

Antioxidant and Anti-inflammatory Activity of *Tinospora sinensis*

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ABSTRACT

Throughout thousands of years, humans have used different methods and approaches for curing health disorders, and herbal medicine has emerged as a major approach due to its benefits and fewer side effects compared with conventional medicine. Within this background, this article investigates the medicinal uses of the herbal drug *Tinospora sinensis*, which has been widely employed in traditional medicine systems. The use of *Tinospora sinensis* as a medicinal herb will be explored on the basis of its antioxidant and anti-inflammatory properties, with special emphasis on its role as a scavenger of free radicals and membrane stabilization. By employing various in vitro methods, such as the DPPH radical scavenging assay, reducing power assay, and membrane stabilization assay, it can be determined whether methanol and aqueous extracts of *Tinospora sinensis* have useful effects against oxidative stress and inflammation, which are major components within various disease conditions such as cancer, diabetes, and cardiovascular disease. The findings clearly bring out that methanol extracts of leaves and stems possess significant antioxidant and anti-inflammatory properties, and methanol extracts perform better compared with aqueous extracts.

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Introduction

People have fought ailments, agony, and difficulties across millennia employing a range of approaches [1,2]. One of the many methods used in the fight against these conditions is the use of therapeutic plants for treating various illnesses [3,4]. Despite the creation of several important therapies, the tilt towards herbal medicine is gathering momentum due to growing concerns about the increasing toxicities connected with conventional line treatments [5-7]. Medicinal plants are nowadays viewed as a supplementary and alternative therapy in conjunction with other medicines [8]. Conditions including cancer, Alzheimer's disease, Parkinson's disease, inflammatory disease, lipid peroxidation, DNA damage, celiac disease, stroke, cardiovascular disease, protein oxidation, and diabetes are mostly caused by free radicals. [9]

Free radicals are molecules with an unpaired electron that may exist independently [10]. Because of the unpaired electron in their atomic orbitals, free radicals are extremely unstable and reactive, therefore functioning as either oxidants or reductants by accepting or donating electrons [11]. Because the unpaired electron is in the atomic orbital, free radicals

are very unstable and reactive; this lets them act as oxidants or reductants by donating or accepting electrons [11]. The main free radicals related to health problems are reactive oxygen species (ROS), reactive nitrogen species (RNS), and reactive sulfur species (RSS), which include oxygen singlets, superoxides, hypochlorite, hydrogen peroxide, hydroxyl radicals, nitric oxide, and peroxy nitrite radicals [10]. Crucial to a range of biological processes, including cell signalling, non-specific host defense mediators, and tumour cell death, are free radicals. Though their abundance hurts physiologically important substances like carbohydrates, proteins, lipids, and nucleic acids [11,12], which leads to cellular damage and homeostatic imbalance. Free radicals are produced in the human system as a result of both enzymatic and non-enzymatic reactions during the course of normal essential metabolic processes, but exposure to physical agents like X-rays, ozone, nicotine, air pollutants, and certain industrial chemicals [13,14] can cause them. From the activity of free radicals on molecular oxygen come reactive oxygen species (ROS), which rise significantly during inflammation, thereby unbalancing the body's antioxidant system with

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oxidizing molecules. This oxidative stress causes inflammatory cascades that injure the cellular components.

Inflammation is an adaptive response brought on by infection, tissue damage, and other irritants [15,16]. Well established [17] is the complex and strong relationship between oxidative stress and the inflammatory response. For immunological cell activation and/or dysfunction, changes in the redox condition of the endocellular environment are crucial. Originally thought to be "health-promoting" because of their radical-scavenging activity and/or direct antioxidant effects on cellular biomolecules [19], such compounds are now thought to disrupt cell functions by intercepting reactive species at the level of critical cell signalling pathways. In accordance with this, the plant kingdom is a rich source of secondary metabolites called phytochemicals (PhC), which have been demonstrated to effectively regulate the inflammatory response via their redox-modulating effects [18]. Moreover, recent studies have shown how these molecules react with enzymes, receptors, and transcription factors [20, 21]. Secondary metabolites from a few medicinal plants are useful in alleviating pain and inflammation.

As medical prices keep rising and concerns over the safety and efficacy of synthetic drugs grow, interest in the therapeutic advantages of plants is renewed. Modern scientific verification combined with old knowledge shapes healthcare's future. This study seeks to close the gap between conventional wisdom and modern biomedical research by providing a holistic view of the use of medicinal plants in both ancient healing methods and their possible impact on future medical treatments.

Plant Description

Family: Menispermaceae

Vernacular names include Amudam (Tamil), Vatsadani (Sanskrit), Pothamruthu, Kattamruthu, (Malayalam), Giloe (Hindi).

India to Indo-China distribution:

Woody climbers are characterized. Usually ovate leaves with a sharp apex. Male blooms have pedicels; petals in female blooms are similar to those in male flowers. 3 carpels; stigmas bilobed; 6 staminodes; clavate; glabrous; ovoid drupes red.

Climbers, lianas, are a frequent behavior.

Habitat: Holy groves in the plains, as well as evergreens and wet deciduous forests

From February to June is when blooming and fruit generation takes place.

Used parts: The entire plant

Tonic and stomachic properties and uses. Stem treats diabetes, piles, skin problems, neurological illnesses, and urinary disorders, as well as fever, jaundice, burning. An effective gonorrhoea therapy is honey and leaf juice in combination. The whole plant is used to treat piles, ulcerated sores, and liver disorders. Fresh leaves and stalks are used to treat chronic rheumatism. Folk Medicinal Systems:

Conservation Level: Virtually threatened.

DPPH radical scavenging activity assay

The in vitro free radical scavenging ability of the fractions was evaluated using the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) test, as previously described. 24 mg of DPPH were dissolved in 100 ml of methanol to produce a stock solution that was kept at 20 degrees Celsius until needed. Using a spectrophotometer at 517 nm, methanol diluted the DPPH solution to a concentration of roughly 0.98 ± 0.02 . 3 mL of this stock solution were combined with 100 l of the sample at various concentrations (10–500 g/ml). The reaction mixture was well mixed and kept in the dark for fifteen minutes at room temperature. Following then, the absorbance at 517 nm was noted. The control itself was created same way but without any sample. The percentage of DPPH radical scavenged was used to , the scavenging activity using the following equation:

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

Reducing power

The reducing ability of the solvent fractions was derived from their capacity to convert Fe (III) to Fe (II). The creation of Perl's Prussian blue at 700 nm can be used to distinguish Fe(II). Various sample (2 ml) concentrations were mixed with 2 mL of potassium ferricyanide (10 mg/ml) and 2 mL of phosphate buffer (0.2 M, pH 6.6). Following a 20-minute incubation period at 50°C, 2 mL of trichloroacetic acid (100 mg/L) was added to the mix. Centrifuging the mixture at 3000 rpm for 10 minutes provided the upper layer of the solution. 2 ml from each of the above stated mixtures was combined with 2 ml of distilled water and 0.4 ml of 0.1% (w/v) freshly made ferric chloride. The absorbance at 700 nm was recorded ten minutes following reaction. Increased absorption in the reaction mixture suggests a greater reduction capacity. Using a range of standard Trolox solutions ranging from 31.25 µg/mL to 1.0 mg/mL, a Trolox curve was created for the FRAP experiment with triplicate results (the standard curve equation: $y = 0.0007x + 0.0645$, $R^2 =$

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0.9998). TEAC in mM/L Trolox was stated as the outcome.

Anti-inflammatory activity - Membrane Stabilisation Assay

Preparation of suspensions of Red Blood Cells (RBCs)

To perform a membrane stabilisation test, the blood from a sheep's red blood cells (SRBC) was extracted and placed into centrifuge tubes (filled with EDTA, an anticoagulant). Three washes of the blood were carried out with an isotonic buffer solution of 154 mM NaCl in 10 mM sodium phosphate buffer pH 7.4. The blood was spun five minutes at 3000 rpm after each wash.

Hemolysis brought on by heat

Various concentrations of the extract (10–150 µg/mL) were combined with 2 mL of PBS (pH 7.4–10 mM/154 mM NaCl), then 200 µL of a 10% RBC suspension was added. PBS replaced the test sample in the control test tube. The tubes holding the reaction mixture were incubated for 30 minutes in a water bath at 56°C. The tubes were cooled under running tap water after incubation. The absorbance of the resulting supernatants at 560 nm was measured following centrifugation of the reaction mixture for 5 minutes at 2500 rpm. Standard reference medication aspirin was used. The formula for the percentage inhibition of hemolysis follows:

$$\text{Haemolysis inhibition (\%)} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100$$

Results

1. DPPH radical scavenging activity assay of *Tinospora sinensis*

Table 1 displays the % inhibition of methanol, chloroform, ethyl acetate, and hexane *Tinospora sinensis* leaf extracts, along with a standard, all at different concentrations of 20, 40, 60, 80, and 100 g/mL. Considering the % inhibition of each extract and reference at these doses helps us to understand the data as follows: From a concentration of 20 µg/mL to 100 µg/mL, the % inhibition rises for every leaf extract and the standard. This finding suggests a dose-dependent effect, whereby the inhibition becomes more pronounced as the concentration of extracts and standards increases. Generally speaking, the efficacy of bioactive components rises with their concentration.

Table 1. Comparison of % of inhibition of the standard with the *Tinospora sinensis* leaf extracts (Concentration in µg/ml).

S. No	Concentration (µg/ml)	% of inhibition				
		ML	CL	EL	HL	Standard

1.	20	22.6 ±0.1 8	11.2 6±0. 58	13.2 9±0. 55	10.5 9±0. 16	48± 0.51
2.	40	29.6 8±0. 42	18.6 5±0. 41	19.6 9±0. 48	16.3 4±0. 25	88.0 8±1. 0
3.	60	38.5 5±0. 18	25.3 1±0. 24	31.4 8±0. 25	26.8 9±0. 68	90.6 8±0. 35
4.	80	49.6 8±0. 02	36.2 8±0. 75	42.8 8±0. 47	32.2 6±0. 57	93.6 3±0. 54
5.	100	59.8 9±0. 33	45.6 9±0. 73	51.6 7±0. 95	39.9 ±0.2 4	94.2 1±0. 34

With inhibition levels ranging from 48% at 20 µg/mL to 94.21% at 100 µg/mL, the standard a pharmaceutical or known active ingredient exhibits the most significant degree of inhibition across all the concentrations. Among the leaf extracts, the methanol leaf extract is the most powerful inhibitor; it rises from 22.6% at 20 µg/mL to 59.89% at 100 µg/mL. Though it is very active, it often shows lower values than usual. With values ranging between 10.59% at 20 g/mL and 39.9% at 100 g/mL, hexane leaf extract exhibits the least degree of inhibition. Ethyl acetate and chloroform leaf extracts show moderate inhibitory activity. Ethyl acetate leaf extract inhibits from 13.29% at 20 g/mL to 51.67% at 100 g/mL, whereas chloroform leaf extract inhibits from 11.26% at 20 g/mL to 45.69% at 100 g/mL.

Table 2 shows the % inhibition of four different *Tinospora sinensis* stem extracts-methanol, chloroform, ethyl acetate, and hexane-and a standard compound, probably a reference molecule against which results are compared, at various concentrations of 20, 40, 60, 80, and 100 g/mL. The dose-dependent effect is seen in the % inhibition increases with greater concentration for every extract. The higher the concentration of the extracts, in general, the higher their inhibitory effect, suggesting a typical bioactivity trend. Methanol and ethyl acetate stem extracts regularly provide the most inhibition throughout a range of concentrations. Conversely, the chloroform and hexane stem extracts exhibited the least inhibitory activity across most of the concentrations examined. The norm was, as always, the most successful at stopping the intended process, displaying the most significant % inhibition at every tested concentration. The hypothesis of extract concentration being dose-dependent with respect to the inhibitory activity seems

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to be supported by the fact that all extracts have greater inhibition as increases.

Table 2. Comparison of % of inhibition of the standard with the *Tinospora sinensis* stem extracts (Methanol, Chloroform, Ethyl acetate and Hexane) (Concentration in $\mu\text{g/ml}$).

S. No	Concentration($\mu\text{g/ml}$)	% of inhibition				
		MS	CS	ES	HS	Standard
1.	20	12.6 8 \pm 0. 24	11.4 8 \pm 0. 21	14.5 3 \pm 0. 18	8.34 \pm 0.1 6	48 \pm 0.51
2.	40	22.3 3 \pm 0. 67	19.2 3 \pm 0. 29	28.3 4 \pm 0. 52	17.3 4 \pm 0. 25	88.0 8 \pm 1. 0
3.	60	31.2 6 \pm 0. 41	28.3 9 \pm 0. 56	39.6 2 \pm 0. 22	23.8 9 \pm 0. 68	90.6 8 \pm 0. 35
4.	80	38.4 3 \pm 0. 62	32.4 6 \pm 0. 27	48.3 9 \pm 0. 33	29.2 6 \pm 0. 57	93.6 3 \pm 0. 54
5.	100	47.1 3 \pm 0. 7	39.6 4 \pm 0. 45	60.3 2 \pm 0. 58	37.9 \pm 0.2 4	94.2 1 \pm 0. 34

Of all the extracts, the methanol extract of the stem constantly showed the highest at all concentrations. Through methanol extraction, the bioactive components in the plant responsible for the inhibitory action were maybe more successfully separated. Among the extracts for most of the concentrations, the hexane extract of the stem often showed the least inhibition, showing that hexane extraction likely yields worse extractive efficiency for the active ingredients than methanol. Unlike the extracts, the standard exhibits a vastly greater inhibitive activity for all doses, suggesting that the reference chemical applied in this study is far more effective than *Tinospora sinensis* stem extracts. The reference displayed maximum suppression at 100 g/mL concentration to the tune of 94.21%, suggesting it is a particularly potent chemical that may be employed as a baseline for testing the strength of plant extracts.

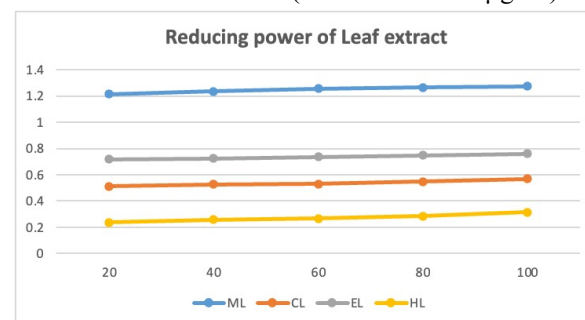
2. Reducing power

One of the most often used techniques in assessing the ability of an antioxidant to give an electron [21] is the reducing power experiment. The ability of the extracts to decrease Fe^{3+} to Fe^{2+} was assessed in this study. Antioxidants found in the extracts caused the reduction of the ferric cyanide complex (Fe^{3+}) to the ferrous cyanide form (Fe^{2+}). The reducing capacity test assesses the degree of reduction in the Fe^{3+} to Fe^{2+} , which produces a green to blue color depending on the

reducing power of the compound [22]. Perl's Prussian blue color, which absorbed at 700 nm, was generated by potent reducing agents.

Figure 1 shows the reducing ability of several *Tinospora sinensis* leaf extracts using ascorbic acid as a benchmark. The absorptive value of the reaction mixture increases with reducing ability. The methanol extract of the leaf showed some capacity to provide electrons. The rising concentration of several extracts reduced their potency. Methanol leaf extracts were discovered to display a greater degree of Fe^{3+} reduction than the hexane extract did. The order of reducing power from ethyl acetate extract, chloroform stem extract, and hexane stem extract was methanol extract.

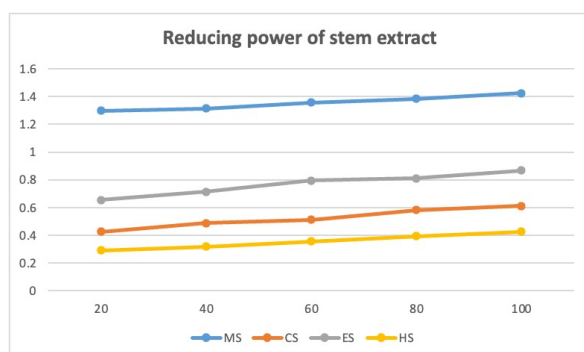
Figure 1: Comparison of absorbance at 700nm for standard and leaf extracts (Concentration in $\mu\text{g/ml}$).



Ascorbic acid served as a standard to show the reductive ability of several *Tinospora sinensis* stem extracts in Figure 2. The ethyl acetate extract, chloroform stem extract, and hexane stem extract were discovered to have their own decreasing order of potency. Surprisingly, the rate at which methanol extracts of both leaf and stem reduced power initially increased with concentration before it fell. Thus, it has more reductones than hexane extracts of leaf and stem. Thus, methanol extracts from stems and leaves might serve as electron donors, interacting with free radicals to transform them to more stable compounds before stopping the chain reactions of free radicals. Table 3 compares the activities of the methanol extract, aqueous extract, and a diclofenac solution (a frequently used anti-inflammatory drug) to show the anti-inflammatory properties of *Tinospora sinensis* leaf extracts at different dosages. The values are stated as averages plus or minus standard deviations; the latter represent the data's spread as well as the average anti-inflammatory action at each concentration.

Figure 2: Comparison of absorbance at 700nm for standard and stem extracts (Concentration in $\mu\text{g/ml}$).

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3. Anti-inflammatory activity

Table 3 shows the anti-inflammatory activity of *Tinospora sinensis* leaf extracts at several concentrations, contrasting the effects of the methanol extract and the aqueous extract with those of a diclofenac solution, that of a frequently used anti-inflammatory medication. Depicting the average anti-inflammatory impact at each concentration as means \pm standard deviations, the values also show the data's variance or spread.

Table 3. Anti-inflammatory results of *Tinospora sinensis* leaf extracts (Concentration in $\mu\text{g/ml}$).

Concentration	Methanol extract	Aqueous extract	Diclofenac solution
10	9.89 \pm 1.19	6.72 \pm 1.29	20.18 \pm 1.24
25	19.78 \pm 1.39	21.58 \pm 1.62	43.58 \pm 1.44
50	39.81 \pm 1.58	25.65 \pm 1.56	58.13 \pm 1.75
100	62.36 \pm 1.35	47.65 \pm 1.23	77.41 \pm 1.23
150	87.98 \pm 1.23	78.6 \pm 1.36	92.26 \pm 1.41

Although, as expected diclofenac is generally more powerful, the methanol extract has the greatest anti-inflammatory action at lower concentrations (10 g/mL) at a value of 9.89, followed by the aqueous extract (6.72) and diclofenac solution with a value of 20.18. At 150 g/mL (87.98), the methanol extract showed the best anti-inflammatory capability, followed by diclofenac at 92.26 and the aqueous extract at 78.6. Higher dosages of aqueous and methanol extracts of *Tinospora sinensis* show a remarkable anti-inflammatory action

Methanol extract's anti-inflammatory properties get

Concentration	Methanol extract	Aqueous extract	Diclofenac solution
10	4.65 \pm 1.21	2.85 \pm 1.46	20.18 \pm 1.24
25	10.15 \pm 1.06	8.91 \pm 1.16	43.58 \pm 1.44
50	18.65 \pm 1.22	14.78 \pm 1.32	58.13 \pm 1.75
100	40.29 \pm 1.33	31.89 \pm 1.13	77.41 \pm 1.23
150	76.8 \pm 1.35	65.16 \pm 1.31	92.26 \pm 1.41

stronger as the concentration rises. At 150 $\mu\text{g/mL}$ 87.98, it is extremely high; it is similar to or greater

than diclofenac at the same concentration. Though typically less anti-inflammatory than the methanol extract at a particular concentration, the aqueous extract shows more activity with dosage. As would be expected, diclofenac is the standard by which all else is evaluated. The methanol extract shows modest improvement over it at the highest concentration tested, and its effects start with concentration; it displays strong anti-inflammatory activity at 150 $\mu\text{g/mL}$, 92.26. Table 4 contrasts the anti-inflammatory properties of *Tinospora sinensis* stem's methanol and aqueous extracts at various concentrations with those of diclofenac, a well-known anti-inflammatory medicine. The average anti-inflammatory effect with the variability at each dose is represented by the mean standard deviation.

Table 4. Anti-inflammatory results of *T. sinensis* stem extracts (Concentration in $\mu\text{g/ml}$).

The methanol extract had moderate anti-inflammatory activity, 4.65 at the lower dosage level of 10 g/mL; the aqueous extract had a rather poor activity, 2.85, showing its weak impact compared to the methanol extract concentration.

At mid-range concentrations (50 $\mu\text{g/mL}$), the methanol extract shows a greater increase in anti-inflammatory action (18.65). Methanol extract continues to show greater anti-inflammatory activity at larger dosages (76.8) as compared to the aqueous extract, which has less activity (14.78) but increased proportionally to the 10 $\mu\text{g/mL}$ dose. Aqueous extract showed a major rise in activity (65.16), but less than the methanol extract and diclofenac.

At 150 $\mu\text{g/mL}$, almost equal to diclofenac's level achieved, the methanol leaf extract obviously shows improved anti-inflammatory activity as concentration rises. Anti-inflammatory qualities of the aqueous extract rise with rising concentration, yet they are always less effective than those of the methanol extract.

Compared to the methanol extract of the leaves of the plant (Table 3), the methanol extract of the stem (Table 4) appears to have considerably weaker anti-inflammatory activity. Both extracts showed that the methanol extract always displays the strongest anti-inflammatory activity. *Tinospora sinensis* methanol leaf extract seems to be a little bit stronger than the stem extracts. The anti-inflammatory action of the leaf aqueous extract is stronger than that of the stem aqueous extract. The aqueous extracts, however, are not as efficient as the methanolic ones. Although the aqueous extract of a leaf seems more efficient than the methanolic extract, its potency is still less than that of the stem.

Discussion

The results obtained from this research offer concrete evidence for the utilization of *T. sinensis* as an herbal remedy and its use in combating oxidative stress and inflammation, which are interrelated phenomena that result in a whole host of medical conditions. Oxidative stress, caused by an overload of potentially damaging free radicals like ROS, RNS, and RSS, leads to cellular damage and upsets cellular stability. Not surprisingly, given the antioxidant properties possessed by various medicinal herbs, it has been noticed that *T. sinensis* also has appreciable scavenging activity against free radicals, particularly in methanol forms.

DPPH radical scavenging activity demonstrated a dose-dependent relationship, and the methanol extract of leaves and stems showed better activity compared with other solvents. It appears that the phytochemicals with antioxidant activity, such as phenols and flavonoids, and more polar compounds are better extracted with methanol. The relatively low activity shown by hexane extracts also agrees with this observation because hexane is a nonpolar solvent that cannot effectively extract phenolic compounds known for electron donation and radical neutralization. The superior activity shown by the reference compound underscores the difference that should be observable between pure and isolated bioactive compounds and natural crude plants; yet, still, it still shows high medicinal value.

Similar tendencies were noticed in the reducing power assay, with the methanol extract having a higher ratio of Fe^{3+} -reduced-to- Fe^{2+} compared to the other samples. The reducing power assay is highly correlated with antioxidant activity, and it validates our hypothesis about the presence of reductones and other electron donor phytochemicals that can break the chain reaction associated with free radicals within the leaves of *T. sinensis*. The result confirms that with an increase in concentration, there is an escalation in antioxidant activity.

The anti-inflammatory assays also prove the merits of using *T. sinensis* for medical purposes. Membrane-stabilising activity, which was measured using the heat-induced hemolysis method, showed that extracts of the plant could be capable of preventing the release of lysosomal enzymes, an important player in inflammation. Once again, the methanol extract showed the greatest activity against all concentrations, sometimes even reaching that of diclofenac. Leaf samples were more active compared to stem samples. These findings agree with previous research suggesting that redox-active compounds present in plants have an

anti-inflammatory effect and can alter the redox status of cells and interfere with signaling molecules involved in an inflammatory response.

The dose-dependent anti-inflammatory tendencies shown in leaf and stem extracts emphasize the medical value associated with *T. sinensis*, despite the fact that methanol extract from leaves was more active. Although these results were very encouraging, it should be noted that natural product extracts display mixtures of phytochemicals, and it is these mixtures that have been shown to display bioactivity. Individual compound characterizations would enable specialists to better analyze associated mechanisms.

In essence, based on the findings of this research, it can be argued that the usage of *T. sinensis* as a herbal remedy for reducing inflammation and metabolic disorders and combating oxidative stress is justified. It can be hypothesized that this drug may be an excellent natural source of developing more efficient therapeutic agents and drug molecules because of its pronounced antioxidant and anti-inflammatory properties.

Conclusion

The present study highlights the promising therapeutic potential of *Tinospora sinensis*, particularly its antioxidant and anti-inflammatory properties, which are essential in mitigating oxidative stress and inflammatory responses associated with various diseases. Among the different extracts tested, the methanol extracts from both leaves and stems demonstrated the most significant free radical scavenging and anti-inflammatory activities, making them the most effective among the tested solvents. While the aqueous extracts also exhibited activity, they were generally less potent than their methanol counterparts. These results align with traditional uses of the plant and provide scientific validation for its medicinal benefits. Further research is needed to isolate and identify the specific bioactive compounds responsible for these effects, which could pave the way for the development of natural therapeutic agents for managing oxidative stress-related disorders and inflammation.

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