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Original Research Article

Phytochemical Screening and Antioxidant Potential of Methanol Extract of *Triumfetta cordifolia* A. Rich. (Malvaceae) Leaves

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Abstract

Triumfetta cordifolia is a medicinal plant that has been widely utilized in Africa for its therapeutic properties in treating various ailments. The purpose of this research was to investigate phytochemicals and *in vitro* antioxidant activity of the leaf extract of *T. cordifolia*. Standard methods were employed to conduct qualitative phytochemical screening of the plant extract. This study used the DPPH (1, 1diphenyl-2-picryl hydrazyl) radical scavenging and ferric reducing antioxidant power method to assess antioxidant activity. Phytochemical analysis of *T. cordifolia* leaves indicated the existence of saponins, tannins, terpenoids, cardiac glycosides, alkaloids, flavonoids but no anthraquinones. In the DPPH scavenging test, the IC_{50} value of the methanolic extract was discovered to be 1.29 µg/mL, and the standard reference value for ascorbic acid was 2.14 µg/mL. The leaf extract of *T. cordifolia* displayed stronger inhibition of DPPH activity, showing a higher potency than ascorbic acid. The DPPH scavenging activity was also shown to increase with concentration. In FRAP assay, the *T. cordifolia leaf* extract and the ascorbic acid revealed reducing power of 71.01±0.15 and 548.39±1.62 µM Fe^{2+} per mg of extract, respectively. The FRAP assay demonstrated that ascorbic acid exhibited greater antioxidant activity than the leaf extract of *T. cordifolia.* The presence of flavonoids and phenols in *T. cordifolia* leaves may account for the observed antioxidant activity. In conclusion, the study's findings suggest that that *T. cordifolia* leaf extract could be a source of lead compounds with promising antioxidant activity.

Keywords: *Triumfetta cordifolia*, phytochemicals, flavonoids, antioxidant, DPPH.

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INTRODUCTION

Humans have used plants and plant-based products to prevent and treat illness from the beginning of time. Plants have long been human's best friends, and we rely on a wide range of plant-related products in various fields, including medicine. Plants contribute significantly to the ustainability of the human race in planet earth. In addition to providing food and oxygen for breathing, plants provide bioactive substances that can be used as medicines to improve health. Many people worldwide rely on plants for traditional and natural forms of healing. Studies on how native people use plants in various places worldwide are well documented. Medicinal plants are the most useful to humans. Medicinal plants are among the most important plants in the economy. When referred to as medicinal, a plant can be used as a medication or as an active component in medicinal treatment. Additionally, a plant can only be considered medicinal if its biological properties, as proposed by ethnobotany, have been documented, thoroughly researched, and proven.

According to Arias (1999), a medicinal plant is defined as any plant utilized to alleviate, hinder, or treat an illness or to change physiological processes or any plant used to make drugs or their precursors. This is because of the presence of active chemical compounds called phytochemicals, which have antioxidants, anti-diabetic, antibacterial, and anti-inflammatory properties that cause the human body to respond physiologically (Edeoga *et al.,* 2006). Phytochemicals, which are molecules with definite physiological activity within the human body, give these plants their medical value. Phytochemicals are Bioactive compounds found naturally in stems, fruits, roots, barks, and leaves of medicinal plants that play a role in the defensive mechanisms that shield plants, animals, and people who eat them against a variety of ailments (Ogidi *et al.,* 2019). Flavonoids, alkaloids, phenolic compounds, and tannins are the most significant bioactive components in plants (Ajoko *et al.,* 2020).

Antioxidants are naturally occurring plant chemicals that protect the body from free radicals that are damaging molecules. Free radicals are chemicals formed when the body processes food. (Vasanthi *et al.,* 2014). Environmental exposures, such as cigarette smoke and radiation, can also cause them. Owing to the unpaired electrons in the outermost shell of the oxygen atom, these compounds are extremely reactive. The body constantly generates free radicals owing to the continuous use of oxygen. The body's cells can be damaged by these free radicals, which also contribute to several other health conditions like cardiovascular disease, cancer and diabetes.

Antioxidants play important roles in daily life. Antioxidants help stop oxidation, which can damage cells and possibly accelerate aging. They may boost immune defenses and possibly reduce the risk of cancer, cardiovascular disease, and infection.

Antioxidants act as "free-radical scavengers" to stop chain reactions and stabilize free-radical species. When a free radical receives an electron from an antioxidant, it stabilizes and no longer requires attacking the cell, breaking the oxidation chain reaction. The antioxidant becomes a free radical after donating an electron; however, it remains safe and stable owing to its capacity to tolerate the shift in electrons without becoming reactive. Therefore, reducing chemicals such as thiols and ascorbic acid, and polyphenols are frequently used as antioxidants (Hamid *et al.,* 2010).

Triumfetta cordifolia, a member of the Malvaceae family, is typically available in West Africa, Florida, Bermuda, Central America, and South America. *Triumfetta cordifolia* is a typical herb used in traditional African medicine, and its components are used to treat a variety of ailments. Hepatitis, asthenia, muscle soreness, diarrhea, and other disorders have been treated throughout Africa. Studies conducted earlier has revealed that *T. cordifolia* has antibacterial, antidiabetic, analgesic, antifungal, antidiarrheal, antiantimicrobial, antiulcerogenic, and cytotoxic properties (Akerele, 1992; Borokini & Omotayo, 2012; Nwafor *et al.,* 2011; Sandjo *et al.,* 2010; Ajoko *et al.,* 2020).

There has not been much research on *T. cordifolia* compared to that on other therapeutic plants. Therefore, the aim was to examine the therapeutic potential of methanol *T. cordifolia* leaf extract based on its phytochemical, total flavonoid, total phenolic, and antioxidant activities. The findings of this research could raise the worth of total therapeutic prospect of plants.

MATERIALS AND METHODS

Collection and Identification of the Plant

The leaves utilized in this research were those of *T. cordifolia*, gathered on March 10, 2022, from the Amassoma-Yenagoa road located in the Niger Delta region of Nigeria. The plant's identification was conducted by Professor K.K. Ajibesin, affiliated with the Department of Pharmacognosy and Herbal Medicine at Niger Delta University in Bayelsa State, Nigeria.

Extraction Procedure for the Plant Extract

420 grams of coarsely crushed *T. cordifolia* leaves were immersed in 3 liters of 50% (v/v) methanol. This mixture was agitated regularly and allowed to sit at room temperature for 72 hours. Subsequently, the extract was filtered into a sterile conical flask. After 72 hours of additional maceration, the marc was strained and combined with the original extract. The filtrate underwent vacuum concentration utilizing a rotary evaporator at (30°C). The yield obtained was dark in color. In comparison to the powdered leaves, the crude extract yielded 30.3 g.

Screening for Qualitative Phytochemicals

The existence of chemical components in *T. cordifolia* extract was qualitatively examined. The extract was screened for phytochemicals using standard procedures (Evans, 2009; Sofowora, 1993).

Terpenoids Test (Salkowski Test):

0.5 g plant extract was combined with chloroform (2 mL). Subsequently, concentrated H_2SO_4 (3 mL) was poured into it to generate a layer. A reddish-brown color near the interface was used to identify terpenoids.

Tannins Test:

0.5 g of dried *T. cordifolia* leaf extract was dissolved in 10 mL of distilled water and then filtered. The filtrate was then mixed with a drop of 0.1% ferric chloride. Tannins were detected as blue or green precipitates.

Test for Flavonoids (Shinoda's Test):

In a test tube, 0.5 g *T. cordifolia* extract was mixed with 10 mL ethanol, and a few drops of hydrochloric acid were added, along with four magnesium filings. A reddish-colored precipitate displayed evidence of flavonoids.

Test for Alkaloids:

0.5g of dried T. cordifolia leaf extract was mixed in 5 mL of 1% HCL over a steam bath with continual stirring and filtered. Dragendorffs reagent (potassium bismuth iodide solution) was applied to a test tube containing the filtrate (2 mL), and the color change was monitored. The presence of a reddish-brown precipitation was interpreted as proof of the existence of alkaloid.

Saponins Test (Frothing test):

Plant extract weighing 0.5 g was combined with distilled water (10 mL) and agitated for approximately 2 mins. The emergence of durable foam served as a signal for the existence of saponins.

Cardiac Glycosides Test (Keller-Kiliani):

Added 2 mL glacial acetic acid and one drop ferric chloride to 0.5 g T. cordifolia leaf extract. 1 mL concentrated H_2SO_4 was added on top. The solution was added with a few drops of concentrated H_2SO_4. Within minutes a greenish-blue color appeared, which a sign of the existence of cardiac glycosides.

Test for anthraquinones:

1mL of dissolved plant extract was brought up to 10 mL using distilled water, 5 mL of benzene was added, and 1.5 mL of 10% ammonia solution was gently mixed. The presence of pink, violet or red colouring in the ammonia phase showed the existence of anthraquinones derivatives in the extract.

Antioxidant Activities

Total Flavonoid Content Estimation

Total flavonoid contents of aqueous methanolic T. cordifolia leaf extract estimated utilizing the aluminium chloride colorimetric technique as used by Hossain *et al*., (2011) with slight modifications. 1 mL of aqueous methanolic T. cordifolia leaf extract solution (100 µg/ ml) was combined with 3 mL of CH_3OH, 0.2 mL of 10% $AlCl_3$, 0.2 mL CH_3COOK and distilled water (5.6 mL). At room temperature it was left to incubate for 30 minutes. A spectrophotometer was employed to assess the absorbance at 415 nm in contrast to a blank sample. Quercetin equivalents (mg QE/g dry extract) were used to calculate the total flavonoids. The total flavonoids in the extract were measured twice, and the average of the findings was obtained.

Total Phenolic Content Estimation

Total phenolic concentration in the extract was determined using Folin-Ciocalteu reagent and gallic acid calibration (Hossain *et al.,* 2011). The extract (0.5g) was dissolved in 50 mL water. Then, 0.5 mL of the extract solution and 0.1 mL of Folin-Ciocalteu reagent (0.5 N) were mixed.

2.5 mL of 7.5% Na_2CO_3 was introduced after 15 minutes of shaking; the mixture was left to stand undisturbed at room temperature for 30 minutes. A spectrophotometer was employed to assess the absorbance at 760 nm In contrast to a control sample. Total phenolics were quantified in milligrams of GAE/g of dry extract. The amount of phenolic substances in the crude extract was quantified twice, and the findings were averaged.

Total Antioxidant Capacity Determination

Utilizing the Phosphomolybdenum technique as stated by Saha *et al.,* (2008), the antioxidant activity of *T. cordifolia* leaf extract was assessed. A 1 mL aqueous methanolic *T. cordifolia* leaf extract solution was combined with a 3 mL reagent solution (0.6 M H_2SO_4, 28 mM Na_3PO_4 and 4mM $(NH_4)_6Mo_7O_{24}$). A boiling water bath with 95°C was used to incubate the test tubes holding the reaction solution for 90 minutes. A spectrophotometer was employed to assess the samples absorbance at 695 nm in contrast to a blank as soon as the solution reached room temperature.

DPPH radical scavenging activity assay

To ascertain the extracts radical scavenging activity, the modified method of Saha et al., (2008) was used. 0.5 mL aliquots of the extract in 95% ethanol were prepared at various concentrations (25, 50, 75, 100 µg/mL) and placed in separate test tubes. MeOH was introduced into the quantities to bring them to 100 µg/mL. Next was the addition of 2.0 mL of DPPH reagent solution (0.00004 g/mL in methanol) to the test tubes, shaken thoroughly, and left at room temperature. Using 95% methanol as the blank, a control sample devoid of the extract was created. After duration of 30 minutes, the measurement of absorbance at 517 nm was conducted.

The radical scavenging capability was calculated using the equation below:

$$\% \text{ inhibition} = [A_0 - A_1] \times 100/A_0$$

A_0 in the equation stands for the absorption value of the control sample, and A_1 for the extract.

All tests were performed in duplicate.

The extract concentration at which the production of DPPH radical is 50% inhibited is known as the IC 50 value (Ndhlala *et al.,* 2013) and using a dose-response curve, it was determined as µg/mL.

Measuring Antioxidant Activity through the Ferric Reducing/Antioxidant Power (FRAP) Technique

The FRAP test was performed according to the technique published by Chaves *et al*., (2020).

Aliquots of extract (0.2 mL) were combined with FRAP reagent (3.8 mL) in three duplicates per sample and concentration (0.1, 0.5, 1, and 2 mg/mL). A earlier preparation of this reagent involved Mixing 10 portions of a 300 mM C2H3NaO2 buffer solution with a pH of 3.6, 1 portion of 10 mM TPZT, and one portion of 20 mM FeCl3 hexahydrate (Alfa Aesar, Kandel, Germany). Following 30-minute incubation at 37°C, the absorbance rise at 593 nm was measured utilizing a UV spectrophotometer (Thermospectronic BioMate 3, USA). The same amount of methanol was used to make the blank in place of the diluted extract. The calibration line was made with FeSO4 values of 0.0025, 0.005, 0.01, and 0.02 mg/mL. The findings were expressed as µM Fe 2+ per milligram of extract.

Statistical Analysis

Data analysis was carried out utilizing SPSS version 21.0. One-way analysis of variance (ANOVA) was utilized to determine the mean difference and statistically significant values at $P<0.05$. Values with the same superscripts were considered to have no statistical significant difference.

RESULTS AND DISCUSSION

Qualitative Phytochemical Analysis

Qualitative tests are useful for determining the existence or lack of specific phytochemicals as well as their distribution in various plant parts. Furthermore, they enable semi-quantitative examination of phytochemicals and aid in the identification of potentially pharmacologically active substances. The antioxidant properties of the T. cordifolia leaf may therefore be due to phytochemicals, which are well-known to promote bioactive actions in medicinal plants. The existence of several secondary metabolites was discovered during qualitative phytochemical analysis of T. cordifolia methanolic leaf extract, namely alkaloids, tannins, terpenoids, cardiac glycosides, flavonoids, saponins, but anthraquinones were not present (Table 1).

Table 1: Phytochemical composition of methanolic leaf extract of *T. cordifolia*

Parameter	Methanolic *T. cordifolia* leaf extract
Alkaloids	+
Terpenoids	+
Tannins	+
Cardiac glycosides	+
Saponins	+
Anthraquinones	-
Flavonoids	++

+ = present, ++ = strongly present, - = not detected

This finding supports previous research that the plant contains phytochemicals including saponins, flavonoids, terpenoids, alkaloids, cardiac glycosides, and tannins (Borokini *et al.,* 2012; Odewo & Adeyemo, 2018). Several biological processes have been connected to these substances.

Antioxidant Activities

Total flavonoids and phenolics, as well as total antioxidant capacity and DPPH were measured in T. cordifolia crude extract. According to reports total phenolic content (TPC) and antioxidant activity are closely related. These substances are regarded as strong chain-breaking antioxidants (Chaouche *et al*., 2014). From Table 2, the findings indicated that *T. cordifolia* leaf extract had a total phenolic content of 17.450.28 mg GAE/g dry weight and a considerably high level of flavonoid concentration of 139.300.99 mg QE/mg dry weight. These two measurements suggest that the crude extract has a stronger antioxidant activity. The high concentration of flavonoids allows one to infer that antioxidant capability of the leaf extract of *T. cordifolia* is mainly due to 139.30±0.99 mg QE/mg of flavonoids. This is in line with the crude extract having high antioxidant effect.

The total antioxidant capacity was discovered to be 28.13±0.52 mg/100 g. The TAC yielded a total value equal to the total of all antioxidants (Prio and Cao, 1999). The value of the crude T. cordifolia extract's total antioxidant capacity as reported in this research may indicate that the extract has high antioxidant content.

One of the most popular techniques for testing plant extracts antioxidant properties is the DPPH assay (Amos-Tautua *et al.,* 2017). This test was established on the capacity of a persistent free radical called 1,1-diphenyl-2-picrylhydrazyl (DPPH) to change color in the presence of antioxidants, indicating the plant has the potential to act as an antioxidant. Graph 1 depicts the percentage of DPPH free radical scavenging by the plant extracts at 517 nm. The study's findings showed that the scavenging activity of DPPH increased with an increasing concentration (Table 3 and Fig 1). Although the extracts activity to scavenge DPPH radicals was higher than that of the standard at 25 µg/mL, 50 µg/mL, and 75 µg/mL but was lesser at 100 µg/mL. At minimum concentration (25 µg/ml), the extract showed a better inhibition (46.09±0.17%) compared to that of the ascorbic acid (25.95±0.50%) (Table 3).

In this study, the IC 50 values for the methanolic *T. cordifolia* leaf extract and the ascorbic acid was discovered to be 1.29 µg/mL and 2.14 µg/mL respectively (Table 4). The T. cordifolia methanolic extract had a high efficacy in comparison to ascorbic acid and showed the highest suppression of DPPH effect. A lower IC 50 value, which also denotes greater antioxidant capacity, suggests better effectiveness at scavenging DPPH. The results of this study therefore depict that *T. cordifolia* leaf extract is more effective than the Ascorbic acid and it can be inferred that *T. cordifolia* leaf extract acts as a primary antioxidant based on its capability to scavenge DPPH free radicals. This property of *T. cordifolia* leaf extract may account for its application in treating various kinds of afflictions especially those that are associated with free radical generation such as inflammation.

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Table 2: Total Flavonoids, Total Phenolics, and Total Antioxidant Capacity in methanolic leaf extract *T. cordifolia*

Component	Crude aqueous methanol extract of *T. cordifolia* leaves
Total phenolics (mg GAE/g dry wt.)	17.45±0.28
Total flavonoids (mg QE/mg dry wt.)	139.30±0.99
Total Antioxidant Capacity (mg/100 g)	28.13±0.52

Values are shown as mean ± standard deviation (n=2)

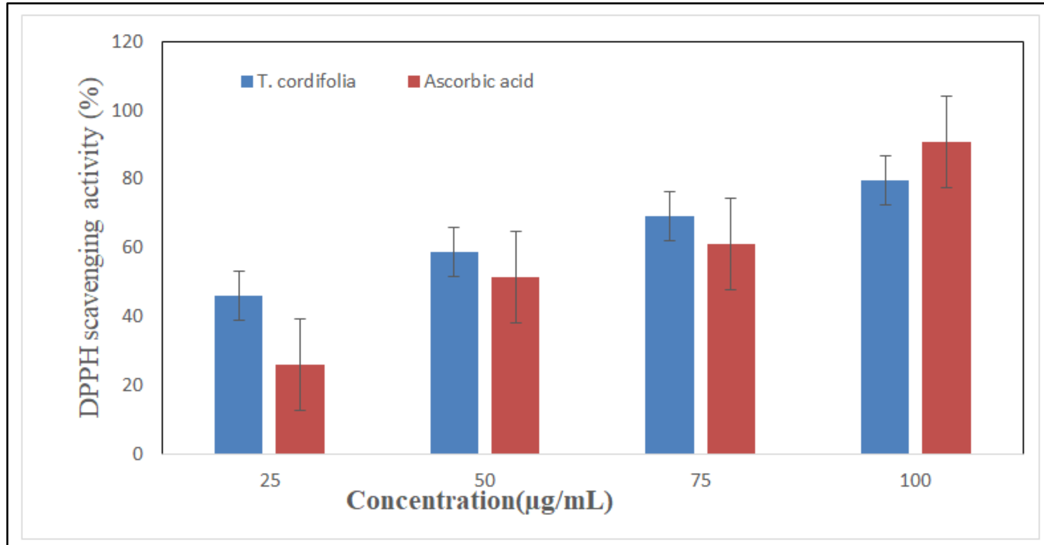

Fig 1: DPPH free radical scavenging activity of *T. cordifolia* leaf extract

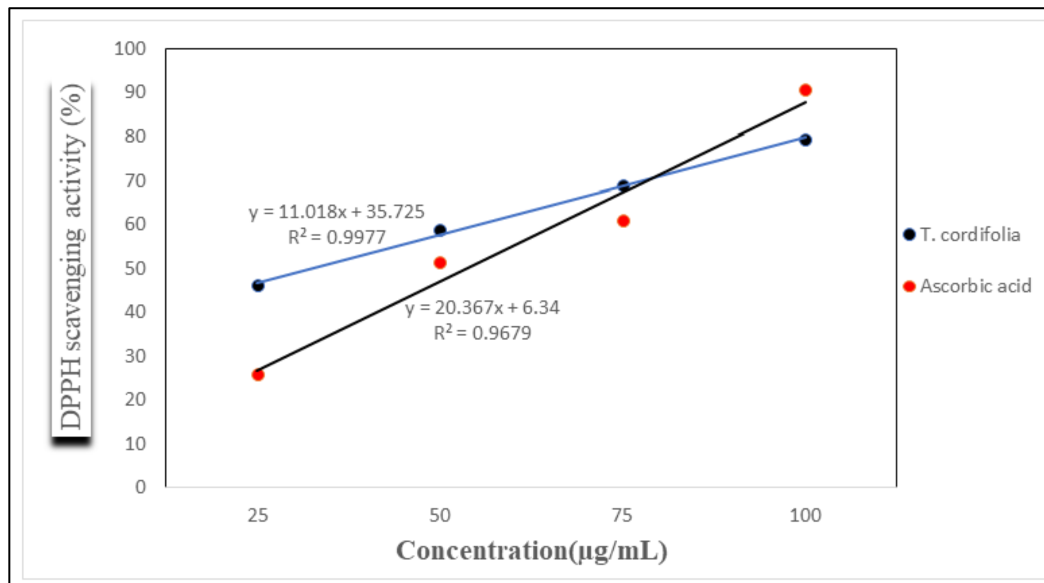

Fig 2: Dose Response Curve of DPPH scavenging potential of *T. cordifolia* and Ascorbic acid

Table 3: DPPH radical scavenging effect of *T. cordifolia* leaf extract and the standard antioxidant, Ascorbic acid

Concentration (µg/mL)	Percentage inhibition (%) for Ascorbic acid	Percentage inhibition (%) for Crude Extract of *T. cordifolia* leaves
25	25.95 ± 0.50^a	46.09 ± 0.17^a
50	51.42 ± 1.34^b	58.65 ± 0.50^b
75	61.02 ± 0.50^c	68.96 ± 0.67^c
100	90.64 ± 0.17^d	79.38 ± 01.00^d

Values are presented as mean ± standard deviation (n=2); the numbers in the same column with various superscripts vary significantly (p<0.05).

Table 4: The IC50 value of DPPH Scavenging activity of *T. cordifolia* leaf extract and Ascorbic Acid (Standard)

Parameter	IC_{50} Value (µg/mL)
T. cordifolia leaf extract	1.29
Ascorbic acid (Standard)	2.14

The study also demonstrates that *T. cordifolia* leaf extract has proton-donating effects and may operate as an antioxidant by scavenging or inhibiting free radicals. It is thought that antioxidants ability to give out hydrogen is what causes them to affect DPPH (Adedapo *et al.,* 2009). On the basis of the data from this research *T. cordifolia* leaf is a potent antioxidant attributable to the high flavonoids content as revealed from the study (Table 2). The chemical structure of flavonoid molecules, which contains hydroxyl groups that can act as a radical scavenger, may potentially be responsible for the high scavenging ability of *T. cordifolia* (Pourmorad *et al.,* 2006).

Table 5: Antioxidant capacity of *T. cordifolia* leaf extract and the standard antioxidant, Ascorbic acid by ferric reducing (FRAP) assay

Parameter	concentration (µM Fe^{2+} per mg of extract)
T. cordifolia leaf extract	71.01±0.15
Ascorbic acid	548.39±1.62

Values are presented as mean ± standard deviation (n=3)

The FRAP test is a widely employed method for assessing the ability of plant extracts to function as antioxidants. In this test, the reductants present in the antioxidant sample convert the Fe 3+ /ferricyanide complex to its Fe^{2+} /ferrous form (Raham *et al*., 2015). The FRAP assay involves mixing an antioxidant sample with the TPTZ (2,4,6-Tris-(2-pyridyl)-s-triazine) solution, leading to the development of a blue color as TPTZ is reduced. The FRAP assay provides quick and repeatable findings (Kishan *et al.,* 2022).

Table 5, displays the outcomes of the FRAP test. The ascorbic acid, with a FRAP value of 548.39±1.62 µM Fe^{2+} per mg of extract, exhibits a significantly stronger antioxidant capacity compared to the *T. cordifolia* leaf extract (71.01±0.15µM Fe^{2+} per mg of extract). This suggests that the standard ascorbic acid possesses a higher ability to reduce ferric ions and act as an antioxidant. In contrast, *T. cordifolia* leaf extract shows a moderate antioxidant activity, indicating a relatively lower capacity to reduce ferric ions.

CONCLUSION

The study revealed that *T. cordifolia* leaves contain a high concentration of phytochemicals with significant medicinal value, which confirms and justifies its application in the treatment of numerous human ailments. It was also discovered that *T. cordifolia* leaf extract showed potent antioxidant effect therefore, it may be a source of fresh natural antioxidants in the future. The presence of flavonoid and phenolic components in this plant may be responsible for its antioxidant activity.

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