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Assessment of phytochemicals, antimicrobial, antioxidant and cytotoxicity activity of methanolic extract of *Tinospora cordifolia* (Gurjo)

Tara Shrestha, Janardan Lamichhane
Department of Biotechnology, Kathmandu University, Dhulikhel, Kavre, Nepal. *Received: 23 Feb 2021; Revised: 14 Jul 2021; Accepted: 19 Jul 2021; Published online: 31 Jul 2021*

Abstract

Traditionally used medicinal plants are the major resources of biologically active metabolites which are widely used for the cure of numerous diseases especially in developing countries where health facilities are rare. Many plants are in use for centuries but there is not enough scientific evidence and exploration. This research is focused on phytochemicals, antibacterial, antioxidant and cytotoxicity activity analysis of one of the most commonly used ethnomedicine *Tinospora cordifolia* collected from the Kavrepalanchok district of Nepal. Phytochemicals analysis of methanol extract of *T. cordifolia* showed the presence of alkaloids, coumarin saponins, glycosides, reducing sugar, and triterpenes. Antibacterial activity performed by disc diffusion method exhibited the highest activity against *Streptococcus* with a zone of inhibition are 10.3mm, 8.5mm, 6.5mm, and 6mm at 200mg/ml, 100mg/ml, 50mg/ml, and 25mg/ml of concentration respectively. DPPH radical scavenging activity increased with increasing concentration of extract. When compared with ascorbic acid at equivalent concentration, the extract shows a lower scavenging profile (56.07% for the extract and 98.01% for ascorbic acid at 320 ppm). Cytotoxicity was evaluated in terms of LC_{50} (lethality concentration). The result showed that the extract of *T. cordifolia* was found to be toxic with an LC_{50} value of 232.64µg/ml. The bioactive component present in the plants could be the result of its pharmacological effects that support the traditional use of plants.

Keywords: *Tinospora cordifolia*, Antioxidant activity, Antibacterial activity, Phytochemicals, DPPH, Brine Shrimp bioassay.

Corresponding author, email: tarashrestha2@gmail.com

Introduction:

Herbal medicines are the biological resource of medicines that have been used in different cultures around the world as safe therapeutic drugs. In addition, they are also the enormous resource of dietary supplements, popular drugs, pharmaceutical intermediaries, nutraceutical drugs, and chemical entities for synthesized drugs [1]. The activity of medicinal plants is focused on the rich experiences of countless healers over centuries, whether inherited from ancestors, passed down from healer to healer, or established over time through personal experiences [2]. Secondary metabolites are non-nutrient plant chemical compounds or bioactive components that protect the plant from microbial infections or insect infestations. They are also known as phytochemicals or phytoconstituents [3].

T. cordifolia, also known as "Guduchi," is well-known in traditional ayurvedic literature for its extensive use in the treatment of various diseases like jaundice, urinary diseases, rheumatism, anemia, fever, vomiting, diabetes, skin disease, etc. It is found mostly throughout It is a big deciduous climbing shrub with a variety of coiling branches that spreads widely. The plant's stem is succulent, with long, fleshy, and climbing tendencies [4, 5].

The discovery of active components of the plant and their biological role in disease control has generated intense interest in the plant [6]. A wide number of chemical compounds like aporphine alkaloids, diterpenes, berberine, palmatine, tembertarine, magniflorine, choline, and tinosporin, etc. have been isolated from this plant. [7]. T. cordifolia methanol extracts of stem [8] are effective against microbial infections. It is reported that aqueous, ethanol, and acetone extract of leaves and stem of T. cordifolia showed inhibitory activity against urinary pathogens such as Klebsiella pneumoniae and Pseudomonas aeruginosa [9]. Methanolic, ethanolic and water extracts [10] of T. cordifolia has found to be significant

antioxidant compared to other solvents. It is suggested that T. cordifolia methanol extract when taken orally has increased the erythrocytes membrane lipid peroxide and catalase activity [11]

T. cordifolia is a well-known medicinal plant in traditional medicine, and several recent scientific investigations have highlighted its potential value in modern medicine. There have been several studies are carried on, but T. cordifolia research in Nepal is limited. The purpose of this research is to document T. cordifolia medicinal characteristics as well as its potential for further scientific inquiry in the production of effective therapeutic molecules. Isolation of phytochemicals, antibacterial activity, antioxidant activity, and cytotoxicity analysis of phytoconstituents derived from methanol extract stem of T. cordifolia all are addressed in this research. The decision of solvent is made due to the idea of solvent to separate wide assortments of hydrophilic and lipophilic substance constituents.

Materials and Methods

Sample collection

The sample of *T. cordifolia* stems was collected from Bhakundebesi, Kavrepalanchok district of Nepal situated at an altitude of 1120 msl with Latitude 27.560677° and Longitude 85.6409178° shown in **Figure 1**. The identification of the plant was done by Tirtha Maya Shrestha, taxonomist, Department of Pharmacy, Kathmandu University.

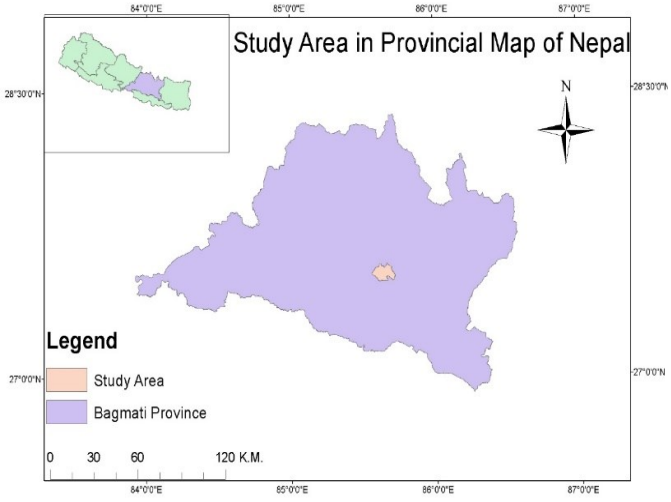

Figure 1: The sample collection site, Kavrepalanchok District, Nepal.

Sample preparation and extraction:

The collected plant was shade dried at 25°C and finely powdered using a grinder. The sample extract was prepared using a cold methanol maceration process [12] in which 50 g of finely ground powder was infused with 200 ml of methanol. The mixture was then stirred for approximately half an hour and stored for 48 hours. The solution was filtered using the Whatman No. 1 filter paper after 48 hours. A rotary evaporator was used to evaporate the filtrate sample. The extraction yield was calculated using the formula:

$$Extraction\ yield(\%) = \frac{weight\ of\ extract\ obtained}{total\ weight\ of\ sample\ used} \times 100$$

Phytochemical screening

Phytochemical screenings were performed for basic alkaloids, coumarins, saponins, glycosides, reducing sugars, sterols, triterpenes, and flavonoids [13].

Antimicrobial Screening

Antimicrobial analysis was done by the disc diffusion method [14]. Test solution of 200mg/ml, 100mg/ml, 50mg/ml, and 25mg/ml concentration of plant was prepared for antimicrobial test against the bacterial pathogens namely *Staphylococcus aureus, Klebsiella pneumoniae, Bacillus subtilis, and Streptococcus* in Muller Hinton Agar. The human pathogenic strain was obtained from Dhulikhel Hospital, Kavrepalanchok, Nepal. The plant extract was prepared in the No.1 Whatman filter. CIP30: Ciprofloxacin-30 µg/disc, T30: Tetracyclin-30 µg/disc, and A10: Ampicillin-10 µg/disc were used to equate the zone of inhibition with regular antibiotic discs.

DPPH free radical scavenging assay

The DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging method was used to test antioxidant activity [15, 16]. Ascorbic acid was used as a standard scavenger**.** Dimethyl sulfoxide (DMSO) was used as a solvent to prepare a solution of ascorbic acid and plant extracts at concentrations of 0.5, 2, 4, 8, 16, and 32 µg/ml. Then, using a UV spectrophotometer set to 517nm, the IC_{50} (the sample needed to scavenge 50% of the DPPH free radical) was measured for each concentration of the sample. The equation for IC_{50} calculation:

Table 1: Qualitative phytochemical screening of *T. cordifolia* from methanol extract shows the presence of alkaloids, coumarin, saponins, glycosides, reducing sugar and triterpenes

Phytochemicals	Alkaloids	Coumarins	Saponins	Glycosides	Reducing Sugar	Sterols	Triterpenes	flavonoids	Tannins and polyphenols
	+	+	+	+	+	-	+	-	-

Present(+) Or Absent(-)

$$PercentageScavenging(IC50) = \frac{A0 - A1}{A0} \times 100$$

where, A0 = DPPH solution absorbance, A1= DPPH absorbance in relation to various extract concentrations. The IC_{50} was determined by plotting a graph of concentration versus percent inhibition and using a linear trend line equation. Higher free radical scavenging activity was demonstrated by a lower absorbance of the reaction mixture.

Brine shrimp lethality assay

The toxic property of *T. cordifolia* methanolic extract was determined using the brine shrimp lethality [17] technique. To obtain various concentrations, the extracts were dissolved in DMSO and diluted with artificial sea saltwater. The total volume was adjusted to 10 ml with aerated seawater (1000, 100, and 10ppm). Every tube held ten nauplii, which were incubated for 24 hours. After that, the tubes were examined under a magnifying glass, and the number of survivor's nauplii in each tube was counted. Experiments were carried out in a series of three tubes per dose, with a monitor (DMSO in seawater) and various concentrations of the test substances. The logarithm of the extract concentration was used (to make dealing with a wide variety of values simpler). Death percentage was calculated by:

$$Death\ percentage = \frac{Death\ nauplii}{Initial\ nauplii} \times 100.$$

% death corrected vs. log extract conc (ppm) was plotted and the Lethal Dose 50 (LD_{50}) from the graph from the equation of straight-line. Lethal concentration was determined from the equation of the straight line.

Results

Phytochemical Screening

The term "phytochemical investigation" refers to the process of identifying and characterizing crude pharmaceuticals in terms of their phytochemical ingredients. Chemical elements of the plant were assessed. The results for the different types of phytochemicals presence are shown in **Table 1**. The presence of these phytochemicals explains why plants are used to treat ailments such as diabetes, anticancer drugs, jaundice, etc.

Antibacterial activity

The antibacterial susceptibility test showed that bacterial strains are susceptible to standard antibiotics. The zone of inhibition (ZOI) shown by standard antibiotics CIP^{30}: Ciprofloxacin-30mcg/disc, T^{30}: Tetracycline-30mcg/disc, and A^{10}: Ampicillin-10mcg/disc are shown in **Table 2.**

Triplicates of the experiment were carried out, and the ZOI values are the average in millimeters (mm). The activity was carried out against four disease-causing bacteria, including, *Klebsiella pneumoniae, Bacillus subtilis, Staphylococcus aureus,* and *Streptococcus*. In comparison to standard antibiotics. *Streptococcus* was found to be a more susceptible comparison to other bacterial strains showing zone to inhibition 10.3mm, 8.5mm, 6.5mm, and 6mm at 200mg/ml, 100mg/ml, 50mg/ml, and 25mg/ml

Table 2: Antibacterial activity shown by the standard antibiotics, DMSO and plant extract against B. subtilis, K. pneumonia, S. aureus and Streptococcus species. Standard antibiotics Ampicillin, Ciprofloxacin and Tetracycline were taken as positive control while DMSO was taken as negative control. Four different concentration of T. cordifolia sample (Tc-200mg/ml, 100mg/ml. 50mg/ml and 25mg/ml) was tested for its bactericidal activity and corresponding values represent the respective zone of inhibition.

Bacterial Strain	Zone of Inhibition(mm)			
Test sample	*Bacillus Subtilis*	*Klebsiella pneumoniae*	*Staphylococcus aureus*	*Streptococcus*
Ampicillin(10µg)	11±1	14±0.5	12	10±1
Ciproflaxin(30µg)	26±1	27	26	24
Tetracycline(30µg)	19±1	22±1	19±1.08	19
DMSO	0	0	0	0
Tc - 200mg/ml	8	6.5	6	10.3
Tc- 100mg/ml	7	6.5	6	8.5
Tc - 50mg/ml	6.5	6	6	6.5
Tc - 25mg/ml	6	6	6	6

of concentration respectively. Similarly, *T. cordifolia* was found to be mildly inhibitory *Bacillus subtilis* showing a zone of inhibition 8mm, 7mm, 6.6mm and 6mm at 200mg/ml, 100mg/ml, 50mg/ml, and 25mg/ml respectively. *T. cordifolia* did not show a prominent zone of inhibition for *Klebsiella pneumoniae* and *Staphylococcus aureus*. The zone of inhibition against all the microorganisms is shown in **Figure 2, Figure 3**, and **Table 2**.

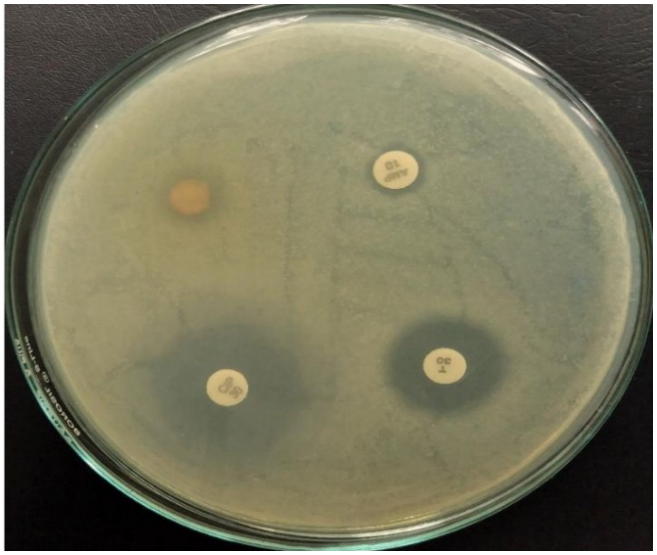

Figure 2. Comparision of ZOI of *T.Cordifolia* with standard antbiotic discs Amp10, CIP30 and T30.

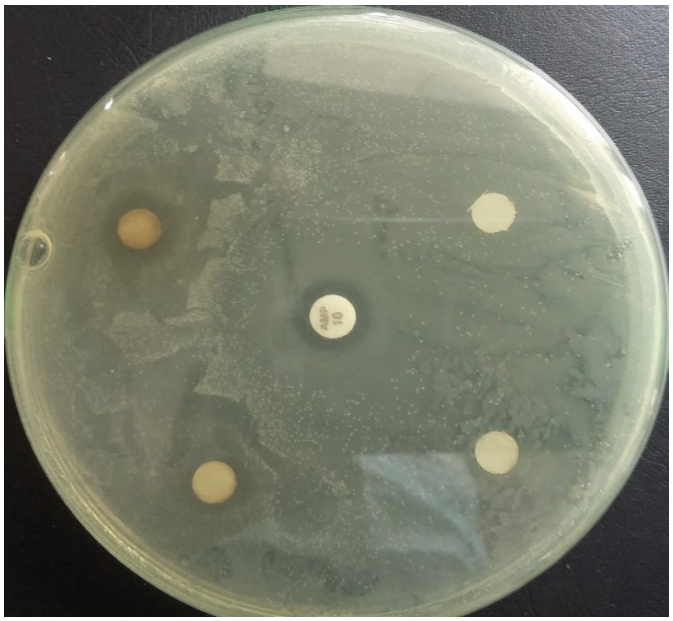

Figure 3. *T. cordifolia* showing ZOI against *Streptococcus spp.* at 200mg/ml, 100mg/ml, 50mg/ml and 25mg/ml.

Assay for scavenging DPPH free radicals

DPPH scavenging percentage was found to be concentration-dependent. The absorbance was determined using a spectrophotometer at 517nm for the calculation of %scavenging of *T. cordifolia*. Ascorbic acid was taken as a standard analyte. The scavenging percentage of ascorbic acid and extract of *T. cordifolia* is compared in **Figure 4**. From, the linear regression analysis the IC_{50} value of *T. cordifolia* and ascorbic acid was observed to be 238µg/ml and 4.76µg/ml respectively.

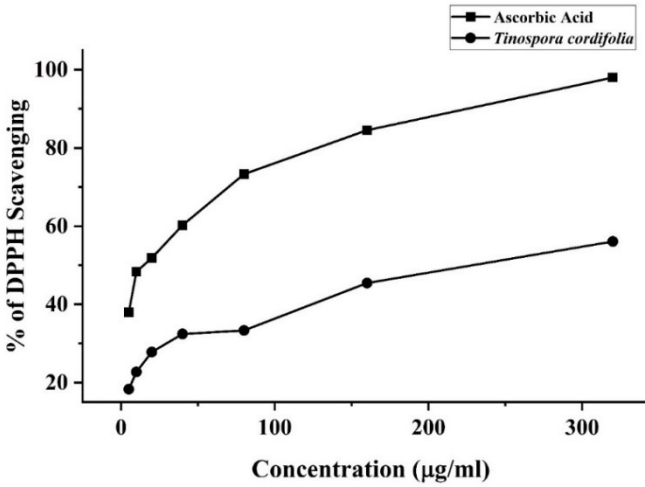

Figure 4. The graph shows the DPPH scavenging activity of methanol extract of T. cordifolia. The percent scavenging activity is compared with Ascorbic acid.

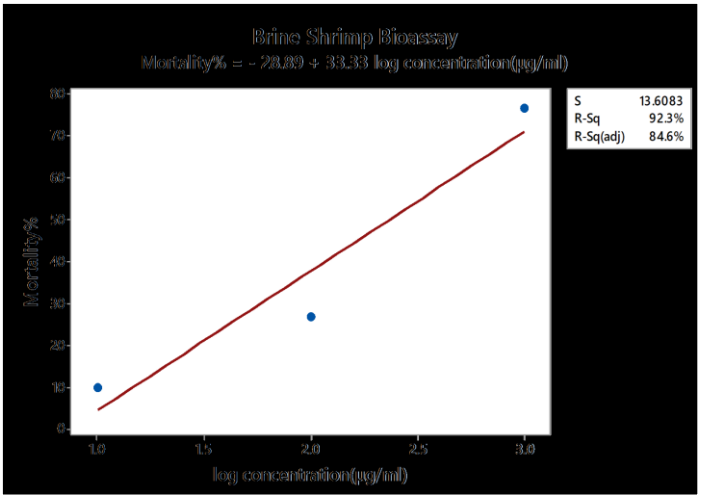

Figure 5. Toxicity effects of T. cordifolia Shown by Brine shrimp Bioassay. The trend line equation gives lethal concentration (LC50) of 232.64 ppm.

Brine shrimp bioassay

The methanol extract of *T. cordifolia* showed good brine shrimp larvicidal activity. The regression trend line graph was plotted using the concentration and death

percentage. The lethality concentration (LC_{50}) obtained from regression analysis (**Figure 5**)was found to be 232.64 ppm.

Discussion

The presence of phytochemicals alkaloids, coumarin, saponins, glycosides, reducing sugar and triterpenes gives evidence of its anti-diabetic, anticancer, anti-inflammatory infections, anti-viral infections. Many of these active compounds have various immunomodulatory and physiological functions [18]. The presence of these compounds may be the reason for the antibacterial, antioxidant, and cytotoxic activity of plants. The antibacterial activity was found to be susceptible mostly to gram-positive bacteria. The presence of triterpenes shows that it has antibacterial activity. A previous study suggests that ethanol extract *T. cordifolia* showed good inhibition against both gram-positive and gram-negative bacteria. Present findings support the applicability of *T. cordifolia* in traditional systems for its claimed uses like fever inflammations, urinary and skin diseases [19]. Methanol was employed to extract antioxidant-rich fractions from the stem of *T. cordifolia* in this investigation. Methanol is a better solvent for the extraction of chemicals than other solvents, according to previous research. The presence of polyphenols and tannins in plant extracts can contribute to their antioxidant activity [20].

According to Meyer's toxicity index [21], extracts with $LC_{50} < 1000$ µg/ml are considered as toxic, while extracts with $LC_{50} > 1000$ µg/ml are considered as non-toxic . The lethal concentration of the extract was found to be 232.64 which indicates the extract is toxic and worthy of further investigation. The early toxicity information acquired from the Brine Shrimp Lethality Assay provides LC_{50} values, which can be used as a starting point for further toxicity research. [22].

Conclusion

Herbal medicines are critical in the restoration of modern civilization's health. T. cordifolia has been utilized pharmacologically in the treatment of a variety of diseases, according to the literature review. The findings of the phytochemicals study back up the common use of T. cordifolia to treat several ailments like jaundice, rheumatism, urinary tract infections, dermal diseases, anemia, inflammation, diabetes, etc. [7]. The main constituents of T. cordifolia are tinosporine, tinosporide, tinosporaside, cordifolide, cordifol, heptacosanol, clerodane furano diterpene, diterpenoid furanolactone tinosporidine, columbin, and b-sitosterol. Berberine, Palmatine, Tembertarine, Magniflorine, Choline, and Tinosporin are reported from its stem. This chemical constituent plays a major role in the antibacterial, antioxidant, and cytotoxicity role of T. cordifolia [23].

T. cordifolia has gain attention due to its immense application in traditional as well as modern medicine. Tinospora cordifolia is seasonal and geographically dependent. At the same time, the organic and aqueous extracts of Tinospora cordifolia could be used in the pharmaceutical sector in the future as a source of important phytochemical substances.

Author's contributions

Janardan Lamichhane contributed to designing the experiment. Tara Shrestha contributed to all the laboratory works and data analysis work. JL and TS contributed equally in drafting and reviewing the manuscript.

Competing Interests

No competing interests were disclosed.

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Ethical Approval and Consent

Not applicable.

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