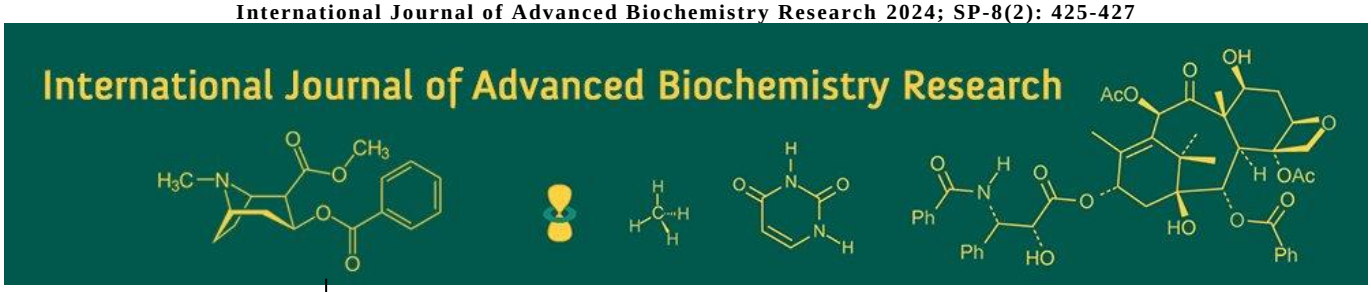

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Antioxidant potential of *Tinospora cordifolia*: Insights into its therapeutic significance

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Abstract
This research investigates the bioactive antioxidant potential of *Tinospora cordifolia*, a plant renowned for its medicinal properties in traditional systems of medicine. Employing a systematic approach, the study focuses on extracting and evaluating the antioxidant compounds present in *Tinospora cordifolia* through various analytical techniques. The research methodology involves the extraction of bioactive components using appropriate solvents, followed by the application of advanced analytical tools such as spectrophotometry. The study delves into the *in vitro* estimation of the antioxidant activity, providing insights into the efficacy of *Tinospora cordifolia* in combating oxidative stress. The research aims to contribute valuable data to knowledge on natural antioxidants, emphasizing the potential therapeutic applications of *Tinospora cordifolia* in mitigating oxidative damage associated with various health conditions. The outcomes of this study hold significance for researchers, healthcare professionals and the pharmaceutical industry, offering a foundation for further exploration of *Tinospora cordifolia* as a rich source of bioactive components especially, antioxidants with potential applications in preventive and therapeutic interventions.

Keywords: *Tinospora cordifolia*, oxidative stress, antioxidant, spectroscopy

Introduction
In recent years, the quantitative estimation of the bioactive component of the natural sources with potential health-promoting properties has gained interest of the researchers. Among the diverse array of botanicals, *Tinospora cordifolia* has emerged as a promising candidate due to its rich traditional use in various systems of medicine. Renowned for its multifaceted therapeutic attributes, *Tinospora cordifolia*, commonly known as Guduchi or Giloy, has garnered attention for its antioxidant potential, suggesting a possible role in combatting oxidative stress-related disorders.

Tinospora cordifolia is a climbing shrub native to the Indian subcontinent and has been an integral part of Ayurveda, the traditional Indian system of medicine, for centuries. Traditionally used to enhance vitality and treat various ailments, this botanical reservoir has piqued scientific curiosity, particularly in the context of its antioxidant properties. Oxidative stress is linked to the pathophysiology of many chronic diseases, including neurological ailments and cardiovascular disorders. It arises from an imbalance between the body's antioxidant defences and the creation of reactive oxygen species (ROS). Hence, the exploration of natural antioxidants, such as those present in *Tinospora cordifolia*, holds substantial promise for preventive and therapeutic interventions.

This research paper aims to provide a comprehensive examination of the bioactive antioxidant potential of *Tinospora cordifolia*. By employing a multidisciplinary approach, we seek to elucidate the chemical composition of *Tinospora cordifolia* extracts, identify specific antioxidant compounds, and evaluate their efficacy in mitigating oxidative stress. The study encompasses a range of analytical techniques, from solvent extraction to *in vitro* assays, with the overarching goal of unraveling the intricate relationship between *Tinospora cordifolia* and its antioxidant properties.

As the scientific community continues to grapple with the challenges posed by oxidative stress-related disorders, a deeper understanding of the bioactive components in *Tinospora cordifolia* could pave the way for novel therapeutic strategies.

The plant extracts are rich source of antioxidants serves as a substantial source of essential nutrients, including vitamins, antioxidants, minerals, flavonoids, carotenoids, anthocyanins, carbohydrates, fats, and proteins. It not only provides a flavorful option for direct consumption but also contributes to the production of diverse food items and beverages with added nutritional value (Sharma *et al*., 2023) [8, 9]. This research contributes to the ongoing dialogue surrounding natural antioxidants, offering insights that may have implications for the development of pharmaceuticals or nutraceuticals aimed at bolstering the body's defense mechanisms against oxidative damage. In doing so, this investigation aligns with the broader pursuit of harnessing nature's potential to address contemporary health challenges.

Materials and Methods

Plant material for extract preparation

The bright and fresh leaves of *T. cordifolia* plant were obtained from local cultivators. After a meticulous cleansing with distilled water and air drying in the shade to eliminate surface moisture, the leaves underwent further drying in a hot air oven at 60 °C for 42 hours. After that, the dried leaves were ground into a fine powder and used in the extraction procedure.

Methanolic extract preparation

Methanol was used in turn to extract ten grams of powdered, shade-dried leaf material until the plant material's colour was gone. The final mixture was poured into a clean conical flask, and the filtrate was poured into the lyophilizer plates after being filtered with sterile Whatman filter paper. For later usage, the extracted materials were weighed and aseptically stored in airtight bottles at 4 °C.

Total Phenolic Count

The Folin-Ciocalteu test was used to calculate the total phenolic content. In a 25 ml volumetric flask with 9 ml of distilled water, an aliquot (1 ml) of the extracts or a standard solution of gallic acid (20, 40, 40, 60, 80, and 100 µg/ml) was added. Distilled water was used to create a blank for the reagent. After adding and shaking the mixture, one millilitre of Folin-Ciocalteu phenol reagent was added. The mixture was mixed with 10 ml of 7% Na_2CO_3 solution after 5 minutes. Subsequently, the volume was adjusted appropriately. Using a UV/Vis spectrophotometer, the absorbance against the reagent blank was measured at 550 nm following a 90-minute incubation period at room temperature. The amount of total phenolics was reported in milligrammes of gallic acid equivalents (GAE).

Total Flavonoid Content

To determine total flavonoid content, four millilitres of distilled water were mixed with one hundred microliters of extract. 0.3 millilitre of 5% sodium nitrite was then added. 0.3 cc of 10% aluminium chloride was added after 5 minutes. After 6 minutes, 2 millilitres of 1 M sodium hydroxide were added to the blend. The mixture was blended well and immediately diluted by adding 3.3 millilitres of distilled water. At 510 nm, the absorbance was measured in relation to a blank. The calibration curve's standard was Catechin. The extract's total flavonoid content was reported as mg/g, or milligrams catechin equivalents per gram of sample.

Ferric Reducing Antioxidant Power Assay

The ferric reducing ability of plasma FRAP assay, developed by Benzie and Strain (1999) [11] as a measure of antioxidant power, was used to calculate a sample's total antioxidant potential. The FRAP reagent was made by combining 20 mM $FeCl_3$, 300 mM acetate buffer (pH 3.6), and a solution of 10 mM TPTZ in 40 mM HCl at a ratio of 10:1:1 (v/v/v). After adding the reagent (3.400 µL) and sample solutions (100 µL) to each well, they were thoroughly mixed. Following, the absorbance was measured at 593 nm. A standard curve was created utilising various trolox concentrations. The day of preparation saw the application of every remedy. The data was presented in terms of µmol trolox equivalent/g dw. On each extract, analyses were done in triplicate.

DPPH radical scavenging activity

The diphenyl-picrylhydrazyl (DPPH) assay was used to quantify the capacity to scavenge free radicals. One millilitre of the methanolic extract solutions (was mixed with five millilitres of an 80 mM DPPH radical solution. A spectrophotometer was used to measure the absorbance at 515 nm after the reaction had been running for 30 minutes and the value was compared with that of BHA that was taken as standard. The experiment was repeated three times, and the percentage of DPPH scavenging was calculated using the provided formula:

$$\text{Antioxidant activity percentage} = [(Ac - As) \div Ac] \times 100$$

As is the absorbance of the testing specimen; Ac is the control reaction absorbance.

Results

This study explores various biological activities of *T. cordifolia* leaves. Methanol extract was subjected to assays to determine their total phenolic and flavonoid contents, as well as their ferric reducing antioxidant power employing diverse *in vitro* models.

Total Phenolic and Flavonoid Content

The findings from this study indicate that the methanol extract of *T. cordifolia* leaves exhibited the polyphenol level at 46.4±2.32 mg GAE. The amount of total flavonoids in the methanolic extract was represented as 28.35±1.23 milligrams of catechin equivalents per grams of extract

Ferric Reducing Antioxidant Power Assay

The capacity of plant extract to reduce ferric ions was assessed through the FRAP assay The FRAP values for the methanolic extract was observed to be 11.69±5.53 µmol trolox/g.

DPPH radical scavenging activity

The DPPH radical scavenging activity of *T. cordifolia* leaf methanolic lyophilized exhibited scavenging activity of 63.72 ±3.61%.

Discussion

Total Phenolic and Flavonoid Content

Medicinal plants play a crucial role as a source of antioxidants. Natural antioxidants contribute to increased plasma antioxidant capacity, thereby reducing the risk of certain diseases. Polyphenols, particularly phenolic acids

and flavonoids, are key plant compounds known for their antioxidant activity (Demiray *et al*., 2009) [3]. Phenolics, including phenolic acids and flavonoids, are reported to be major contributors to the variation in the antioxidant activity of plants. These phenolics exhibit antioxidant activity by neutralizing lipid free radicals and preventing the decomposition of hydroperoxides into free radicals. The flavonoids, a subclass of phenolic acids, are significant antioxidants present in various medicinal plants and related phytomedicines (Pietta, 1998) [6]. The flavonoids have the ability to both scavenge and prevent the production of free radicals, which is the source of their antioxidant action. Overall, the presence of polyphenols, particularly phenolic acids and flavonoids, highlights the valuable antioxidant potential of medicinal plants. The addition of the different plant extract-enriched cheese to the rats' diet improved the condition of the wistar rats, alleviating the effects of oxidative stress attributing to their antioxidant potential (Sharma *et al*., 2023) [8, 9].

Ferric Reducing Antioxidant Power Assay

In the presence of 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), antioxidants have the ability to decrease Fe3+ to Fe2+, generating a brilliant blue Fe2+ -TPTZ complex with an absorption peak at 593 nm. This process is the basis for the FRAP assay. The ideal pH for this pH-dependent reaction is 3.6. According to Benzie and Strain (1996) [2], the absorbance drop is directly correlated with the antioxidant concentration. Antioxidant activity was shown to be higher in the current investigation, and this rise was correlated with the polyphenol concentration. Similar results were observed in the leaf and stem extracts of *Calpurnia aurea*; because of its higher polyphenol content, the methanolic stem extract demonstrated more antioxidant activity than the leaf extract (Adedapo *et al*., 2008) [1]. This emphasises the relationship between polyphenol concentration and antioxidant action.

DPPH radical scavenging activity

When assessing primary antioxidant activity, the stable radical DPPH is frequently used. The foundation of the DPPH antioxidant assay is the ability of DPPH, a persistent free radical, to become colourless when antioxidants are present. The DPPH radical scavenging ability of *T. cordifolia* aerial parts was shown in a study (Hasan *et al*., 2009) [4]. The distribution of secondary metabolites, which might change between various plant organs, could be the cause of the values (Lissiewska *et al*., 2006) [5].

The differences in antioxidant activity seen in various studies may be due to the chemical diversity of plant components.

Conclusion

This study provides valuable insights into the biological activities of *T. cordifolia* leaves, with their extracts displaying significant antioxidant capabilities. The high levels of phenolic and flavonoid compounds contribute to their ferric reducing power and radical scavenging activities, marking them as promising candidates for further research and development in health-related applications.

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