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Original Article

Evaluation of the Antioxidative and Qualitative Properties of the *Tinospora cordifolia*

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ABSTRACT

Background: *Tinospora cordifolia* is a perennial shrubby creeper, a member of the Menispermaceae family. It is a plant containing various phytochemical compounds and is used in traditional medicine. **Materials and Methods:** In the following study the qualitative analysis of the phytochemical compounds found in the stem of *T. cordifolia* is studied and FRAP, DPPH and ABTS assay is carried out to analyse the antioxidative activity of the three solvents including aqueous, ethanol and acetone of *T. cordifolia* stem. **Results:** In a qualitative analysis of the *T. cordifolia* stem the compounds carbohydrates, alkaloids, flavonoids, reducing sugar, phenolic compounds, tannins, saponins, phytosterol, lignin, quinone, anthocyanin and coumarins are found present. In FRAP among the three solvent extracts ethanolic extract of *T. cordifolia* stem performed better followed by acetone and aqueous. In DPPH the aqueous extract of *T. cordifolia* stem exhibited better antioxidative activity with IC_{50} 136 µg/mL followed by acetone and ethanol extract. In the ABTS assay too the aqueous extract showed better antioxidative properties with IC_{50} 48.150 µg/mL followed by acetone and ethanol extract of the plant. **Conclusion:** The above study indicates that *T. cordifolia* has medicinal properties due to the existence of different phytochemical constituents. In antioxidative activity analysis, the aqueous extract of the *T. cordifolia* stem performs better than the other two extracts, which indicates that the aqueous extract has good antioxidative properties. However, further analysis is needed.

Keywords: *Tinospora cordifolia*, Antioxidant, ABTS, FRAP, DPPH.

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INTRODUCTION

Herbal plants have been utilised for treating many ailments for a prolonged time in many different regions worldwide. Their immense healing properties against different diseases have made the researchers study more to find the beneficial phytochemical compounds in the plants. Most of the healing effects of these plants are due to the existence of these phytochemical constituents. These plants are used in old traditional medicines such as Traditional Chinese Medicine, Ayurveda, Unani Medicine, Traditional African Medicine and Indigenous Australian Medicine. These medicinal plants have a long history of curing various serious illnesses and are found to play a major part in maintaining the health of humans. The diverse range of the phytochemical compounds contained in these plants has a significant contribution to doing the same. Some of these phytochemical compounds found in the plants are flavonoids, alkaloids, terpenoids and others and their significance lies not only in their diverse richness but also their various roles such as antioxidant, anti-cancer, cardio-therapeutic, neurotherapeutic and other. These plants are essential for human health and are a significant source for many regions which has limited access to modern healthcare. The cultivation of these plants is more environmentally friendly than the synthetic ones. The use of these plants also allows for personalized medicine considering factors like genetics, lifestyle and environment.[1-3] These medicinal plants were the primary source in ancient times, but over time the benefit of these plants for therapeutic purposes has changed. In modern pharmacology, the essential components from the plants are extracted and many valuable medicines are produced from these valuable phytochemical compounds. Most of the world's population depends on medicines that are plant-based.[4] According to the World Health Organization (WHO), more than 80% of the population of the developing country uses conventional therapy and herbal medicines derived from medicinal plants that

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have pain relief and have been exploited for disease treatment. These medicinal plants may be of great promise in the future for the treatment of different diseases.[5,6]

Many phytochemical compounds are present in the extracts prepared from plants, these compounds have therapeutic efficiency against a wide range of diseases including viral conditions to malignant neoplasms. Several of the compounds present in these plants have potential synergistic effects. Because of these effects, these compounds are the basis of the traditional pharmacology. *Tinospora cordifolia* is a perennial shrubby creeper which belongs to the family of Menispermaceae and is native to various regions.[7] *T. cordifolia* commonly known as *Guduchi* is a therapeutic plant which has a rich history in conventional medicine and has reported many pharmacological activities.[8] Also known as *Amrita* or *Giloy* this plant is used as an Ayurvedic medicine and has medicinal properties against several health conditions like anaemia, jaundice, inflammation, rheumatism, diabetes, urinary disorders, skin disease, allergic conditions and others. This plant is considered an essential herbal plant of the Indian system of Medicine.[9,10] *T. cordifolia* is a large considerably spreading deciduous climber that grows in a broad range of hedges and trees and is documented to be dioecious, to have separate male and female flowers with stem bark of succulent nature covered by thin bark of colour brown and studded with warty lenticels. In Ayurveda along with *Sushrut Sam*hita and *Charak Samhita*, this plant is described with various properties that include *Chhinnaruha* which means this plant can be multiplied by vegetative propagation, *Vatsadini* which means eaten by grazing animals, *Pittaghni* or able to destroy bile, *Amruta* or have the ability to impart immortality, *Rasayana* that means able to improve the primordial tissue that strengthens other tissues, *Jwaranashi* means antipyretic, *Vayastha* means able to prevent ageing, *Saumya* explains that it is harmless also bitter. The plant stems, roots and leaves have a medicinal effect.[11-13] In the following study, the *T. cordifolia* stem is used for the identification of the different compounds by qualitative analysis and the antioxidative properties of the *T. cordifolia* stem extract.

MATERIALS AND METHODS

The *T. cordifolia* stem samples are collected from the University campus of Bharathiar, Coimbatore. The plant is authenticated by the Botanical Survey of India branch at Tamil Nadu Agricultural University, Coimbatore, India. The stem collected is dried and powdered into fine particles. The powdered stem is used further for study.

Qualitative test for phytochemicals in the stem of *T. cordifolia*

Three extracts aqueous, ethanol and acetone are utilised for the preparation of *T. cordifolia* stem extract. The qualitative analysis is carried out in the three extracts to analyse the presence of the phytochemical compounds like carbohydrates, flavonoids, phenolic compounds, tannins, alkaloids, reducing sugar, saponins, phytosterol, lignin, quinone, anthocyanin and coumarin.

Estimation of antioxidants

Ferric Reducing Antioxidant Power Assay

The Ferric Reducing Antioxidant Power (FRAP) is a methodology for determining the reducing power of the extract to reduce the Fe^{3+} to Fe^{2+}. The reducing ability is measured by determining the capability of the plant to act as the electron donor in reducing ferricyanide to ferrocyanide. The following antioxidative assay is carried out in the three solvent extracts, aqueous, ethanol and acetone prepared from the *T. cordifolia* stem. The following assay is carried out by assimilating 0.3 M acetate buffer, 10 mM 2,4,6-tripyridyl-s-triazine in HCl of 40 mM and $FeCl_3$ of 20 mM in the ratio of 10:1:1 and then to the above assimilation, different volume of plant extracts is added and the reactions are kept at room temperature for 15 min and absorbance are taken in a UV/vis Spectrophotometer at 595 nm. In a similar manner, ascorbic acid is prepared for different concentrations ranging from 200-1000 µg and readings are taken.[14]

2,2-Diphenyl-1-Picrylhydrazyl Assessment

The 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) activity of the plant extracts is carried out by adding 4.5 mL of 80 µM of DPPH prepared with ethanol with different concentrations ranging from 200-1000 µg of each of the extracts. The assimilations are incubated in a dark condition at room temperature for 30 min and the readings are taken at 517 nm in a UV/vis Spectrophotometer. In a similar manner, the standard ascorbic acid is prepared for different concentrations ranging from 200-1000 µg and readings are taken.[15] The formula used to calculate scavenging activity is:

$$\% \text{ inhibition} = [\text{Absorbance control} - \text{Absorbance sample}] \times 100$$

2,2'-Azino-Bis (3-Ethylbenzthiazoline-6-Sulphonic Acid) Assessment

The 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) radicle decolourising assay is carried out by preparing a reaction of ABTS solution of 7 mM with 2.45 mM $K_2S_2O_8$. The mixture prepared is allowed to stand for 16 hr in dark conditions at normal room temperature. The mixture is diluted with phosphate-buffered saline of pH 7.4 and readings are taken at 734 nm until the absorbance value reaches .700. The diluted ABTS reagent of 4.5 mL is added to different concentrations ranging from 200-1000 µg of each of the extracts and readings are taken. In a similar manner to the standard ascorbic acid, the assay is carried out with concentrations ranging from 200-1000 µg and readings are taken.[15] The percentage of inhibition for ABTS assay is carried out using the following formula:

$$\% \text{ inhibition} = [\text{Absorbance control} - \text{Absorbance sample}] \times 100$$

RESULTS

Qualitative analysis

In all three solvent extracts of *T. cordifolia* stem the constituents that are found to be present are listed in Table 1.

Antioxidant assay

In the FRAP method, the reduction of Fe^{3+} to Fe^{2+} activity of the solvent extracts is carried out along with the standard ascorbic acid. The reduction activity of different concentrations ranging from 200-1000 µg is performed in which the ethanolic extract showed better FRAP activity than the aqueous and the acetone extract of *T. cordifolia* stem as presented in Figure 1.

The DPPH assay of the different solvent extracts in the stem of *T. cordifolia* was found to have antioxidative properties in different concentrations by calculating the inhibitory percentage of the extracts of *T. cordifolia* stem. The IC_{50} values of the three solvent extracts are found to be: in aqueous 136 µg/mL, in ethanol 140 µg/mL and in acetone 139 µg/mL as presented in Figure 2.

The ABTS assay of the different solvent extracts in stems of *T. cordifolia* was found to have antioxidative properties in different concentrations by discolouring the ABTS reagent after the addition of different concentrations of the extracts of *T. cordifolia* stem. The IC_{50} values of the solvent extracts are found to be: in aqueous 48.150 µg/mL, in ethanol 60 µg/mL and, in acetone 51.2 µg/mL as presented in Figure 3.

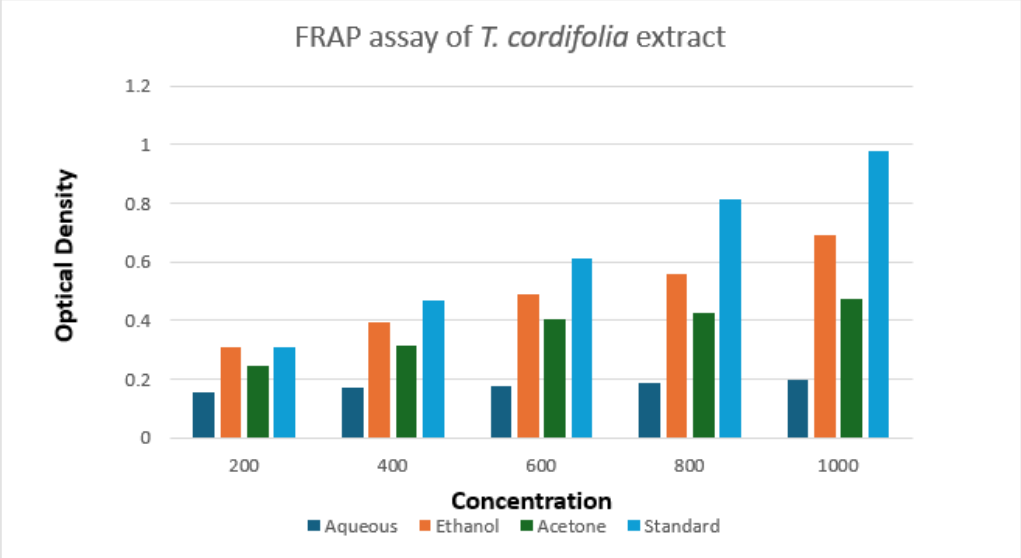

Figure 1: FRAP assay for different concentration of different solvent extract of *T. cordifolia*.

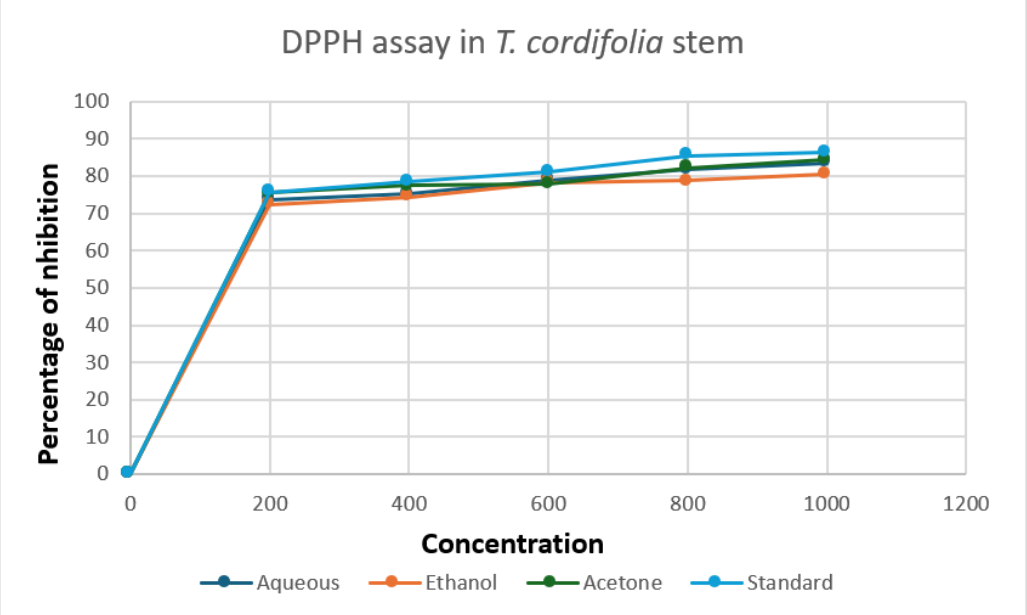

Figure 2: DPPH assay for different concentration of different solvent extracts of *T. cordifolia*.

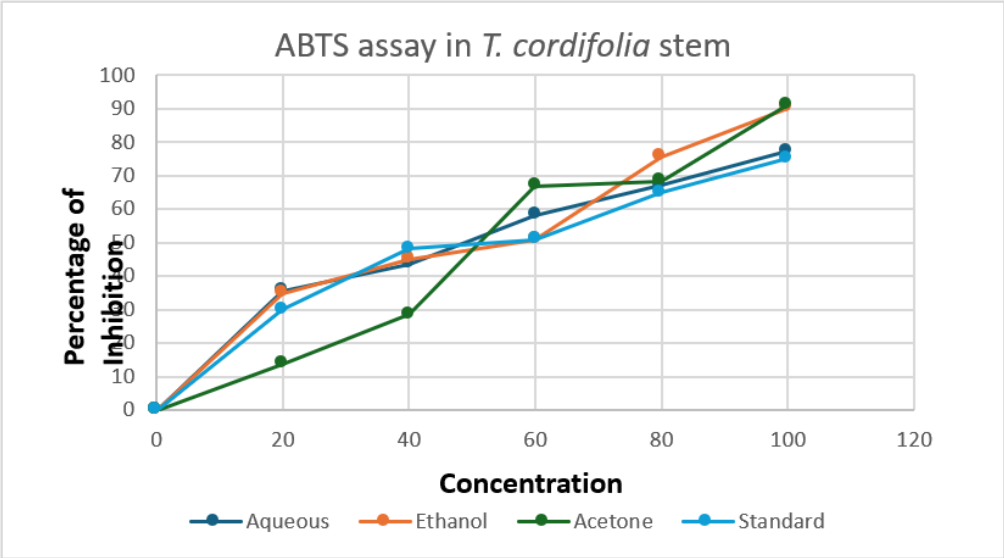

Figure 3: ABTS assay for different concentration of different solvent extracts of *T. cordifolia*.

Table 1: Qualitative analysis of the *T. cordifolia* stem.

Compounds	Present/ Absent (+/-)	Properties
Carbohydrate	+	Appearance of violet-red ring at the bottom.
Alkaloids	+	Formation of white precipitate at the bottom.
Reducing sugar	+	Formation of green colour.
Flavonoids	+	Formation of intense yellow colour.
Phenolic compounds	+	Formation of white precipitate.
Tannins	+	Formation of white precipitate.
Saponins	+	Persisted foam for over 10 min.
Phytosterol	+	Formation of red colour.
Lignin	+	Appearance of olive-green colour.
Quinones	+	Formation of green colour.
Anthocyanins	+	Formation pink red colour.
Coumarins	+	Appearance of yellow colour.

DISCUSSION

T. cordifolia is a dioecious plant, a characteristic climbing shrub found in tropical parts of the Indian subcontinent and found to climb many trees. *T. cordifolia* is generally known as *Amrita* or *Guduci*. It is a medicinal plant prescribed for conditions like fever, diabetes, jaundice and urinary problems as well as used for anti-spasmodic, anti-inflammatory and anti-allergic.[16] This plant is an essential part of the Indian system of medicine and has been practised in the Ayurvedic system for its therapeutic properties. *T. cordifolia*'s root is used as an emetic drug and the starch present in this plant is a beneficial remedy for chronic fever, relieves a burning sensation, increases appetite and energy.[9] It is present in a wide variety of soil from acid to alkaline and needs fair moisture for growth. It is a plant of tremendous promise.[17-19]

In the following study, the plant *T. cordifolia* stem is studied, for its different qualitative and antioxidative properties. In qualitative analysis compounds like carbohydrates, alkaloids, flavonoids, reducing sugar, phenolic compounds, tannins, saponins, phytosterol, lignin, quinone, anthocyanin and coumarins are found to be present in all three extracts of *T. cordifolia* stem extracts of ethanol, acetone and aqueous. In the FRAP antioxidative assay, the ethanolic extract is seen to show higher antioxidation, in the DPPH and ABTS antioxidative assay, the IC_{50} values of aqueous are found to be lower than the other two extracts of 136 µg/mL and 48.150 µg/mL. From both the DPPH and ABTS analysis, it may be indicated that the aqueous extract of *T. cordifolia* stems may have potential antioxidative properties and also have medicinal effects due to the existence of phytoconstituents.

CONCLUSION

In the following, the stem of *T. cordifolia* is studied to identify different phytochemical compounds present and also to quantify the antioxidative activity using DPPH, FRAP and ABTS. In the qualitative analysis the phytocompounds, carbohydrates, alkaloids, flavonoids, reducing sugar, phenolic compounds, tannins, saponins, phytosterol, lignin, quinone, anthocyanin and coumarins are indicated to be present in the three solvent extracts of *T. cordifolia* stem. In an antioxidative study among the three extracts aqueous extracts are found to perform better than the ethanolic and acetone extract, in both DPPH and ABTS assay. From the above study it can be indicated that aqueous extract of

the *T. cordifolia* stem may have potential antioxidative properties but to conclude further, studies are needed to be done.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ABTS: 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid); **DPPH:** 2,2-Diphenyl-1-Picrylhydrazyl; **FRAP:** Ferric Reducing Antioxidant Power; **WHO:** World Health Organization.

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