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Possible Wound Healing Potential and Ethnopharmacological Profiling of Solvent-Fractionated Stem Extracts of *Tinospora cordifolia*: a Comparative Study with Marketed Dosage Forms in Bangladesh

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Abstract

Tinospora cordifolia is a prominent medicinal plant used in traditional systems like Ayurveda to treat chronic conditions. This study evaluated the ethno-pharmacological activities of its stem and seed extracts across varying solvent polarities to validate its traditional uses. Extracts were prepared using n-hexane and methanol concentrations (0, 40, 80%). Assessments included phytochemical screening, GC-MS analysis, antioxidant assays (DPPH, ABTS), anti-diabetic assays (alpha-amylase inhibition), anti-inflammatory assays (HRBC membrane stabilization), and *in vivo* wound-healing assays using an excision model. The extraction yielded 42.31%. Phytochemical analysis identified alkaloids, flavonoids, phenols, tannins, and saponins. Quantitatively, the n-hexane extract showed a total phenolic content of 21.61 ± 3.84 mg gallic acid equivalent/g and a flavonoid content of 0.236 ± 0.02 mg quercetin equivalent/g. The 80% M-Ex proved most potent for antioxidant (IC_{50}, 768 mg/ml) and anti-inflammatory (IC50, 717.23 mg/ml) activities. Conversely, the n-hexane stem extract exhibited the strongest anti-diabetic effect (IC_{50} 651.31mg/ml). In wound healing, the 80% methanolic extractachieved 100% wound contraction by day 14. Solvent polarity significantly influences the therapeutic profile of *T. cordifolia*. Methanol-rich extracts are most effective for oxidative stress and wound repair, while non-polar fractions show specific promise for diabetes management.

Key words: Wound-healing, HRBC, Excision, Oxidative stress, *T. cordifolia.*

Introduction

Medicinal plants continue to play an important role in healthcare and drug discovery because of their rich content of biologically active secondary metabolites including alkaloids, flavonoids, phenolics, tannins, terpenoids and glycosides (Harborne, 1998; Evans, 2009). These phytochemicals possess diverse pharmacological properties and have contributed significantly to the development of modern therapeutics. Inflammation, oxidative stress and diabetes mellitus are closely associated pathological conditions that contribute to the progression of numerous chronic diseases. Persistent inflammation and excessive production of reactive oxygen species may lead to cellular damage and impaired tissue repair, thereby delaying wound healing (Medzhitov, 2008; Liguori *et al*., 2018). Although several synthetic drugs are available for the management of these conditions, their long-term use is often associated with adverse effects, highlighting the need for safer and more effective alternatives derived from natural sources (Forbes and Cooper, 2013). *Tinospora cordifolia* (Willd.) Miers (Menispermaceae), commonly known as Guduchi or Giloy, is a climbing shrub widely distributed throughout South Asia. In traditional Ayurvedic

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medicine, it is recognized as a valuable Rasayana and is used for the treatment of fever, diabetes, inflammation, jaundice and immune-related disorders (Singh *et al*., 2003). Phytochemical investigations have revealed the presence of alkaloids, diterpenoid lactones, glycosides, polysaccharides, steroids and phenolic compounds, which are responsible for its antioxidant, antidiabetic, anti-inflammatory, immunomodulatory and wound-healing activities (Upadhyay *et al*., 2010). Although several studies have reported the pharmacological potential of *T. cordifolia*, comparative information regarding the influence of solvent polarity on the biological activities of stem extracts remains limited. Therefore, the present study was undertaken to evaluate the phytochemical composition, antioxidant, anti-inflammatory, antidiabetic and wound-healing activities of solvent-fractionated stem extracts of *T. cordifolia*.

Materials and Methods

Plant collection and identification: Fresh stems of *Tinospora cordifolia* (Willd.) Miers were collected from Chattogram, Bangladesh. The plant material was taxonomically identified by Professor Dr. Shaikh Bokhtear Uddin, Department of Botany, University of Chittagong, Bangladesh. A voucher specimen was deposited in the Herbarium of the Department of Pharmacy, University of Science and Technology Chittagong (USTC), Bangladesh.

Extraction process: The collected stems were washed thoroughly, shade-dried and pulverized into coarse powder using a laboratory grinder. The powdered stem material was macerated separately in n-hexane and methanol-water mixtures (40 and 80%) for 72 hrs with continuous stirring. The extracts were filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator following standard extraction procedures (Harborne, 1998).

Experimental animals: All experimental procedures involving animals were conducted according to institutional guidelines and approved by the appropriate ethical review committee.

Determination of yield: With the help of an electric balance, the dried pure extracts were weighed individually [4]. The yield was determined by using the given formula-

Yield (%) = Dry extract weight/Dry powder weight × 100

Hytochemical screening (Qualitative): Phytochemical screening refers to the initial tests that are done to ascertain the existence of primary and secondary metabolites in the plant extract. Various qualitative methods have been used to determine such compounds as alkaloids, tannins, flavonoids, saponins, flavones, sterols, terpenes, cardiac glycosides, proteins, carbohydrates and fats. Qualitative phytochemical analysis was done following standard methods, such as the ferric chloride test for phenols, Wagner's test for alkaloids, hydrochloric acid test for flavonoids, Salkowski test for terpenoids, Salkowski reaction for steroids, shake (foam) test for saponins, and Molisch's test for carbohydrates. The occurrence of these compounds was determined based on typical color changes in each test. (Harborne (1998) and Evans (2009))

Phytochemical screening (Quantitative):

Total phenolic content (TPC): It was determined using the Folin–Ciocalteu method, with the absorbance recorded at 760 nm. Gallic acid is the calibration standard. Phenolic content was expressed as gallic acid equivalents (GAE) per gram dry plant material, using a standard curve of gallic acid (Y = 0.0019x + 0.0135). Methanol was added to a 250 µl sample (2 mg/ml), followed by 250 µl Folin–Ciocalteu reagent, before incubation was kept for 6 minutes after vortexing . Then, 2.5 ml of 7% sodium carbonate solution and double-distilled water were included. Then, after incubating the mixture for 90 minutes and absorbance was measured at 760 nm (Singleton *et al*., 1999)

Total Flavonoid Content (TFC): The aluminum chloride colorimetric method was used to determine the total flavonoid content. Absorbance was measured at 510 nm. Quercetin acid was used as the standard, and flavonoid contents were measured as quercetin acid equivalent QAE/g of dry plant material based on a standard curve of Quercetin acid (5 = 0.0013x + 0.0451). 0.5 ml of extract solution in

methanol was combined with 0.3 ml of 5% sodium nitrite and incubated for 5 min. Addition of 0.3 ml of 10% Aluminum Chloride immediately. 2 ml of 1 M Sodium Hydroxide was combined, and the absorbance was taken at 510 nm (Chang *et al*., (2002)

GC-MS analysis: The chemical composition of *T. cordifolia* seed extract was analyzed using mass spectrometry with an Agilent Technologies 7890A capillary gas chromatography system (Santa Clara, CA, USA). A 90 m × 0.25 mm fused silica capillary column (HP-5MSI) coated with a 0.25 µm film of 95% dimethyl-polysiloxane and 5% phenyl were used. Approximately 6 µL of the crude extract (1% w/v), diluted in a solution of methanol : chloroform: water (2.5 : 1 : 1), was injected. Helium (99.999%) was the carrier gas at a flow rate of 1 mL/min. The source temperature was set at 250°C, and the MS quad was operated at 150°C. The chemical components were identified by matching the mass spectra to the National Institute of Standards and Technology (NIST) database (Adams (2007)

In vitro antioxidant activity:

DPPH radical scavenging activity: The Radical scavenging activity of *T. cordifolia* was done via stable DPPH radicals following the technique described by Sayah et a. Extract solution was prepared at different concentrations in Methanol. DPPH was prepared in Methanol and 3 ml added to all the test tubes (Brand-Williams et al. 1995)

The mixed solution was vortexed and kept for Dark incubation for 30 min, and the absorbance was taken at 517 nm. The percentage of DPPH free radical scavenging was determined by using the following formula:

DPPH radical scavenging (%) = [1 = (A/A*o*) × 100]

A = the absorbance of the extract or standard.

where, Ao = the absorbance of the control

2.8.2 ABTS radical scavenging activity:

The TEAC test (Trolox Equivalent Antioxidant Capacity) or discoloration test of ABTS·+ was carried out according to the method described by (Re et al. 1999) Briefly, the cationic radical (ABTS+) was produced by the reaction between 10 mL of ABTS (2 mM) in H2O and 100 µl of potassium persulfate (K2S2O8) (70 mM). The mixture was incubated in the dark for 12-16 hrs at room temperature .ABTS is prepared by mixing with Potassium Persulfate and adjusting the absorbance at 734 nm after incubation for 12-16 hrs. Finally, the ABTS is added to the extract and incubated for 30 min, and the absorbance is taken at 734 nm

In vitro anti-diabetic activity: Alpha-amylase inhibitory assay (DNSA method): By dissolving 0.1 g of starch in 100 ml of sodium acetate buffer with 16 mmol of sodium acetate, a 0.1 % w/v starch solution was prepared. 27.5 mg of alpha-amylase enzyme were dissolved in 100 mL of distilled water to prepare an alpha-amylase enzyme solution. 96 mmol of sodium potassium tartrate and 3,5-dinitrosalicylic acid were combined to prepare the colorimetric reagent. Equal amounts of starch and alpha- amylase solutions were combined with test samples at concentrations of 250, 500, 750, and 1000 µg/ml that were prepared by using vehicle Tween 80 and distilled water, and the mixture was incubated for 10 minutes at 37°C. After 3,5-dinitrosalicylic acid was added to halt the process, the absorbance at 540 nm was determined. The standard was acarbose. The test was conducted in triplicate, and the mean value was measured from the three replicates to ensure the accuracy and reliability of the results. (Miller 1959), (Bernfeld 1955)

% Inhibition = $[(OD_{control} - OD_{test})/OD_{control}] \times 100$

Where: OD = Optical density

HRBC membrane stabilization assay: To begin, we created a suspension of human red blood cells (HRBC) by collecting 2 ml of fresh human blood from willing participants who provided written consent. An equal amount of Alsever solution (2 grams dextrose, 0.8 grams sodium citrate, 0.05 gm citric acid, and 0.42 gm sodium chloride dissolved in 100ml water) was added to the blood. The mixture was then subjected to centrifugation at 3000 rpm for 10 minutes, and the liquid above the packed cells, known as the supernatant, was discarded (Shinde et al. (1999) The cell suspension was rinsed multiple times using a sterile isosaline solution (0.9% w/v

NaCl) until the supernatant became clear and colorless, leaving the red blood cells settled at the bottom of the tube. The volume of the cellular component was measured and subsequently adjusted to reach a 40% concentration using phosphate-buffered saline (PBS) with a pH of 7.4 and a concentration of 10 mM

In-vivo wound healing activity test: Excision wound model: Rats were anesthetized with ketamine (30 mg/kg, ip), and an area of about ≈500 mm2 was marked on the back of the rat with a standard ring. The full thickness of the marked skin was then cut carefully. Wounds were traced on 1 mm2 graph paper on the day of wounding and subsequently at a gap period of 4 days till the 14th day, then on the alternate days until healing was complete (Morton and Malone 1972) Changes in wound area were measured regularly, and the rate of wound contraction was calculated as given in the formula below. Significance in wound healing of the test groups is derived by comparing the healed wound area on respective days with the healed wound area of the control group. The period of epithelization, that is, the day of the fall of the eschar and the scar area, was also noted down.

Results and Discussion

Yield percentage: After extraction, the percentage yields of *T. cordifolia* were determined. The result showed that the extract yield was 42.31%.

Qualitative analysis: The qualitative analysis of *T. cordifolia* identified the presence of various secondary metabolites, including alkaloids, carbohydrates (Table-1) Table-1: Qualitative analysis of phytochemical constituents present in *T. cordifolia* Stem.

Quantitative analysis: The M-EX showed a TPC of 20.98 ± 0.18 mg GAE/g, while the *n*-hexane extract exhibited 21.61 ± 3.84 mg GAE/g. The TFC was higher in the *n*-hexane extract (0.236 ± 0.02 mg QE/g) compared to the M-Ex (0.069 ± 0.026 mg QE/g) (Table 1).

In-vitro antioxidant activity test (DPPH method): The IC_{50} values of *T. cordifolia* methanol-EX at 80, 40, and 0% concentrations were 768.00, 1754.17, and 1769.13 µg/ml, respectively, while the *n*-hexane extract showed an IC_{50} of 1262.31 µg/ml. The standard control exhibited an LC_{50} value of 258.88 µg/ml. (Figure 1).

In-vitro anti-inflammatory activity: The IC_{50} values of *T. cordifolia* methanolic-EX at 80, 40, and 0% were 768.00, 1418.88, and 1657.14 µg/ml, respectively, with a standard value of 338.29 µg/ml. The *n-hexane* extract showed an IC_{50} of 992.01 µg/ml.

Anti-diabetic activity: The Stem extract showed an IC_{50} of 651.31 µg/ml, while the standard exhibited an IC_{50} of 54.36 µg/ml.

Table 1. Qualitative analysis of phytochemical constituents present in the extract of T. cordifolia stem.

Secondary metabolites	Name of test	Name of the extract	
		Methanol	N-hexane
Alkaloids	Wagner's test	+	+
Tanin	Ferric chloride	+	+
Steroid	Salkowski's	+	+
Terpenoid	Libermann–burchard's test	+	+
Flavonoid	Hydrochloric acid	+	+
Saponin	Shake or foam test	+	-
Phenols	Ferric chloride	+	+
Carbohydrates	Molish	+	+

Extract	TPC (mg GAE/g)	TFC (mg QE/g)
Methanol Ex	20.98 ± 0.18	0.069 ± 0.026.
n-hexane Ex	21.61 ± 3.84	0.236 ± 0.02.

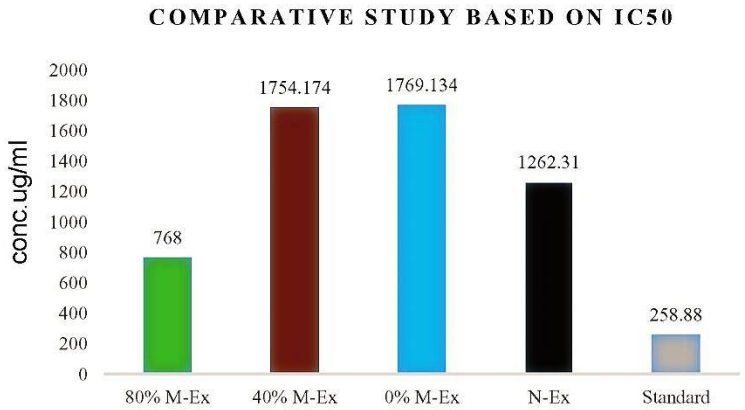

Figure 1. Antioxidant activity of methanol-ex, n-hexane ex and standard of *T. cordifolia.*

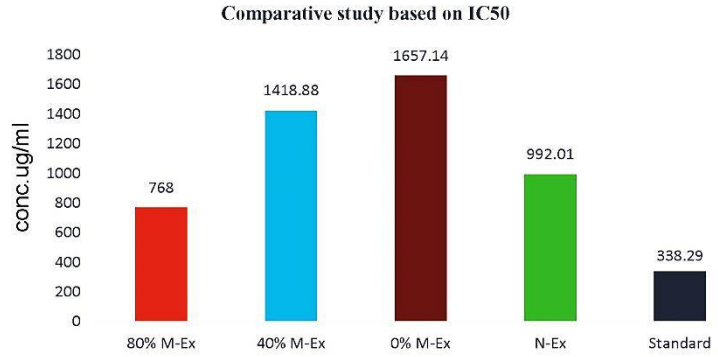

Figure 2. Anti-inflammatory activity of m-ex and n-ex extracts of *T. cordifolia.*

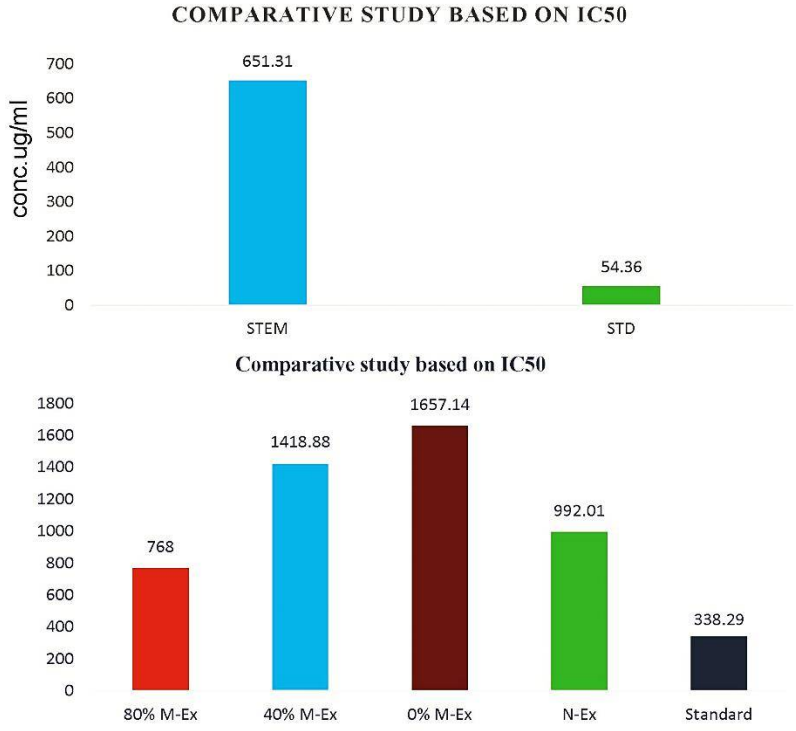

Figure 3 . Antidiabetic activity of the stem ex of *T. cordifolia* and the standard.

Wound Healing Activity

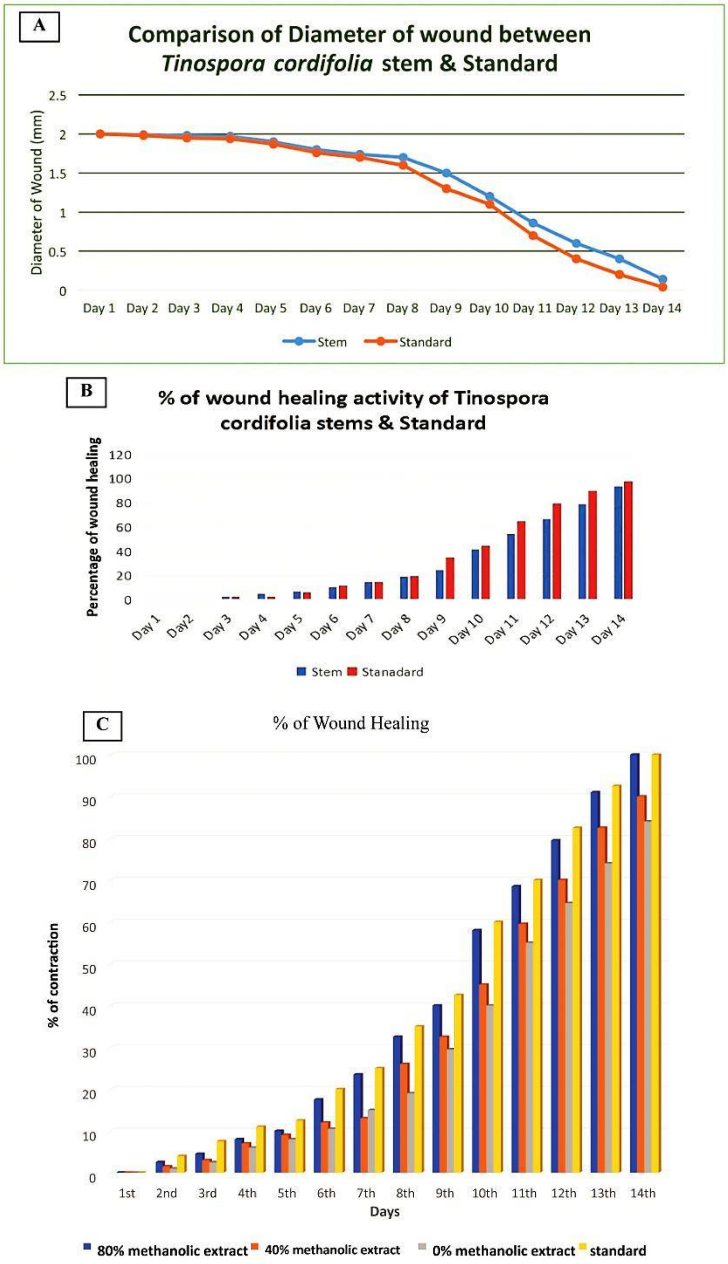

Figure 4. (A) M-Ex (B) N- Ex of *T.Cordifolia* were employed to examine (C) Various percentage of M-EX of *T.Cordifolia* examine wound healing activity.

Qualitative phytochemical screening revealed the presence of alkaloids, flavonoids, phenolics, tannins, terpenoids, steroids and carbohydrates in the stem extracts of *Tinospora cordifolia*. These secondary metabolites are well known for their diverse pharmacological activities, particularly antioxidant properties (Harborne, 1998; Evans, 2009). The antioxidant assays demonstrated that the 80% methanolic extract exhibited the strongest free radical scavenging activity among the tested extracts. This observation may be attributed to the enhanced extraction of phenolic and flavonoid compounds by methanol-rich solvents. The superior antioxidant activity of methanolic extracts is consistent with

previous reports indicating that phenolic constituents of *T. cordifolia* contribute significantly to its antioxidant potential (Singh *et al*., 2003; Upadhyay *et al*., 2010). In contrast, the n-hexane extract showed comparatively weak antioxidant activity, suggesting that non-polar fractions contain lower concentrations of antioxidant constituents.

The anti-inflammatory and antidiabetic investigations revealed distinct effects of solvent polarity on biological activity. The 80% methanolic extract showed the highest membrane stabilization activity, indicating the effective extraction of polar anti-inflammatory constituents such as flavonoids, tannins and phenolic compounds. These phytochemicals are known to inhibit inflammatory mediators and stabilize biological membranes (Shinde *et al*., 1999). Conversely, the n-hexane extract exhibited the strongest α-amylase inhibitory activity, suggesting the presence of lipophilic constituents capable of modulating carbohydrate metabolism. Although methanolic extracts also demonstrated α-amylase inhibition, the superior activity of the n-hexane fraction indicates that non-polar compounds may contribute substantially to the antidiabetic potential of *T. cordifolia.* Similar pharmacological properties of the plant have been reported previously (Singh *et al*., 2003; Upadhyay *et al*., 2010).

Wound-healing evaluation demonstrated that the 80% methanolic extract produced the highest percentage of wound contraction, achieving complete wound closure by day 14. This enhanced healing response may be associated with the combined antioxidant and anti-inflammatory effects of the extract, which promote fibroblast proliferation, collagen synthesis, angiogenesis and tissue remodeling (Guo and DiPietro, 2010). The findings indicate that solvent polarity plays a crucial role in determining the pharmacological profile of *T. cordifolia* stem extracts. Overall, the 80% methanolic extract showed superior antioxidant, anti-inflammatory and wound-healing activities, whereas the n-hexane extract demonstrated promising α-amylase inhibitory activity. These results support the traditional medicinal use of *T. cordifolia* and suggest that the stem may serve as a valuable source of bioactive compounds for the development of plant-based therapeutic agents.

Conclusion

This study demonstrates that solvent polarity and plant part selection markedly influence the pharmacological potential of *T. cordifolia*. The 80% ME stem extract showed superior antioxidant, anti-inflammatory, and wound-healing activities, likely due to its high polyphenol and flavonoid content. Conversely, the n-hexane stem extract exhibited notable α-amylase inhibitory activity, indicating the presence of non-polar constituents with antidiabetic potential.

Overall, these findings suggest that methanol-rich extracts are more suitable for managing oxidative stress, inflammation, and wound repair, whereas non-polar extracts may be beneficial for diabetes management. Further investigations should focus on isolating active constituents, clarifying their mechanisms of action, and developing standardized formulations to support the therapeutic application of *T. cordifolia*.

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