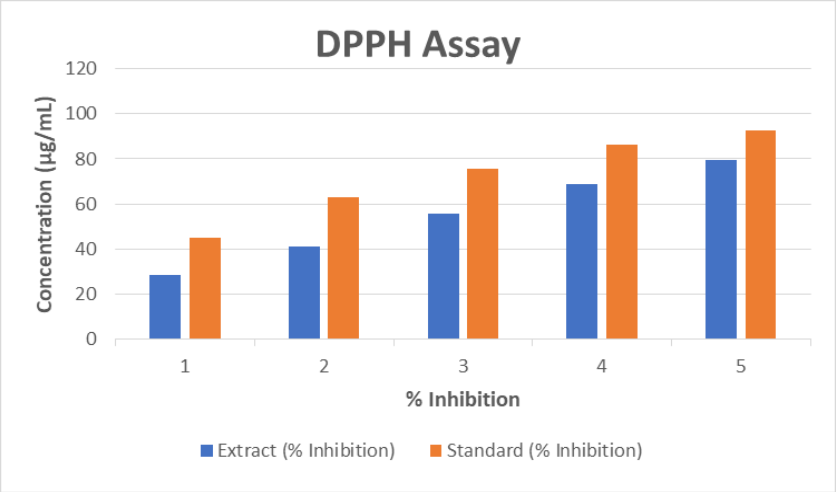

Figure 1: DPPH radical scavenging activity of *Verbena officinalis* root extract compared with ascorbic acid. Data are expressed as mean ± SEM (n = 3). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. Significant differences were observed between extract-treated groups and the standard antioxidant ($p < 0.05$).

3.4 ABTS Radical Cation Scavenging Activity

The extract exhibited marked ABTS radical scavenging activity in a concentration-dependent manner. The inhibition increased from 32.1 ± 1.1% at 20 µg/mL to 84.6 ± 1.5% at 100 µg/mL. Trolox showed greater activity across all concentrations (Table 4).

Table 4: ABTS Radical Cation Scavenging Activity of *Verbena officinalis* Root Extract

Concentration (µg/mL)	Extract (% Inhibition)	Trolox (% Inhibition)
20	32.1 ± 1.1	50.4 ± 1.3
40	47.8 ± 1.4	66.9 ± 1.5
60	61.5 ± 1.7	79.8 ± 1.2
80	73.2 ± 1.9	88.7 ± 1.1
100	84.6 ± 1.5	94.3 ± 1.0

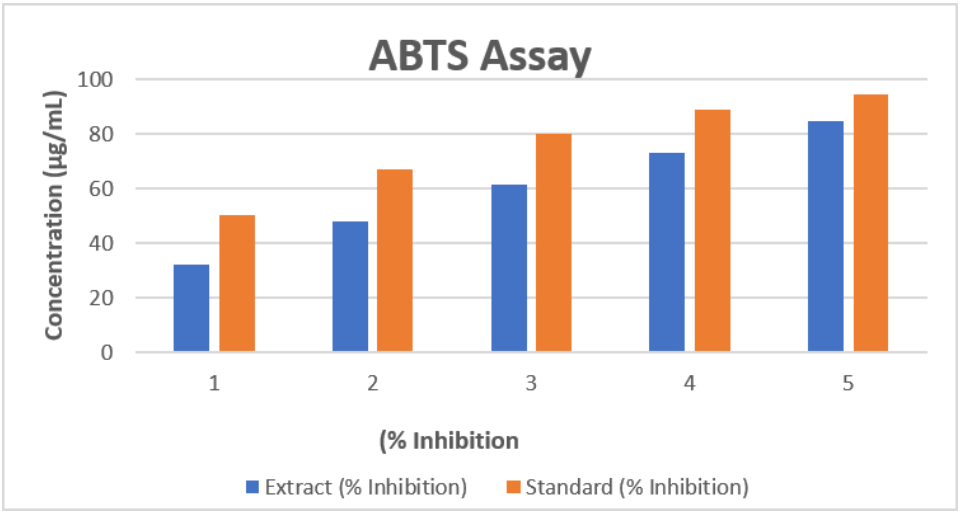

Figure 2. ABTS radical cation scavenging activity of *Verbena officinalis* root extract compared with Trolox. Values are presented as mean ± SEM (n = 3). One-way ANOVA followed by Tukey's post hoc test revealed significant concentration-dependent increases in antioxidant activity ($p < 0.05$).

3.5 Ferric Reducing Antioxidant Power (FRAP)

The ferric reducing capacity of the extract increased progressively with concentration. Absorbance values increased from 0.312 ± 0.01 at 20 µg/mL to 0.978 ± 0.03 at 100 µg/mL, indicating enhanced reducing power at higher concentrations (Table 5).

Table 5: Ferric Reducing Antioxidant Power of *Verbena officinalis* Root Extract.

Concentration (µg/mL)	Absorbance (593 nm)
20	0.312 ± 0.01
40	0.487 ± 0.02
60	0.655 ± 0.02
80	0.821 ± 0.03
100	0.978 ± 0.03

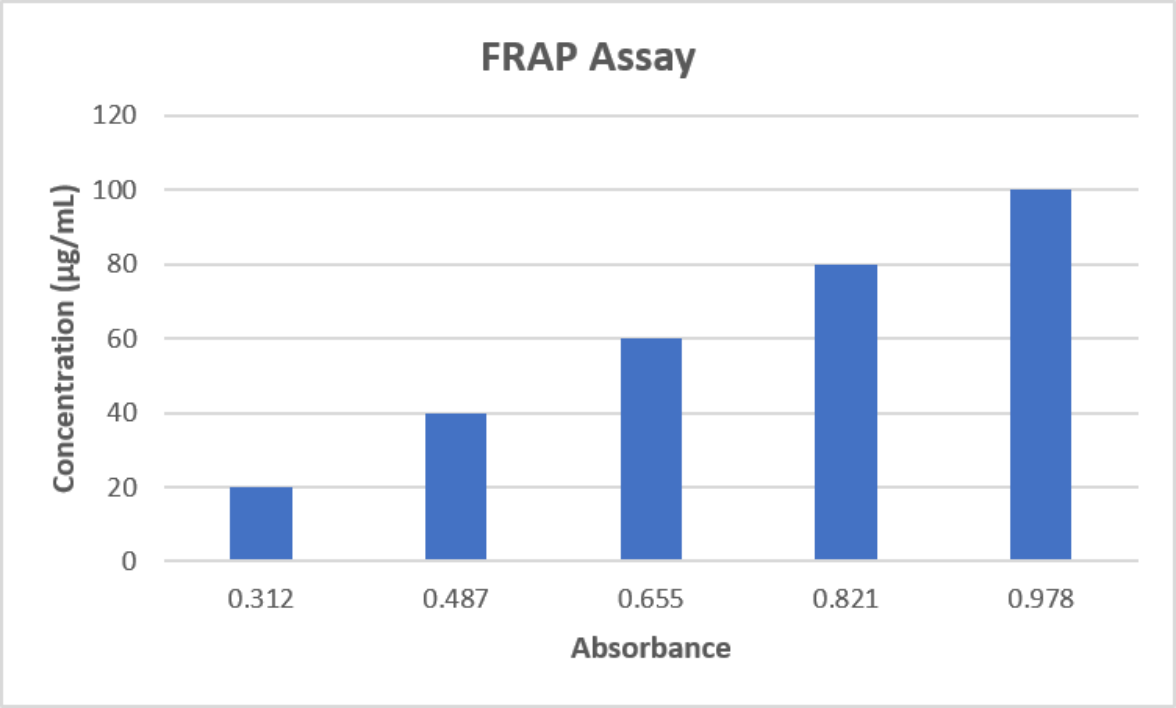

Figure 3: Ferric reducing antioxidant power of *Verbena officinalis* root extract. Data are expressed as mean ± SEM (n = 3). Statistical analysis showed a significant concentration-dependent increase in reducing activity ($p < 0.05$).

3.6 Total Antioxidant Capacity

The total antioxidant capacity of the extract increased significantly with increasing concentration. Absorbance values ranged from 0.285 ± 0.01 at 20 µg/mL to 0.889 ± 0.02 at 100 µg/mL (Table 6), demonstrating substantial antioxidant potential.

Table 6: Total Antioxidant Capacity of *Verbena officinalis* Root Extract.

Concentration (µg/mL)	Absorbance (695 nm)
20	0.285 ± 0.01
40	0.426 ± 0.02
60	0.598 ± 0.02
80	0.742 ± 0.03
100	0.889 ± 0.02

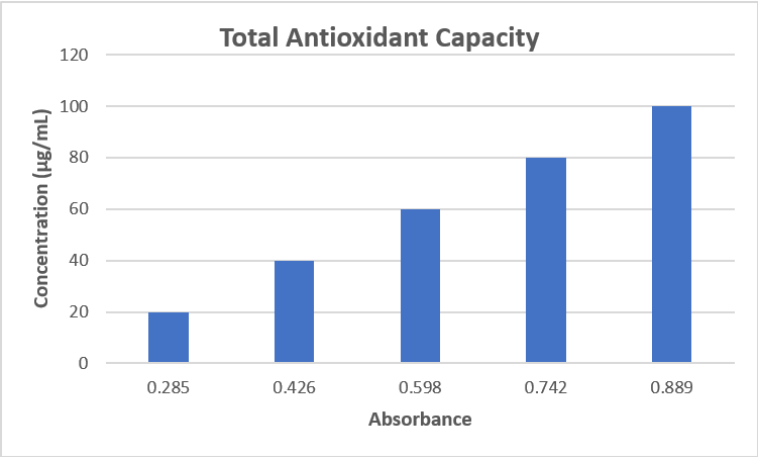

Figure 4: Total antioxidant capacity of *Verbena officinalis* root extract determined by the phosphomolybdenum method. Values are presented as mean ± SEM (n = 3). Significant concentration-dependent increases were observed ($p < 0.05$).

3.7 Effect of *Verbena officinalis* Root Extract on Carrageenan-Induced Paw Edema

Administration of carrageenan produced a marked increase in paw volume in the control group, reaching a maximum value of 1.32 ± 0.06 mL at 3 h. Treatment with *Verbena officinalis* extract significantly reduced paw edema compared with the control group. The 400 mg/kg extract exhibited greater anti-inflammatory activity than the 200 mg/kg dose and was comparable to diclofenac sodium (Table 7).

Table 7: Effect of *Verbena officinalis* Root Extract on Carrageenan-Induced Paw Edema.

Group	0 h	1 h	2 h	3 h	4 h
Control	0.52 ± 0.03	0.84 ± 0.04	1.15 ± 0.05	1.32 ± 0.06	1.28 ± 0.05
Diclofenac (10 mg/kg)	0.51 ± 0.02	0.68 ± 0.03*	0.72 ± 0.04*	0.58 ± 0.03***	0.54 ± 0.03***
Extract (200 mg/kg)	0.52 ± 0.03	0.76 ± 0.04	0.92 ± 0.05*	0.81 ± 0.04**	0.73 ± 0.04**
Extract (400 mg/kg)	0.51 ± 0.03	0.72 ± 0.03*	0.82 ± 0.04**	0.69 ± 0.03***	0.61 ± 0.03***

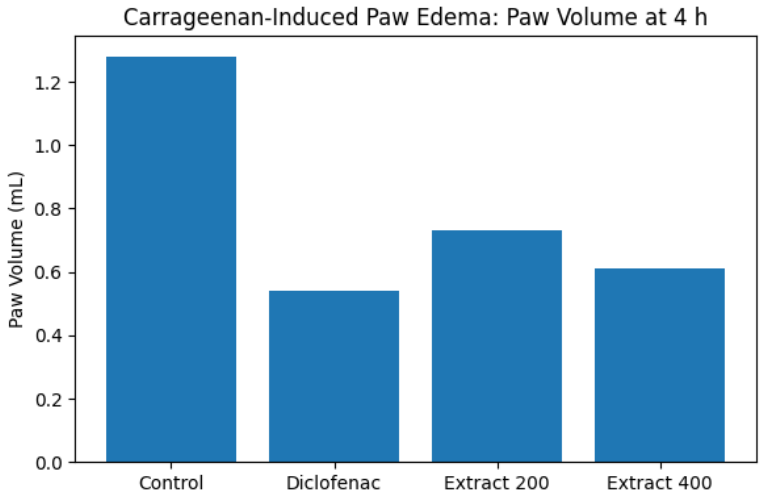

Figure 5: Effect of *Verbena officinalis* root extract on carrageenan-induced paw edema in Wistar rats. Data are expressed as mean ± SEM (n = 6). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. Significant reductions in paw volume were observed in extract-treated groups compared with the control group ($p < 0.05$).

3.8 Percentage Inhibition of Edema

The percentage inhibition of edema was calculated at the 4 h time point. Diclofenac sodium produced the highest inhibition (57.81%), followed by the extract at 400 mg/kg (52.34%) and 200 mg/kg (42.97%). The results indicate that the extract exhibits dose-dependent anti-inflammatory activity (Table 8).

Table 8: Percentage Inhibition of Carrageenan-Induced Paw Edema.

Group	Paw Volume (mL)	% Inhibition
Control	1.28	0.00
Diclofenac (10 mg/kg)	0.54	57.81
Extract (200 mg/kg)	0.73	42.97
Extract (400 mg/kg)	0.61	52.34

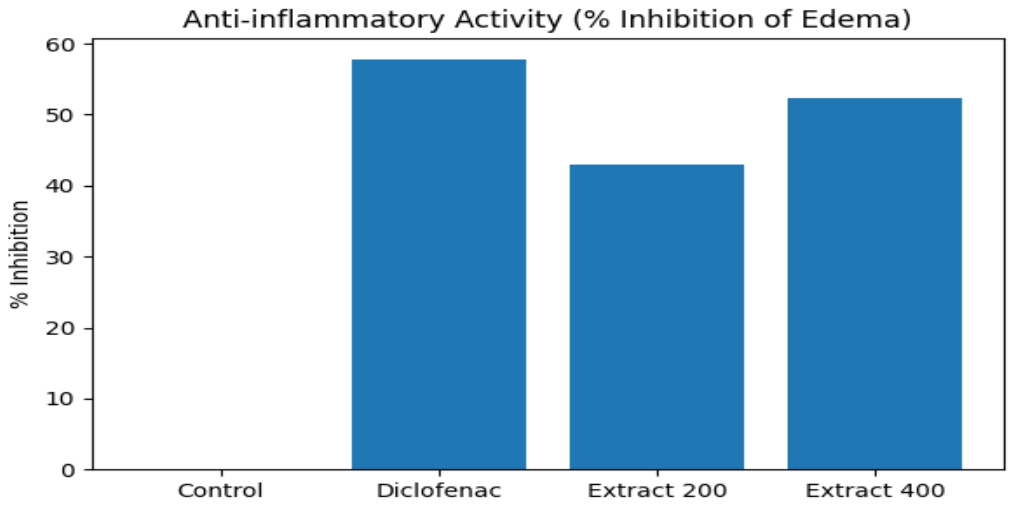

Figure 6: Percentage inhibition of carrageenan-induced paw edema following treatment with *Verbena officinalis* root extract and diclofenac sodium. Data represent mean values calculated from paw volume measurements at 4 h post-carrageenan administration.

4. DISCUSSION

The present study investigated the antioxidant and anti-inflammatory potential of *Verbena officinalis* root extract using in vitro antioxidant assays and the carrageenan-induced paw edema model in Wistar rats. The findings demonstrated that the extract possesses significant free radical scavenging activity and effectively attenuates acute inflammation in experimental animals. These effects may be attributed to the presence of bioactive phytoconstituents such as flavonoids, phenolic compounds, iridoid glycosides, and terpenoids, which have previously been reported in *Verbena officinalis* and are known to exert antioxidant and anti-inflammatory activities.[1-3]

Oxidative stress is recognized as a critical contributor to the pathogenesis and progression of inflammatory disorders, including rheumatoid arthritis and osteoarthritis. Excessive generation of reactive oxygen species (ROS) can promote lipid peroxidation, protein oxidation, cartilage degradation, and activation of inflammatory signaling pathways.[4,5]

Therefore, compounds capable of scavenging free radicals may provide therapeutic benefits by reducing oxidative damage and limiting inflammatory responses.

In the present study, the extract exhibited concentration-dependent DPPH and ABTS radical scavenging activity. The DPPH assay is widely used to evaluate the hydrogen-donating ability of antioxidant compounds, whereas the ABTS assay measures the capacity of antioxidants to neutralize radical cations. The increasing percentage inhibition observed with increasing extract concentration suggests that the extract contains potent electron-donating molecules capable of neutralizing reactive free radicals. Similar antioxidant activity has been reported for *Verbena officinalis* extracts rich in phenolic compounds and flavonoids.[3,6] Phenolic constituents are known to stabilize free radicals through hydrogen atom transfer and electron donation mechanisms, thereby preventing oxidative chain reactions.[7]

5. CONCLUSION

Overall, the findings provide scientific support for the traditional medicinal use of *Verbena officinalis* and indicate that its root extract may serve as a promising natural candidate for the development of plant-based anti-inflammatory and antioxidant formulations. Further investigations involving bioactivity-guided isolation of active compounds, molecular mechanism studies, chronic arthritis models, toxicity assessment, and clinical evaluation are warranted to establish its safety, efficacy, and potential therapeutic application in inflammatory diseases.

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