Asian Journal of Green Chemistry 10 (2026) 742-755

Asian Journal of Green Chemistry

Original Research Article View Article Online | View Journal

Exploration of Antioxidant and Anti-Inflammatory Activities in Various Parts of *Thunbergia fragrans*

Baratam Kasinath, Boddu Veeraswami*

Department of Chemistry, GITAM School of Science, GITAM Deemed to Be University, Visakhapatnam–530045, Andhra Pradesh, India

ARTICLE INFORMATION

Submitted: 2025-11-25
Revised: 2026-01-03
Accepted: 2026-02-07
Published: 2026-02-12
Manuscript ID: AJGC-2511-1875
DOI: 10.48309/ajgc.2026.559072.1875

KEYWORDS

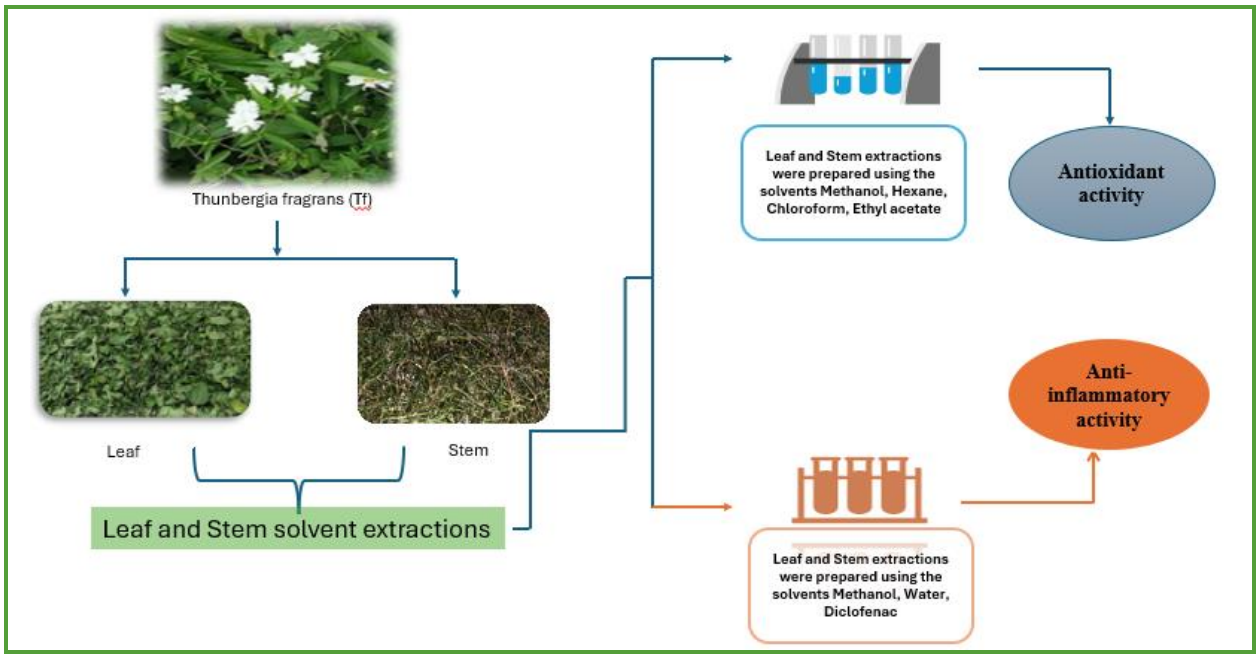
Thunbergia fragrans
Antioxidant
Anti-inflammatory
ROS
DPPH

ABSTRACT

Oxidation and inflammation are key contributors to chronic diseases such as cancer, cardiovascular disorders, and neurodegeneration. Reactive oxygen species (ROS) cause cellular damage, while chronic inflammation can worsen health outcomes. Antioxidants, though protective against ROS, may have adverse effects when excessively supplemented. This research highlights the importance of natural sources for the extraction of *Thunbergia fragrans*, a medicinal plant, was studied for its antioxidant and anti-inflammatory properties using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and membrane stabilization assays. Methanol extracts of both leaf and stem showed strong bioactivity (51.63% and 60.32%, respectively), with the leaf extracts nearing the efficacy of diclofenac, the standard anti-inflammatory drug. In contrast, aqueous extracts showed lower activity (79.26), underscoring the role of methanol (97.6) in extracting bioactive compounds. These findings suggest that *Thunbergia fragrans* is a promising source of natural therapeutic agents. Further research is needed to isolate and characterize the specific compounds responsible for the observed effects.

Corresponding author, E-mail: veeraswamiboddu@gmail.com (B. Veeraswami)
Tel.: +919493939469

Graphical Abstract



Introduction

The concept of oxidation refers to a chemical reaction in which electrons are transferred, resulting in the generation of ROS. These ROS, if accumulated in excess, can cause cellular damage, contributing to diseases such as cancer, cardiovascular disease, and neurodegenerative disorders [1]. The historical background of antioxidants dates back to the early 20th century, with the first discoveries related to free radicals. The term "free radical" was first used by the German chemist Hermann Alexander in 1900, but it was not until the 1950s that the importance of these species in biological systems gained attention [2]. In the following decades, the study of antioxidants was enriched by many important contributors. The work of Paul Berthold and later Nobel laureate Linus Pauling greatly emphasized the role of specific vitamins as antioxidants. Pauling's promotion of high-dose vitamin C as a cure for many diseases sparked extensive debate and research [3]. His proposal, although controversial, led to much research on the role of vitamin C in reducing oxidative stress and improving immune function. Despite strong support for the positive effects of antioxidants, a growing body of literature highlights potential negative aspects. The dual nature of antioxidants is essential to understanding their different effects on health. While antioxidants can neutralize free radicals and prevent oxidative damage, excessive supplementation can lead to adverse effects. High levels of vitamin E, for instance, have been linked to a higher risk of hemorrhagic stroke [4]. In addition, some studies have shown that antioxidants can promote tumor growth in certain contexts, suggesting that the complex interplay between oxidative stress and cell signaling pathways inherently complicates the antioxidant narrative [5]. Antioxidants work in several ways to provide their protective benefits. They can scavenge free radicals, chelate metals that catalyze oxidation, and promote the repair of oxidative damage. Many compounds have been identified as antioxidants, including polyphenols found in fruits and vegetables,

flavonoids, and carotenoids. Eating foods rich in these compounds has been associated with various health benefits. According to epidemiological research, diets high in fruits and vegetables are linked with a reduced occurrence of chronic diseases [6]. Although dietary antioxidant intake is beneficial, the role of supplements remains controversial. The usefulness of antioxidant supplements in reducing the incidence of chronic diseases has been the subject of conflicting clinical studies. Some studies have shown that certain antioxidants, when used alone, may not reproduce the beneficial effects of whole foods, suggesting that the synergistic properties of nutrients in their natural context may be important for their effectiveness [7]. Therefore, the general consensus among nutritionists and health professionals favors eating a diet rich in antioxidants derived from whole foods rather than relying on supplements. Inflammation is a complex biological response of the body's immune system to eliminate harmful stimuli, such as pathogens, damaged cells, and irritants. Although inflammation is a protective mechanism, chronic inflammation is increasingly recognized as a factor in many diseases, including arthritis, cardiovascular disease, and even some cancers. The search for anti-inflammatory agents has led to significant advances in medical science, nutrition, and pharmacology. This research article explores the historical background of anti-inflammatory research, highlights key figures in the field, and considers the positive and negative aspects associated with anti-inflammatory treatments. With advances in biochemistry and molecular biology, researchers have gained a better understanding of the complex pathways of inflammation. Notably, the discovery of cytokines, such as interleukin-6 and tumor necrosis factor alpha, has highlighted the role of inflammation in chronic diseases [8]. The advent of biologic therapies aimed at targeting specific pathways in the inflammatory response has revolutionized the treatment of autoimmune and inflammatory diseases. Although the benefits of anti-inflammatory treatments are significant, they do have negative aspects. For example, long-term use of traditional NSAIDs (Non-Steroidal Anti-Inflammatory Drugs) has been associated with gastrointestinal complications, renal failure, and increased cardiovascular risk [9]. Aspirin, although beneficial for its anti-inflammatory and antithrombotic effects, can lead to gastrointestinal bleeding and ulceration. Additionally, the increased prescription of opioids for the management of severe inflammatory pain raises another level of concern; the risk of addiction and the public health crisis surrounding opioid misuse have raised awareness of the importance of balancing pain relief with the risk of addiction. The exploration of dietary anti-inflammatory agents has become popular in recent years. Research has highlighted the role of anti-inflammatory foods—such as fruits, vegetables, nuts, and omega-3 fatty acids—in reducing the risk of chronic inflammation. The Mediterranean diet, rich in these foods, has been associated with lower rates of inflammatory diseases and has attracted attention for its potential health benefits [10]. However, the effectiveness of dietary interventions can be inconsistent, and individual responses to dietary changes can vary considerably. Additionally, the availability and accessibility of anti-inflammatory foods can be problematic for low-income individuals, which may lead to disparities in health outcomes. The primary objective of this research article is to investigate the antioxidant and anti-inflammatory activity of *Thunbergia fragrans*. DPPH radical scavenging assay and reducing power assay tests were performed to determine antioxidant activity. The membrane stabilization

assay was performed to determine anti-inflammatory activity.

Objectives

1. To investigate the antioxidant activity of *Thunbergia fragrans*.
2. To determine the anti-inflammatory properties of *Thunbergia fragrans*.

Experimental

Material and methods

DPPH radical scavenging assay

The DPPH radical scavenging assay is the most commonly employed technique for exploring the antioxidant potential of different compounds, both natural and synthetic. This approach can evaluate the antioxidant activity of plant extracts and isolated molecules that are either hydrophilic or hydrophobic. Within this assay, DPPH functions as a stable free radical. The antioxidant capacity of the tested molecule is established by the extent of DPPH reduction in methanol due to the hydrogen-donating action of the antioxidant test molecule, converting DPPH into a nonradical form [11]. In the procedure, 2.9 mL of a 0.3 mM DPPH solution in methanol was added to 0.1 mL of the test compound at different concentrations, namely 1 g/mL, 5 g/mL, 10 g/mL, and 20 gm/mL (n = 3). The mixture was thoroughly agitated and allowed to sit at room temperature for 30 min undisturbed. As a standard, 10 µg/mL of ascorbic acid was utilized, while methanol acted as a blank. A negative control consisted of 3 mL of DPPH in methanol. A spectrophotometer at 517 nm was used to measure the decrease in absorbance. Equation 1 is utilized for calculating the scavenging activity:

$$\%\text{Inhibition} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample)}}{\text{Absorbance of Control}} \times 100 \quad (1)$$

The half-maximal inhibitory concentration (IC_{50}) is calculated by plotting percent inhibition as a function of the sample (antioxidant).

Reducing power assay

The reducing ability of *Thunbergia fragrans* extracts was assessed using the potassium ferricyanide approach [12]. One mL of methanol extract at varying concentrations (20-120 µg/mL) was combined with phosphate buffer (1 mL, 0.2 M, and pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (1 mL, 1 % w/v). The resulting mixtures were then incubated in a water bath at 50 °C for 20 min. To each mixture, 500 µL of trichloroacetic acid (10 % w/v) was introduced, followed by the addition of 100 µL of ferric chloride (0.01 %, w/v) and mixed thoroughly. The absorbance was measured with a UV-Visible spectrophotometer at a wavelength of 700 nm. Ascorbic acid served as the standard reference for this experiment.

Anti-inflammatory activity: Membrane stabilization assay

Preparation of red blood cell (RBC) suspension

To perform a membrane stabilization assay, the blood was collected from a sheep's red blood cell (RBC) and was transferred into centrifuge tubes (containing the anticoagulant EDTA). The blood underwent a washing process three times with an isotonic buffer solution of 154 mM NaCl in 10 mM sodium phosphate buffer at a pH of 7. 4. Each washing required centrifugation of the blood at 3,000 rotations per minute for 5 min.

Heat-induced hemolysis

Various concentrations of the extract (10-150 µg/mL) were combined with 2 mL of PBS (pH 7.4; 10 mM/154 mM NaCl), followed by the addition of 200 µL of a 10 % RBC suspension. In the control test tube, PBS was added as a substitute for the test sample. All tubes containing the reaction mixture were incubated in a water bath at 56 °C for 30 min. Following incubation, the tubes were cooled under running tap water. The reaction mixture was then centrifuged at 2500 rpm for 5 min. The absorbance of the resulting supernatants was measured at 560 nm [13].

Aspirin was used as the standard reference drug. The percentage inhibition of hemolysis was calculated using Equation 2:

Hemolysis inhibition (%) =

Control-Sample / Control × 100 (2)

Statistical analysis

The mean data were analyzed statistically by applying the Analysis of Variance procedure, treating them as a mixed factorial randomized block design with two factors, where the first factor had three levels and the second factor had five levels. The statistical analysis was carried out as per the method suggested by Panse and Sukhatme [14]. The critical difference to examine the means for their significance was calculated at a 5 % level of significance.

Results

Table 1 represents the percentage of inhibition of different leaf extracts i.e., methanol (MTL), chloroform (CTL), ethyl acetate (ETL), hexane (HTL) and a standard at different concentrations (20, 40, 60, 80, and 100 µg/mL). Here's an interpretation of the data based on the percentage of inhibition for each extract and the standard at these concentrations. For all leaf extracts and the standard, the percentage of inhibition increases as the concentration rises from 20 µg/mL to 100 µg/mL. This shows a dose-dependent response, meaning that higher concentrations of the extracts and standard lead to stronger inhibition. This is typical for bioactive compounds, where efficacy improves as the concentration increases. The comparison of the percentage of inhibition standard with the leaf extracts results is shown in a graphical representation in Figure 1, and the reported values are shown in Table 1.

Concentration (µg/mL)

The standard (likely a pharmaceutical or known active compound) consistently shows the highest inhibition at each concentration, with values ranging from 48 % at 20 µg/mL to 94.21 % at 100 µg/mL. Methanol leaf extract shows the strongest inhibition among the leaf extracts. It steadily increases from 16.25 % inhibition at 20 µg/mL to 51.63% inhibition at 100 µg/mL. While it shows significant activity, it still lags behind the standard.

Table 1. Comparison of % of inhibition of the standard with the leaf extracts (concentration in µg/mL)

Sr./No.	Concentration	% of Inhibition				
	(µg/mL)	MTL	CTL	ETL	HTL	Standard
1	20	16.25±0.15	11.26±0.58	11.3±0.12	8.21±0.36	48±0.51
2	40	23.18±0.72	18.65±0.41	20.6±0.72	16.34±0.26	88.08±1.0
3	60	31.6±0.52	25.31±0.24	30.4±0.52	24.63±0.15	90.68±0.35
4	80	40.73±0.18	36.28±0.75	39.7±0.16	34.21±0.61	93.63±0.54
5	100	51.63±0.36	45.69±0.73	48.27±0.22	43.19±0.67	94.21±0.34

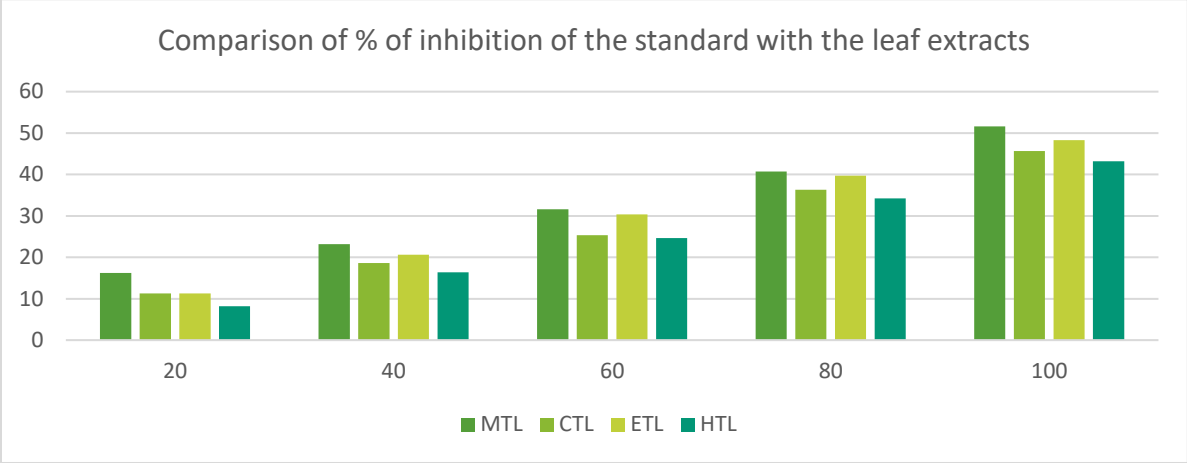


Figure 1. Comparison of inhibition (%) of the standard with the leaf extracts

Hexane leaf extract exhibits the lowest inhibition at all concentrations, ranging from 8.21 % at 20 µg/mL to 43.19 % at 100 µg/mL. The leaf extract of ethyl acetate and chloroform showed moderate inhibition. The percentage of inhibition for ethyl acetate leaf ranges from 11.3 % at 20 µg/mL to 48.27 % at 100 µg/mL, and for chloroform leaf extract, it ranges from 11.26 % at 20 µg/mL to 45.69 % at 100 µg/mL.

Table 2 compares the percentage of inhibition at varying concentrations (20, 40, 60, 80, and 100 µg/mL) for four different stem extracts of methanol, chloroform, ethyl acetate, and hexane, and a standard substance, likely a reference compound used for comparison. As the concentration of each extract increased, a corresponding rise in the percentage of inhibition was observed, indicating a dose-dependent effect. This is a common pattern in bioactivity assays, where higher concentrations of an extract result in stronger inhibitory effects. Methanol stem extract consistently shows the strongest inhibition across all concentrations, followed by ethyl acetate stem extract, chloroform stem extract, and hexane stem extract, which has the lowest inhibitory activity at most concentrations. The standard remains the most effective in inhibiting the target process, showing the highest percentage of inhibition across all concentrations. All extracts show increased inhibition as the concentration increases, supporting the idea of a dose-dependent relationship between extract concentration and inhibitory activity. The comparison of the percentage of inhibition standard with steam extracts with various solvents like Methanol, Chloroform, Ethyl acetate, and Hexane results were shown in a graphical representation in Figure 2, and the reported values are shown in Table 2.

Table 2. Comparison of % of inhibition of the standard with the stem extracts (methanol, chloroform, ethyl acetate, and hexane) (concentration in µg/mL)

Sr./No.	Concentration	% of inhibition				
	(µg/ML)	MTL	CTL	ETL	HTL	Standard
1.	20	14.53±0.18	11.48±0.21	12.68±0.24	8.34±0.16	48±0.51
2.	40	28.34±0.52	19.23±0.29	22.33±0.67	17.34±0.25	88.08±1.0
3.	60	39.62±0.22	28.39±0.56	31.26±0.41	23.89±0.68	90.68±0.35
4.	80	48.39±0.33	32.46±0.27	38.43±0.62	29.26±0.57	93.63±0.54
5.	100	60.32±0.58	39.64±0.45	47.13±0.7	37.9±0.24	94.21±0.34

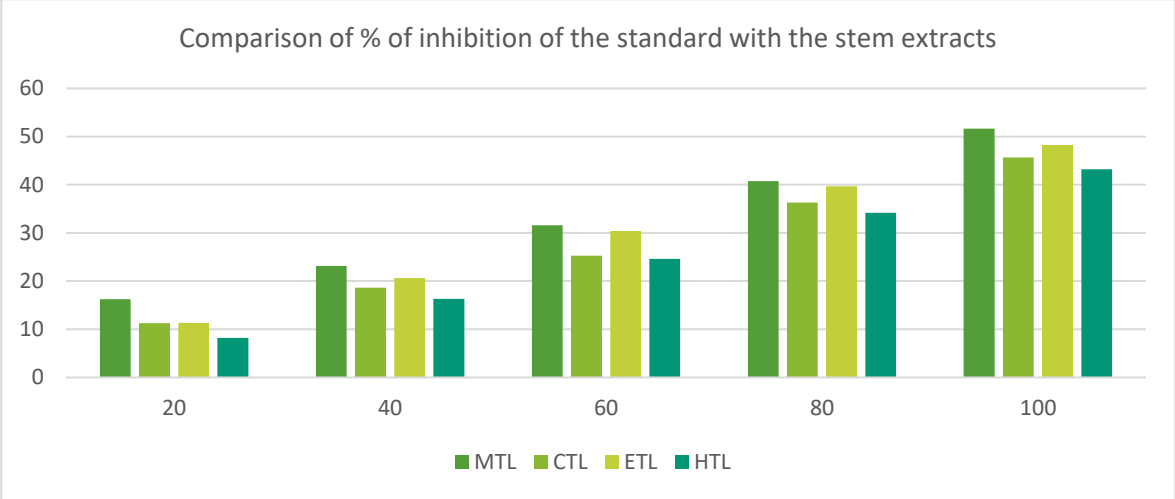


Figure 2. Comparison of % of inhibition of the standard with the stem extracts (methanol, chloroform, ethyl acetate, and hexane) (concentration in µg/mL)

Methanol stem extract consistently outperforms the other extracts at every concentration. This suggests that methanol extraction may be more effective at extracting the bioactive compounds responsible for the inhibition. Hexane stem extract shows the lowest inhibition at most concentrations, indicating that hexane extraction may be less efficient in extracting the active compounds compared to methanol. The standard shows significantly higher inhibitory activity at all concentrations compared to the extracts, indicating that the reference compound used in the experiment is more potent than the stem extracts from *Thunbergia fragrans*. The standard's inhibition is highest at 94.21 % at 100 µg/mL, suggesting it is a potent compound, likely serving as a benchmark for comparing the effectiveness of the plant extracts.

Table 3 presents the anti-inflammatory results of *Thunbergia fragrans* leaf extracts at various concentrations, comparing the effects of methanol extract, aqueous extract, and a diclofenac solution (a common anti-inflammatory drug). The values are presented as means ± standard deviations, which indicate the average anti-inflammatory activity at each concentration along with the variability (spread) of the data. A graphical representation of the results is shown in Figure 3.

At lower concentrations (10 µg/mL) the methanol extract shows the highest anti-inflammatory effect (6.52), followed by the aqueous extract (2.89), and diclofenac solution (20.18).

Table 3. Anti-inflammatory results of *Thunbergia fragrans* leaf extracts

Concentration (µg/mL)	Methanol extract	Aqueous extract	Diclofenac solution
10	6.52±1.58	2.89±1.56	20.18±1.24
25	15.29±1.19	12.08±1.28	43.58±1.44
50	28.56±1.09	19.57±1.6	58.13±1.75
100	60.70±1.39	43.76±1.56	77.41±1.23
150	97.6±1.35	79.26±1.29	92.26±1.41

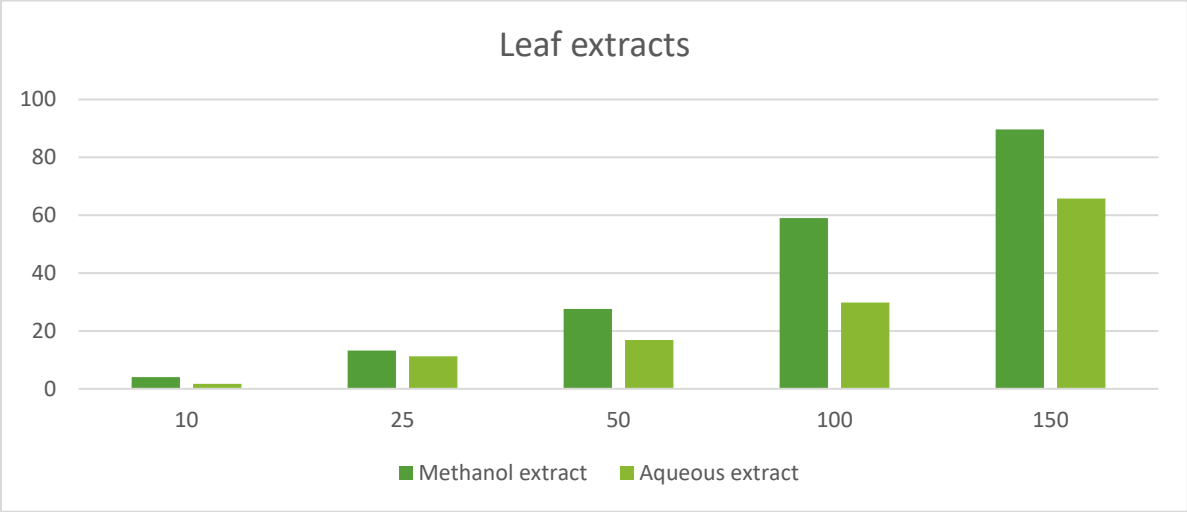


Figure 3. Anti-inflammatory results of *Thunbergia fragrans* leaf extracts

However, diclofenac is typically more potent as it acts as a standard, as expected. At higher concentrations (150 µg/mL), the methanol extract produces the highest anti-inflammatory effect (97.6), followed by the aqueous extract (79.26), and diclofenac (92.26). At higher concentrations, both extracts of *Thunbergia fragrans* exhibit substantial anti-inflammatory effects, approaching the effect of diclofenac. The methanol extract shows a progressive increase in anti-inflammatory activity as the concentration increases. The effect at 150 µg/mL is quite high (97.6), nearly matching or exceeding diclofenac at similar concentrations. The aqueous extract also shows an increase in activity with concentration, though it generally has lower anti-inflammatory effects compared to the methanol extract at each concentration. Diclofenac, as expected, is used as a standard for comparison. Its effects increase with concentration, showing a high anti-inflammatory activity at 150 µg/mL (92.26), though the methanol extract at the highest concentration slightly surpasses it.

Table 4 presents the anti-inflammatory results of *Thunbergia fragrans* stem extracts (methanol and aqueous extracts) at various concentrations, comparing them with the effect of a diclofenac solution (an established anti-inflammatory drug). The values are reported as means ± standard deviations, showing the average anti-inflammatory effects with variability at each concentration. A graphical representation of anti- inflammatory results is shown in Figure 4.

At lower concentrations (10 µg/mL), the methanol extract shows moderate anti-inflammatory activity (4.06). The aqueous extract has relatively low activity (1.69), indicating weaker effects compared to the methanol extract at this concentration.

At mid-range concentrations (50 µg/mL), the methanol extract shows a more significant increase in anti-inflammatory activity (27.63), the aqueous extract continues to show lower activity (16.92), but it increases relative to the 10 µg/mL concentration.

At higher concentrations (150 µg/mL), the methanol extract displays a strong anti-inflammatory effect (89.6), which is quite close to the diclofenac solution. The aqueous extract shows a noticeable increase in activity (65.76), but still remains below the methanol extract and diclofenac.

Table 4. Anti-inflammatory results of *Thunbergia fragrans* stem extracts

Concentration (µg/mL)	Methanol extract	Aqueous extract	Diclofenac solution
10	4.06±1.85	1.69±1.56	20.18±1.24
25	13.21±1.18	11.18±1.38	43.58±1.44
50	27.63±1.23	16.92±1.26	58.13±1.75
100	58.96±1.59	29.86±1.95	77.41±1.23
150	89.6±1.35	65.76±1.29	92.26±1.41

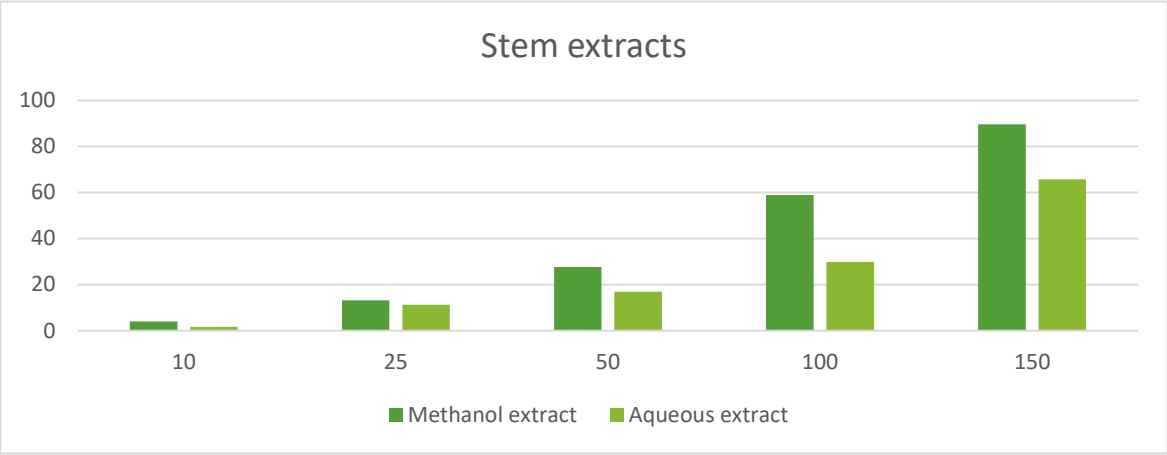


Figure 4. Anti-inflammatory results of *Thunbergia fragrans* stem extracts

The methanol extract shows a clear increase in anti-inflammatory effects with concentration, with the highest result at 150 µg/mL (89.6), nearly matching diclofenac's performance. The aqueous extract also shows an increase in anti-inflammatory effects with increasing concentration, but it is consistently lower in effectiveness compared to the methanol extract.

Methanol extract comparison

The methanol leaf extract shows a steady increase in anti-inflammatory activity from 6.52 at 10 µg/mL to 97.6 at 150 µg/mL. The methanol stem extract also shows an increase in anti-inflammatory effects, but with slightly lower values than in leaf extracts at each concentration. For example, at 150 µg/mL, the activity is 89.6 compared to 97.6 in leaf extracts. The methanol extract from the stem (Table 4) appears to have slightly lower anti-inflammatory effects compared to the methanol extract from leaves of the plant. Both extracts show that the methanol extract has the most potent anti-inflammatory effects among the extracts. The methanol leaf extract from *Thunbergia fragrans* appears to be slightly more effective than in the stem extracts.

Aqueous extract comparison

The aqueous leaf extract has relatively low anti-inflammatory effects compared to the methanol extract. The values range from 2.89 at 10 µg/mL to 79.26 at 150 µg/mL. In Table 3, the aqueous extract also shows lower values compared to methanol extract. However, at higher concentrations (especially 150 µg/mL), the activity is still significant, with a value of 79.26. The aqueous extract from the leaf (Table 3) has a stronger anti-inflammatory effect than the aqueous stem extract (Table 4). However, both aqueous extracts have lower effects than

methanol extracts. The aqueous extract from the leaf appears to be effective than the aqueous stem extract, but it remains less potent than the methanol extracts.

Discussion

Antioxidants are essential in neutralizing ROS, which contribute to the development of numerous diseases, including cancer, cardiovascular conditions, and neurodegenerative disorders. A substance's capacity to scavenge free radicals especially the DPPH radical is widely used as an indicator of its antioxidant activity. *Thunbergia fragrans*, a plant known for its medicinal properties, has demonstrated promising antioxidant activity in various studies, including *in vitro* tests using the DPPH assay. The DPPH assay is commonly employed to assess the free radical scavenging activity of plant extracts, due to its simplicity, sensitivity. DPPH is a stable free radical, and its reduction (by donating hydrogen or electrons) results in a decrease in absorbance that can be quantified spectrophotometrically [11]. The DPPH method is particularly advantageous because it provides quick and reproducible results for screening natural products, like plant extracts, for their antioxidant activity.

Antioxidant potential of Thunbergia fragrans

In this study, the antioxidant activity of *Thunbergia fragrans* leaf and stem extracts was evaluated using the DPPH assay. The extracts exhibited a dose-dependent increase in antioxidant activity, with higher concentrations of the plant extracts resulting in stronger inhibition of DPPH radicals. This suggests that the antioxidant compounds present in *Thunbergia fragrans* function in a concentration-dependent manner, which is consistent with the behavior of most bioactive antioxidants [15]. The methanol leaf extract demonstrated the highest antioxidant activity compared to other extracts (chloroform, ethyl acetate, and hexane) at all tested concentrations (20, 40, 60, 80, and 100 µg/mL). This may be attributed to the high solubility of bioactive compounds, including polyphenols, flavonoids, and other antioxidant metabolites, in methanol. These compounds are known for their ability to donate electrons or hydrogen atoms, thereby neutralizing free radicals [16,17]. Several studies have reported that methanol extracts are effective in extracting antioxidant phytochemicals from plants due to the solvent's ability to dissolve both polar and non-polar compounds [18]. On the other hand, the chloroform extract exhibited the lowest antioxidant activity. Chloroform is a less polar solvent compared to methanol and may not be as effective at extracting the hydrophilic antioxidant compounds present in *Thunbergia fragrans*. This observation is consistent with previous studies showing that the extraction efficacy of antioxidants is highly dependent on the solvent used, with more polar solvents often yielding higher antioxidant activity [19].

Comparison with standard antioxidants

The antioxidant activity of *Thunbergia fragrans* extracts was compared to that of a standard antioxidant (ascorbic acid), which served as a positive control. At all tested concentrations, the standard compound exhibited significantly higher radical scavenging activity than any of the plant extracts, which is expected given that ascorbic acid is a potent antioxidant widely known for its ability to scavenge ROS [1]. However, the results also indicate that *Thunbergia fragrans* has substantial antioxidant potential, particularly in its methanol leaf extract, making it a valuable source of natural antioxidants. The results from this study align with previous findings in which Thunbergia species demonstrated antioxidant properties, suggesting that *Thunbergia fragrans*

can be a promising candidate for natural antioxidant research and applications in nutraceuticals or functional foods [20].

Phytochemical composition and mechanism of action

The antioxidant activity of *Thunbergia fragrans* is likely attributed to its rich phytochemical composition, which includes polyphenols, flavonoids, and terpenoids well-known classes of compounds with proven antioxidant properties [21]. Polyphenols are especially effective at scavenging free radicals, owing to their ability to donate electrons, stabilize reactive species, and protect against damage caused by oxidative stress. Flavonoids including quercetin, catechins, and anthocyanins are powerful antioxidants that play a crucial role in neutralizing ROS and regulating oxidative stress [22]. Terpenoids, which are abundant in many plants, also contribute to antioxidant activity through their ability to interact with and neutralize ROS [23].

In addition to direct free radical scavenging, antioxidants from *Thunbergia fragrans* might also exert protective effects by modulating cellular antioxidant defense mechanisms. For example, they may activate nuclear factor erythroid-2-related factor 2 (Nrf2), a transcription factor that regulates the expression of antioxidant enzymes such as superoxide dismutase (SOD) and catalase, resulting in improved cellular defense against oxidative damage [24]. Thus, the bioactive compounds from *Thunbergia fragrans* might have a dual mechanism of action, both scavenging free radicals directly and upregulating the endogenous antioxidant defense system. The results of the DPPH assay highlight the significant antioxidant potential of *Thunbergia fragrans*, particularly in its methanol leaf extract. The antioxidant activity of the plant is concentration-dependent and suggests that it could serve as a valuable natural source of antioxidants. The findings align with previous studies on other plant species, which emphasize the role of polyphenols and flavonoids in modulating oxidative stress. Given the promising antioxidant activity observed, further investigations are warranted to isolate and identify the specific bioactive compounds responsible for the observed effects. Additionally, *in vivo* studies are necessary to assess the long-term effectiveness and safety of *Thunbergia fragrans* extracts in the prevention and management of diseases associated with oxidative stress. Plant-derived natural products are fundamental to the treatment of various human ailments and have long been utilized in herbal and ethnomedicinal practices worldwide, particularly across Africa and Asia. These remedies are often prepared as decoctions and continue to serve as the first line of defense in managing both acute and chronic health conditions due to their accessibility, efficacy, and generally low incidence of side effects [25,26]. The therapeutic potential of plants largely stems from the diverse phytochemicals they contain, which often act synergistically to produce pharmacological effects. Among the most extensively studied phytochemicals with notable antioxidant and anti-inflammatory properties are alkaloids, polyphenols, terpenoids, and flavonoids such as anthocyanins and flavones. Plants rich in these compounds have been recognized as potent sources of antioxidant and anti-inflammatory agents [27,28]. Despite substantial research, a considerable number of plant species used in traditional Ghanaian herbal medicine remain scientifically uninvestigated [25]. Moreover, there is a critical need for scientific validation of the therapeutic claims associated with many of these traditional remedies, which are currently underrepresented in the literature. The immunomodulatory effects of antioxidants are also evident in their capacity

to affect essential signaling pathways that govern inflammation. NF-kB is a central transcription factor that governs the expression of numerous genes directly implicated in the inflammatory process. Studies indicate that certain polyphenols possess the capability to modulate NF-kB activity and lessen inflammation within cells. Antioxidant activity results in the inhibition of the NF-kB transcription factor, subsequently leading to the downregulation of genes whose protein products contribute to inflammation [29,30]. The suppression of NF-kB driven by polyphenolic compounds and the consequent inhibition of chronic inflammation in neoplastic cells prove to be critically important for tumor growth and proliferation [31,32].

The ability of antioxidants to reduce the production of arachidonic acid, prostaglandins, and leukotrienes is considered a critical mechanism in modulating immune responses and exerting anti-inflammatory effects. Polyphenols, in particular, exert their biological activity on the arachidonic acid signaling pathway primarily through the inhibition of key enzymes involved in this process: cyclooxygenase (COX), lipoxygenase (LOX), and phospholipase A2 (PLA2). These enzymes are essential for the metabolism of arachidonic acid and the subsequent synthesis of inflammatory mediators such as prostaglandins, thromboxane A2, and leukotrienes. Recent *in vitro* and *in vivo* studies have demonstrated that certain polyphenols suppress the expression of these enzymes, leading to a marked reduction in the release of pro-inflammatory cytokines and the downregulation of transcription factors involved in the activation of genes associated with an exaggerated immune response. Notably, the anti-inflammatory actions of flavonoids have also been shown to enhance the anti-tumor efficacy of immune cells, underscoring their potential role in cancer immunomodulation [33-35].

Conclusion

In summary, the methanol leaf extract shows the best anti-inflammatory or inhibitory effect among the leaf extracts, but it is still less potent than the standard compound, which serves as the benchmark for comparison. The methanol stem extract is the most effective stem extract, showing significant improvement as the concentration increases. However, it still does not reach the potency of the standard at the highest concentration. The methanol and aqueous extracts from the stem generally show lower anti-inflammatory effects than the leaf extracts from other parts of the plant. Both indicate that methanol extracts outperform aqueous extracts in terms of anti-inflammatory activity. At higher concentrations (150 µg/mL), methanol extracts approach or even surpass diclofenac, while aqueous extracts remain lower in their effectiveness. These results suggest that methanol extracts may be more promising for bioactive purposes, but further research is required to identify the specific bioactive compounds responsible for the observed effects.

Acknowledgements

None.

Conflict of Interest

All the authors have declared that there are no potential conflicts between them to publish this research work.

ORCID

Boddu Veeraswami
https://orcid.org/0000-0002-4513-0250

References

[1] Halliwell, B., Gutteridge, J.M. Free radicals in biology and medicine. *Oxford University Press*, **2015**.

[2] Alpha-Tocopherol and Beta-Carotene Cancer Prevention Study Group. The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. *The New England Journal of Medicine*, **1994**, 330(15), 1029–1035.

[3] Sinha, K., Sil, P.C. Targeting oxidative stress and inflammation in NSAIDs-induced gastropathy: A plausible therapeutic approach. *Inflammation & Cell Signaling*, **2015**, 2, e763.

[4] Alpha-Tocopherol and Beta-Carotene Cancer Prevention Study Group. The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. *The New England Journal of Medicine*, **1994**, 330(15), 1029–1035.

[5] Sinha, K., Sil, P.C. Targeting oxidative stress and inflammation in nsaids induced gastropathy: A plausible therapeutic approach. *Inflamm Cell Signal*, **2015**, 2(e763), 1-6.

[6] Guigoz, Y., Vellas, B., Garry, P. Mini nutritional assessment: A practical assessment tool for grading the nutritional state of elderly patients. **1997**.

[7] Ding, H., Hu, G.L., Zheng, X.Y., Chen, Q., Threapleton, D.E., Zhou, Z.H. The method quality of cross-over studies involved in cochrane systematic reviews. *Plos One*, **2015**, 10(4), e0120519.

[8] Perico, L., Benigni, A., Casiraghi, F., Ng, L.F., Renia, L., Remuzzi, G. Immunity, endothelial injury and complement-induced coagulopathy in covid-19. *Nature Reviews Nephrology*, **2021**, 17(1), 46-64.

[9] Husby, S., Koletzko, S., Korponay-Szabó, I.R., Mearin, M.L., Phillips, A., Shamir, R., Troncone, R., Giersiepen, K., Branski, D., Catassi, C., Lelgeman, M., Mäki, M., Ribes-Koninckx, C., Ventura, A., Zimmer, K.P. Diagnosis and management of celiac disease in children: ESPGHAN gastrointestinal guidelines. *Journal of Pediatric Gastroenterology and Nutrition*, **2012**, 54(1), 136–160.

[10] Estruch, R., Ros, E., Salas-Salvadó, J., Covas, M.-I., Corella, D., Arós, F., Gómez-Gracia, E., Ruiz-Gutiérrez, V., Fiol, M., Lapetra, J., Lamuela-Raventós, R.M., Serra-Majem, L., Pintó, X., Basora, J., Muñoz, M.A., Sorlí, J.V., Martínez, J.A., Martínez-González, M.A. Primary prevention of cardiovascular disease with a Mediterranean diet. *The New England Journal of Medicine*, **2013**, 368(14), 1279–1290.

[11] Brand-Williams, W., Cuvelier, M.-E., Berset, C. Use of a free radical method to evaluate antioxidant activity. *LWT-Food science and Technology*, **1995**, 28(1), 25-30.

[12] Oyaizu, M. Studies on products of browning reaction antioxidative activities of products of browning reaction prepared from glucosamine. *The Japanese Journal of Nutrition and Dietetics*, **1986**, 44(6), 307-315.

[13] Sachindra, N., Airanthi, M., Hosokawa, M., Miyashita, K. Radical scavenging and singlet oxygen quenching activity of extracts from indian seaweeds. *Journal of Food Science and Technology*, **2010**, 47(1), 94-99.

[14] Panse, V.G., Sukhatme, P.V. Statistical methods for agricultural workers. *Indian Council of Agricultural Research Publication*, **1954**, 87-89.

[15] Abu, N.E., Ezeomeke, S.I., Azegba, P., Davidson, G.I. Phytochemical, nutritional and anti-nutritional properties of leaves, stems bark and roots of trees used in popular medicine for the treatment of malaria in south eastern nigeria. *Journal of Medicinal Plants Research*, **2016**, 10(38), 662-668.

[16] Naguib, A.E.-M.M., El-Baz, F.K., Salama, Z.A., Hanaa, H.A.E.B., Ali, H.F., Gaafar, A.A. Enhancement of phenolics, flavonoids and glucosinolates of broccoli (brassica olaracea, var. Italica) as antioxidants in response to organic and bio-organic fertilizers. *Journal of the Saudi Society of Agricultural Sciences*, **2012**, 11(2), 135-142.

[17] Saxena, M., Saxena, J., Nema, R. Ds-of pharmacognosy and, 2013 undefined. *Phytochemistry of Medicinal Plants. PhytojournalCom*, **2013**, 1, 168-182.

[18] Ravi, H.K., Breil, C., Vian, M.A., Chemat, F., Venskutonis, P.R. Biorefining of bilberry (vaccinium myrtillus l.) pomace using microwave hydrodiffusion and gravity, ultrasound-assisted, and bead-milling extraction. *ACS Sustainable Chemistry & Engineering*, **2018**, 6(3), 4185-4193.

[19] Zheng, Y., Jiang, X., Gao, F., Song, J., Sun, J., Wang, L., Sun, X., Lu, Z., Zhang, H. Identification of plant-derived natural products as potential inhibitors of the mycobacterium tuberculosis proteasome. *BMC Complementary and Alternative Medicine*, **2014**, 14(1), 400.

[20] Ahmed, N., Brahmbhatt, K.G., Khan, S.I., Jacob, M., Tekwani, B.L., Sabde, S., Mitra, D., Singh, I.P., Khan, I.A., Bhutani, K.K. Synthesis and biological evaluation of tricyclic guanidine analogues of batzelladine k for antimalarial, antileishmanial, antibacterial, antifungal, and anti-hiv activities. *Chemical Biology & Drug Design*, **2013**, 81(4), 491-498.

[21] Chukwujekwu, J.C., Amoo, S.O., de Kock, C.A., Smith, P.J., Van Staden, J. Antiplasmodial, acetylcholinesterase and alpha-glucosidase inhibitory and cytotoxicity properties of buddleja saligna. *South African Journal of Botany*, **2014**, 94, 6-8.

[22]. Boudier, A., Kirilov, P., Franceschi-Messant, S., Belkhelfa, H., Hadioui, L., Roques, C., Perez, E., Rico-Lattes, I. Evaluation of biocompatible stabilised gelled soya bean oil nanoparticles as new hydrophobic reservoirs. *Journal of Microencapsulation*, **2010**, 27(8), 682-692.
[23]. Bento, J.F., Noleto, G.R., de Oliveira Petkowicz, C.L. Isolation of an arabinogalactan from endopleura uchi bark decoction and its effect on hela cells. *Carbohydrate Polymers*, **2014**, 101, 871-877.
[24]. Liu, S., Pi, J., Zhang, Q. Signal amplification in the keap1-nrf2-are antioxidant response pathway. *Redox Biology*, **2022**, 54, 102389.
[25] Oguntibeju, O.O. Medicinal plants with anti-inflammatory activities from selected countries and regions of africa. *Journal of Inflammation Research*, **2018**, 307-317.
[26] Welz, A.N., Emberger-Klein, A., Menrad, K. Why people use herbal medicine: Insights from a focus-group study in germany. *BMC Complementary and Alternative Medicine*, **2018**, 18(1), 92.
[27] Fang, J. Bioavailability of anthocyanins. *Drug Metabolism Reviews*, **2014**, 46(4), 508-520.
[28] Liu, R.H. Potential synergy of phytochemicals in cancer prevention: Mechanism of action. *The Journal of Nutrition*, **2004**, 134(12), 3479S-3485S.
[29] Maurya, A.K., Vinayak, M. Quercetin attenuates cell survival, inflammation, and angiogenesis via modulation of akt signaling in murine t-cell lymphoma. *Nutrition and Cancer*, **2017**, 69(3), 470-480.
[30] Sakamoto, Y., Kanatsu, J., Toh, M., Naka, A., Kondo, K., Iida, K. The dietary isoflavone daidzein reduces expression of pro-inflammatory genes through pparα/γ and jnk pathways in adipocyte and macrophage co-cultures. *Plos One*, **2016**, 11(2), e0149676.
[31] Venancio, V.P., Cipriano, P.A., Kim, H., Antunes, L.M., Talcott, S.T., Mertens-Talcott, S.U. Cocoplum (chrysobalanus icaco l.) anthocyanins exert anti-inflammatory activity in human colon cancer and non-malignant colon cells. *Food & Function*, **2017**, 8(1), 307-314.
[32] Ai, X.-Y., Qin, Y., Liu, H.-J., Cui, Z.-H., Li, M., Yang, J.-H., Zhong, W.-L., Liu, Y.R., Chen, S., Sun, T. Apigenin inhibits colonic inflammation and tumorigenesis by suppressing STAT3-NF-KB signaling. *Oncotarget*, **2017**, 8(59), 100216.
[33] Shirakami, Y., Sakai, H., Kochi, T., Seishima, M., Shimizu, M. Catechins and its role in chronic diseases. *Drug Discovery from Mother Nature*, **2016**, 67-90.
[34] Ginwala, R., Bhavsar, R., Chigbu, D.G.I., Jain, P., Khan, Z.K. Potential role of flavonoids in treating chronic inflammatory diseases with a special focus on the anti-inflammatory activity of apigenin. *Antioxidants*, **2019**, 8(2), 35.
[35] Ghiţu, A., Schwiebs, A., Radeke, H.H., Avram, S., Zupko, I., Bor, A., Pavel, I.Z., Dehelean, C.A., Oprean, C., Bojin, F. A comprehensive assessment of apigenin as an antiproliferative, proapoptotic, antiangiogenic and immunomodulatory phytocompound. *Nutrients*, **2019**, 11(4), 858.

HOW TO CITE THIS MANUSCRIPT
B. Kasinath, B. Veeraswami. Exploration of Antioxidant and Anti-Inflammatory Activities in Various Parts of *Thunbergia fragrans*. *Asian Journal of Green Chemistry*, 10 (4) 2026, 742-755.
DOI: https://doi.org/10.48309/ajgc.2026.559072.1875
URL: https://www.ajgreenchem.com/article_240522.html