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# IN VITRO EVALUATION OF ANTIMICROBIAL ACTIVITY OF *TINOSPORA CORDIFOLIA* (GILOY) CREEPED ON *EUGENIA JAMBOLANA* (JAMUN TREE) AND *DALBERGIA SISSOO* (SESAM TREE) IN COMPARISON WITH ITS INDEPENDENT CREEPER ON FENCES

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**Abstract:** The antibacterial and antifungal activity of the aqueous, ethanol, methanol, dimethyl sulfoxide and acetone extracts from the stems of *Tinospora cordifolia* creeped on different trees and its independent creeper on fences was studied using disc diffusion method against *Bacillus subtilis*, *Enterobacter faecalis*, *Streptococcus mutans*, *Pseudomonas aeruginosa and Candida albicans***.** Results suggest that the stem extract has antibacterial activity against tested bacteria and fungus. The present study justifies the claimed uses of *Tinospora cordifolia* in the traditional system of medicine to treat various infectious diseases.

**Key words: Antimicrobial activity, *Tinospora cordifolia*, Inhibition zones, secondary metabolites, Infectious diseases.**

## I. INTRODUCTION

Plant-derived medicines have been part of traditional health care in most parts of the world for thousands for year. More than 80% of the population in developing countries depends on plants for their medical needs. In India, medical plants are widely used by all sections of people either directly as folk remedies or in different indigenous medicinal plants and their therapeutic values (Choudhary et al, 2014). All over the countries, plant and plant based antimicrobial agents were used against the microbial infection for ages. Plant-derived medicines are the major antimicrobial agents for 60 to 80% of populations in the developing countries (Ojiako, 2015). One of the plants known for having many medicinal uses in traditional system of medicine is *Tinospora cordifolia* (Wild) Hook & Thomson. *T. cordifolia* is one of the most widely used herb in Ayurveda, one of the ancient systems of medicine practiced in India. It belongs to Menispermaceae family and commonly called Guduchi in Sanskrit, Giloe in Hindi (Gupta et al, 2016). The plant is of great interest to researchers because of its medicinal properties like anti-bacterial, anti-fungal, anti-cancer, anti-periodic, anti-spasmodic, anti-stress, anti-inflammatory, anti-arthritis, anti-neoplastic, anti-diabetic activity (Sohamsaha, 2012). It is commonly used in traditional system of medicine for its rejuvenating properties. It contains various bioactive compounds including alkaloids, diterpenoid lactones, glycosides, steroids, sesquiterpenoids, phenolics, aliphatic compounds, and polysaccharides (Upadhyay, 2010 and Joshi et al, 2017). These compounds have been reported to have different biological roles in disease conditions thus enabling potential applications in clinical research. Its efficacy has been also recognized by the modern system of medicine. This plant has been known to possess anti-oxidant, anti-diabetic, anti-allergic, immunomodulatory, anti-inflammatory, anti-microbial, anti-cancer and several other properties (Anita, et al, 2013). Plants of economic interest in general and medicinal plants in particular are disappearing at an alarming rate due to various developmental activities. The scientists realize that the effective life span of any antibiotic is limited so new sources especially plant sources have to be investigated. Therefore, the plant *T. cordifolia* can be chosen as a source for the development of industrial products for treatment of various diseases (Preeti, 2011). Considering the uses of *Tinospora cordifolia* in traditional and modern system of medicine, we have proposed to work on this aspect to evaluate its antimicrobial activities.

## II. MATERIALS AND METHODS

### Collection of plant material

The plant material (stems) were collected from Adalhatu, Ranchi (Jharkhand) and identity was confirmed in the Post Graduate Department of Botany, Ranchi University, Ranchi. Three different types of stems were collected from three types of creepers of *Tinospora cordifolia* (*Tc* 1-*Tinospora cordifolia* creeped on *Eugenia jambolana* (Jamun tree), Tc 2-*Tinospora cordifolia* creeped on *Dalbergia sissoo* (Sesam tree) and Tc 3-Independent Creeper of *Tinospora cordifolia* on Fences. From the collected plant materials, Stems were separated and washed with sterile single distil water, dried in shade and crushed to coarse powder with mortar and pestle. Powdered stem were extracted with methanol (1gm powder/10 ml methanol) in shaker incubator for 48 hours. The extracts were then filtered and taken to dryness in front of the fan. These extracts were resuspended in different solvents such as water, ethanol, methanol, acetone and dimethyl sulfoxide (DMSO).

### Culture media and microorganisms

Nutrient Broth and Nutrient Agar medium manufactured by Hi media laboratories, Mumbai, India were used for the cultivation of bacteria. The laboratory microbial strains of test microbes were *Bacillus subtilis, Lactobacillus acidophilus, Enterococcus faecalis, Streptococcus mutans, Pseudomonas aeruginosa and Candida albicans* and from National Centre for Microbial Resource (NCMR), National Centre for Cell Science (NCCS), Pune.

### Sterilization of glassware's and culture media

Glassware's were sterilized in hot air oven at 160°C for 60 minutes. All the media were first prepared in conical flasks and then kept in an autoclave for sterilization at 15 psi pressure,121°C temperature for 15 minutes.

### Determination of zone of inhibition

The antimicrobial activity of the stem extracts of three different giloy creeper (creeped on *Eugenia jambolana* (Jamun tree), *Dalbergia sissoo* (Sesam tree) and independent creeper on fences) was tested in vitro using disc diffusion assay. A diluted 20 µl microbial culture of respective strains poured in sterile petri plates containing 10 ml of semisolid nutrient agar medium and spread over agar plates using sterile glass L-rod, five discs of each extracts in different solvent was applied per filter paper disc (Whatman no. 1) and was allowed to dry before being placed on to the top layer of the agar plates. The plates were incubated at 37°C for 24 hours. The experiments were carried out in triplicate. Plates were observed and the average diameter of zone of inhibitions was recorded.

### III. RESULTS AND DISCUSSION

Results of antimicrobial screening and evaluation of the stem extracts of *Tinospora cordifolia* creeped on different trees were measured in terms of zone of inhibition. The zones of inhibition recorded for ethanol, methanol, DMSO, acetone and aqueous are depicted in figure 1, figure 2 and figure 3 (a, b and c) and table 1. It is revealed that the ethanolic extract of TC 1 stem exhibited maximum zone of inhibition of 104.405 $mm^2$ while no zone of inhibition was observed with aqueous and acetone stem extract against *Candida albicans*. However, these stem extracts of Tc 1 with acetone showed maximum zone of inhibition of 826.605 $mm^2$ against *Lactobacillus acidophilus*. Stem extract of Independent creeper of *Tinospora cordifolia* (Tc3) with acetone showed no zone of inhibition against the same bacterium *Lactobacillus acidophilus.* Aqueous extract of stem of Tc 2 showed no zone of inhibition on *Candida albicans*, *Pseudomonas aeruginosa*, *Streptococcus mutans* and *Lactobacillus acidophilus* but extract with DMSO showed 104.405 $mm^2$ zone of inhibition against *Bacillus subtilis*. Tc 3 stem extract with acetone and ethanol exhibited 226.08 mm2 zone of inhibition against *Streptococcus* mutans and *Lactobacillus acidophilus* respectively. From the results it dictates that the moderate or greater activity resides in ethanolic stem extracts of plant. Extract with aqueous and acetone did not effectively inhibit the growth of the bacteria or very less antibacterial activity. This may be due to the bioactive constituents present in the plant responsible for the antibacterial activity are more soluble in ethanol extracts. It can be interpreted that the antifungal and antibacterial activity against microorganisms is due to any one or more bioactive compounds of the plants.

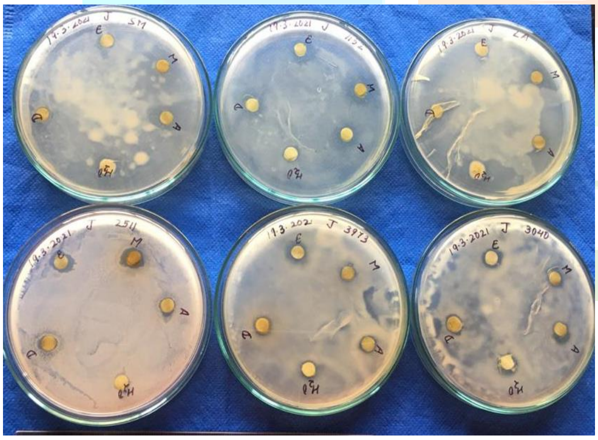

**Figure 1**

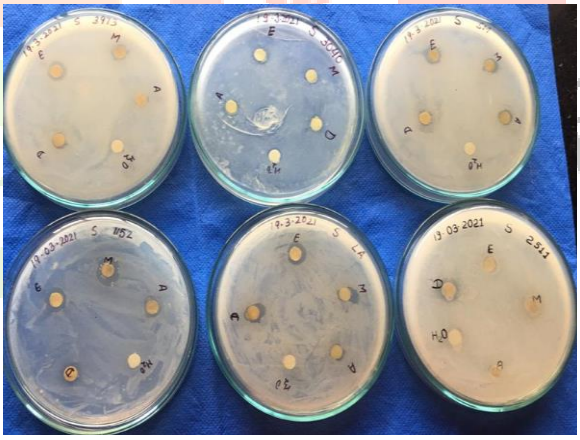

**Figure 2**

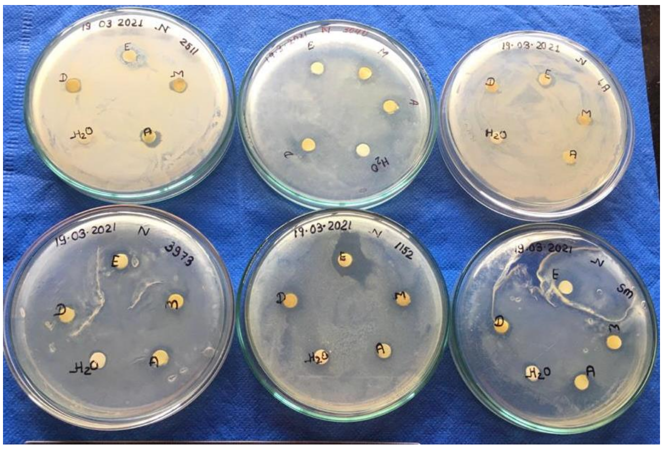

**Figure 3**

**Figure 1.** Zone of inhibition of **Tc1** (*Tinospora cordifolia* creeped on *Eugenia jambolana* (Jamun tree) stem extract in different solvents on different species of bacteria and fungi (*Bacillus subtilis*, *Enterobacter faecalis*, *Streptococcus mutans*, *Lactobacillus acidophilus*, *Pseudomonas aeruginosa* and *Candida albicans*).

**Figure 2.** Zone of inhibition of **Tc2** (*Tinospora cordifolia* creeped on *Dalbergia sissoo* (Sesam tree) stem extract in different solvents on different species of bacteria and fungi (*Bacillus subtilis*, *Enterobacter faecalis*, *Streptococcus mutans*, *Lactobacillus acidophilus*, *Pseudomonas aeruginosa* and *Candida albicans*).

**Figure 3.** Zone of inhibition of **Tc3** (Independent creeper of *Tinospora cordifolia* on fences) stem extract in different solvents on different species of bacteria and fungi (*Bacillus subtilis*, *Enterobacter faecalis*, *Streptococcus mutans*, *Lactobacillus acidophilus*, *Pseudomonas aeruginosa* and *Candida albicans*).

**Table 1.** Zone of inhibition of creepers of *Tinospora cordifolia* (on different trees) stem extract in different solvents against different species of bacteria and fungi (*Bacillus subtilis*, *Enterobacter faecalis*, *Streptococcus mutans*, *Lactobacillus acidophilus*, *Pseudomonas auruginosa* and *Candida albicans*).

| **Tc1 stem extract's inhibition on *Candida albicans* in different solvents** | | | | | |
|---|---|---|---|---|---|
| **Solvents** | **r1 (mm)** | **Area of disc (mm2)** | **r2 (mm)** | **Area of inhibition (mm2)** | **Zone of inhibition (mm2)** |
| Water | 3 | 28.26 | 0 | 0 | 0 |
| Methanol | 3 | 28.26 | 6 | 113.04 | 84.78 |
| Acetone | 3 | 28.26 | 0 | 0 | 0 |
| Ethanol | 3 | 28.26 | 6.5 | 132.665 | 104.405 |
| DMSO | 3 | 28.26 | 4.5 | 63.585 | 35.325 |
| **Tc1 stem extract's inhibition on *Bacillus subtilis* in different solvents** | | | | | |
| Water | 3 | 28.26 | 3.5 | 38.465 | 10.205 |
| Methanol | 3 | 28.26 | 5 | 78.5 | 50.24 |
| Acetone | 3 | 28.26 | 5 | 78.5 | 50.24 |
| Ethanol | 3 | 28.26 | 6 | 113.04 | 84.78 |
| DMSO | 3 | 28.26 | 8 | 200.96 | 172.7 |
| **Tc1 stem extract's inhibition on *Enterococcus faecalis* in different solvents** | | | | | |
| Water | 3 | 28.26 | 4 | 50.24 | 21.98 |
| Methanol | 3 | 28.26 | 4.5 | 63.585 | 35.325 |
| Acetone | 3 | 28.26 | 5 | 78.5 | 50.24 |
| Ethanol | 3 | 28.26 | 6.5 | 132.665 | 104.405 |
| DMSO | 3 | 28.26 | 4.5 | 63.585 | 35.325 |
| **Tc1 stem extract's inhibition on *Pseudomonas aeruginosa* in different solvents** | | | | | |
| Water | 3 | 28.26 | 3.5 | 38.465 | 10.205 |
| Methanol | 3 | 28.26 | 4 | 50.24 | 21.98 |
| Acetone | 3 | 28.26 | 4 | 50.24 | 21.98 |
| Ethanol | 3 | 28.26 | 6 | 113.04 | 84.78 |
| DMSO | 3 | 28.26 | 4.5 | 63.585 | 35.325 |
| **Tc1 stem extract's inhibition on *Streptococcus mutans* in different solvents** | | | | | |
| Water | 3 | 28.26 | 9 | 254.34 | 226.08 |
| Methanol | 3 | 28.26 | 10 | 314 | 285.74 |
| Acetone | 3 | 28.26 | 10.5 | 346.185 | 317.925 |
| Ethanol | 3 | 28.26 | 5 | 78.5 | 50.24 |
| DMSO | 3 | 28.26 | 0 | 0 | 0 |
| **Tc1 stem extract's inhibition on *Lactobacillus acidophilus* in different solvents** | | | | | |
| Water | 3 | 28.26 | 15.5 | 754.385 | 726.125 |
| Methanol | 3 | 28.26 | 10 | 314 | 285.74 |
| Acetone | 3 | 28.26 | 16.5 | 854.865 | 826.605 |
| Ethanol | 3 | 28.26 | 8.5 | 226.865 | 198.605 |
| DMSO | 3 | 28.26 | 11 | 379.94 | 351.68 |
| **Tc3 stem extract's inhibition on *Candida albicans* in different solvents** | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| Water | 3 | 28.26 | 5 | 78.5 | 50.24 |
| Methanol | 3 | 28.26 | 6.5 | 132.665 | 104.405 |
| Acetone | 3 | 28.26 | 4.5 | 63.585 | 35.325 |
| Ethanol | 3 | 28.26 | 7.5 | 176.625 | 148.365 |
| DMSO | 3 | 28.26 | 5 | 78.5 | 50.24 |
| **Tc3 stem extract's inhibition on *Bacillus subtilis* in different solvents** | | | | | |
| Water | 3 | 28.26 | 0 | 0 | 0 |
| Methanol | 3 | 28.26 | 4.5 | 63.585 | 35.325 |
| Acetone | 3 | 28.26 | 5.5 | 94.985 | 66.725 |
| Ethanol | 3 | 28.26 | 7 | 153.86 | 125.6 |
| DMSO | 3 | 28.26 | 3.5 | 38.465 | 10.205 |
| **Tc3 stem extract's inhibition on *Enterococcus faecalis* in different solvents** | | | | | |
| Water | 3 | 28.26 | 0 | 0 | 0 |
| Methanol | 3 | 28.26 | 8 | 200.96 | 172.7 |
| Acetone | 3 | 28.26 | 7.5 | 176.625 | 148.365 |
| Ethanol | 3 | 28.26 | 6 | 113.04 | 84.78 |
| DMSO | 3 | 28.26 | 6.5 | 132.665 | 104.405 |
| **Tc3 stem extract's inhibition on *Pseudomonas aeruginosa* in different solvents** | | | | | |
| Water | 3 | 28.26 | 4 | 50.24 | 21.98 |
| Methanol | 3 | 28.26 | 5 | 78.5 | 50.24 |
| Acetone | 3 | 28.26 | 5.5 | 94.985 | 66.725 |
| Ethanol | 3 | 28.26 | 5 | 78.5 | 50.24 |
| DMSO | 3 | 28.26 | 5 | 78.5 | 50.24 |
| **Tc3 stem extract's inhibition on *Streptococcus mutans* in different solvents** | | | | | |
| Water | 3 | 28.26 | 4 | 50.24 | 21.98 |
| Methanol | 3 | 28.26 | 6 | 113.04 | 84.78 |
| Acetone | 3 | 28.26 | 9 | 254.34 | 226.08 |
| Ethanol | 3 | 28.26 | 7 | 153.86 | 125.6 |
| DMSO | 3 | 28.26 | 6 | 113.04 | 84.78 |
| **Tc3 stem extract's inhibition on *Lactobacillus acidophilus* in different solvents** | | | | | |
| Water | 3 | 28.26 | 0 | 0 | 0 |
| Methanol | 3 | 28.26 | 4 | 50.24 | 21.98 |
| Acetone | 3 | 28.26 | 0 | 0 | 0 |
| Ethanol | 3 | 28.26 | 9 | 254.34 | 226.08 |
| DMSO | 3 | 28.26 | 0 | 0 | 0 |
| **Tc2 stem extract's inhibition on *Candida albicans* in different solvents** | | | | | |
| Water | 3 | 28.26 | 0 | 0 | 0 |
| Methanol | 3 | 28.26 | 6.5 | 132.665 | 104.405 |
| Acetone | 3 | 28.26 | 5 | 78.5 | 50.24 |
| Ethanol | 3 | 28.26 | 5 | 78.5 | 50.24 |
| DMSO | 3 | 28.26 | 4.5 | 63.585 | 35.325 |
| **Tc2 stem extract's inhibition on *Bacillus subtilis* in different solvents** | | | | | |
| Water | 3 | 28.26 | 0 | 0 | 0 |
| Methanol | 3 | 28.26 | 0 | 0 | 0 |
| Acetone | 3 | 28.26 | 0 | 0 | 0 |
| Ethanol | 3 | 28.26 | 0 | 0 | 0 |
| DMSO | 3 | 28.26 | 6.5 | 132.665 | 104.405 |
| **Tc2 stem extract's inhibition on *Enterococcus faecalis* in different solvents** | | | | | |
| Water | 3 | 28.26 | 3.5 | 38.465 | 10.205 |
| Methanol | 3 | 28.26 | 4 | 50.24 | 21.98 |
| Acetone | 3 | 28.26 | 4 | 50.24 | 21.98 |
| Ethanol | 3 | 28.26 | 4.5 | 63.585 | 35.325 |

| | | | | | |
|---|---|---|---|---|---|
| DMSO | 3 | 28.26 | 4 | 50.24 | 21.98 |
| **Tc2 stem extract's inhibition on *Pseudomonas aeruginosa* in different solvents** | | | | | |
| Water | 3 | 28.26 | 0 | 0 | 0 |
| Methanol | 3 | 28.26 | 4.5 | 63.585 | 35.325 |
| Acetone | 3 | 28.26 | 0 | 0 | 0 |
| Ethanol | 3 | 28.26 | 4.5 | 63.585 | 35.325 |
| DMSO | 3 | 28.26 | 4 | 50.24 | 21.98 |
| **Tc2 stem extract's inhibition on *Streptococcus mutans* in different solvents** | | | | | |
| Water | 3 | 28.26 | 0 | 0 | 0 |
| Methanol | 3 | 28.26 | 4.5 | 63.585 | 35.325 |
| Acetone | 3 | 28.26 | 3.5 | 38.465 | 10.205 |
| Ethanol | 3 | 28.26 | 4 | 50.24 | 21.98 |
| DMSO | 3 | 28.26 | 0 | 0 | 0 |
| **Tc2 stem extract's inhibition on *Lactobacillus acidophilus* in different solvents** | | | | | |
| Water | 3 | 28.26 | 0 | 0 | 0 |
| Methanol | 3 | 28.26 | 5 | 78.5 | 50.24 |
| Acetone | 3 | 28.26 | 3.5 | 38.465 | 10.205 |
| Ethanol | 3 | 28.26 | 4.5 | 63.585 | 35.325 |
| DMSO | 3 | 28.26 | 5 | 78.5 | 50.24 |

Tc 1- *Tinospora cordifolia* creeped on *Eugenia jambolana* (Jamun tree).
Tc 2- *Tinospora cordifolia* creeped on *Dalbergia sissoo* (Sesam tree).
Tc 3- Independent creeper of *Tinospora cordifolia* on Fences.

Phytochemicals are biologically active compound found in plants. Naturally they help plants to protect against microbes and these important properties very beneficial for future drug discovery (Banu et al, 2015; Aziz et al, 2014 and Bansal et al, 2012). Antibacterial assay was performed with aqueous, methanolic, ethanolic, chloroform, hexane and acetone extract (Bhalodia et al, 2011; Sen et al, 2012; Boligon et al, 2014; Shah et al, 2014 and Karimi et al, 2015). The outcome found from this experiment is just a uneven estimate in finding the medicinal property in terms of antimicrobial activity of the plant. The phytochemical screening of *T. cordifoila* stem revealed the presence of phenols, flavanoids, alkaloids and saponins. This study concluded that the stem of this plant can used in the pharmacological industries (Karimi et al, 2013). Which may reason for the antimicrobial property of this agent. Comparable results have been observed in earlier studies where this plant extract has shown promising results against the pathogens used in our study (Bansal et al, 2012; Duraipandiyan et al, 2012 and Upadhyay et al, 2011).

**IV. CONCLUSION**

Present findings support the applicability of *Tinospora cordifolia* in traditional systems for its claimed uses like urinary, fever inflammations and skin diseases. The antibacterial activity has been attributed to the presence of some active constituents in the extracts. Data and findings of the proposed work dictates that *Tinospora cordifolia* creeped on *Eugenia jambolana* (Jamun tree), Tc1 has more antibacterial activity against *Lactobacillus acidophilus* and *Streptococcus mutans* as compared to its independent creeper on fences. This may be due to incorporation of medicinal value (antibacterial activity) of *Eugenia jambolana* when Giloy climbs or creeps on it. Further study and research work is required for isolation, purification and quantitative estimation of specific bioactive compounds in *Tinospora cordifolia* extracts (creeped on different trees), which will authenticate and thus allow the researchers and scientists to work with and recommend their utilization. It is hoped that this work would lead to the establishment of some compounds showing their efficacy as a result of synergy effect that could be used to formulate new and more potent antimicrobial drugs of natural origin.

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