



EXTRACTION OF DIFFERENT PARTS OF “*TINOSPORA CORDIFOLIA*” PLANT AND BIOLOGICAL EVALUATION FOR ANTIBACTERIAL ACTIVITY

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ABSTRACT :

In present study, extraction and isolation of the different parts of *Tinospora cordifolia* such as stem, leaves and fruit has been done by Soxhlet method. The antimicrobial activity of methanolic extracts of leaves, stems and fruits of *Tinospora cordifolia* were studied against *Escherichia coli* and *Staphylococcus aureus* by agar well diffusion method showing considerable zones of inhibition. The Antimicrobial properties of various extracts of *Tinospora cordifolia*, were found efficacious as an antimicrobial agent.

KEYWORDS : *Tinospora cordifolia*, stem extract, leaves extract, fruit extract, and antibacterial activity

INTRODUCTION :

The World Health Organization reported that 80% of the world population relies chiefly on traditional medicines involving the use of plant extracts or their active constituents. India with its mega-biodiversity and knowledge of rich ancient traditional systems of medicine (*Ayurveda*, *Siddha*, *Unani*, *Amchi* and local health traditions) provide a strong base for the utilization of a large number of plants in general healthcare and alleviation of common ailments of the people [1].

Tinospora cordifolia known as *Gulvel* or *Guduchi*, has been an extensively used and investigated plant from family *Menispermaceae* for its varied activities. It is a deciduous, fleshy, robust climber growing with support of mango or neem trees, and is also known as *Cocculus cordifolius* Dec, *Menispermum cordifolium* Wild. and *Tinospora glabra* (N.Br.) Merr [2]. “Giloya” the Hindi name of the plant refers in Hindu mythology to a heavenly elixir used to stay off the aging and to stay young forever. The Sanskrit name ‘*Guduchi*’ means that protects from illnesses. Hence the words ‘rejuvenator’ or adaptogen seem to have appeared in literature [3]. It is found in India, China, Myanmar, Sri Lanka, Thailand, Philippines, Indonesia, Malaysia, Borneo, Vietnam, Bangladesh, North Africa, West Africa, and South Africa. In India, it is abundantly found in Maharashtra, Gujarat, Madhya Pradesh, Himachal Pradesh, and some other states in North and South India [4,5,6,7].

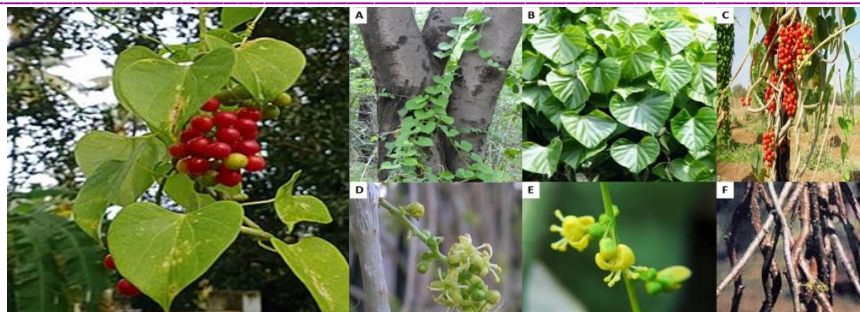


Fig. 1 Morphology of different parts of *Tinospora cordifolia* A. Stem, B. Leaves, C. Fruit, D. Inflorescence, E. Flower, F. Aerial roots

It thrives well in the tropical region, often attains a great height, and climbs up the trunks of large trees. The stem is grey or creamy white, deeply cleft spirally and longitudinally, with the space between spotted with large rosette-like lenticels. The wood is white, soft, and porous, and the freshly cut surface quickly assumes a yellow tint when exposed to air. Leaves are simple, alternate, exstipulate, long petiolate, chordate in shape showing multicoated reticulate venation. Long thread like aerial roots comes up from the branches. Flowers are small and Unisexual. Male flowers are in clusters female flower are solitary. Six sepals arranged in two whorls of three each. Six petals arranged in two whorls, they are obovate and membranous. Aggregate fruit is red, fleshy, with many drupelets on thick stalk with sub terminal style scars, scarlet coloured [8]. *Tinospora cordifolia* is an important drug of Indian systems of medicine and used in medicines since times immemorial. The drug is well known Indian bitter and prescribed in fevers, diabetes, dyspepsia, jaundice, urinary problems, skin diseases and chronic diarrhoea and dysentery. It has been also indicated useful in the treatment of heart disease, leprosy, and helminthiasis. In crude form, it is available in market mostly as dried stem pieces. The starch obtained from the stem is highly nutritive and digestive and used in many diseases [9].

Tinospora cordifolia is a well-known medicinal plant in traditional medicinal system and recent scientific studies have emphasized the possible use of *Tinospora cordifolia* in modern medicine. The rise of diseases caused by MDR (multidrug - resistant) pathogens has been noted as world's most prevalent deaths [10], contributing around 68% of fatalities in the year 2012 [11]. Several pathogens are resistant to synthetic drugs, that has become a significant concern for health infrastructure facilities worldwide; hence, an alternate treatment is required [12]. Journal of Pharmacognosy and Phytochemistry has made Comprehensive phytochemical, pharmacological, and clinical research have all been conducted on the drug, with several intriguing results reported [13-15].

Lots of researchers have researched antimicrobial activity of other plant parts of Giloy, Neem [16], and Tulsi [17,18], which are used to treat numerous infectious illnesses in folk medicines, but unfortunately very limited research has been carried on antimicrobial activities of *Tinospora cordifolia*. To show the efficacy of ayurvedic medicines, the current study examined the antibacterial screening of *Tinospora cordifolia*, leaves, stems and fruits against human microbial infections.

2. MATERIALS AND METHODS

2.1 Collection of sample

The sample was collected from Kandalgaon near Solapur district, Maharashtra in the summer and the authentication of the sample is done by Department of Botany, D. B. F. Dayanand College of Arts and Science, Solapur, Maharashtra.

2.2 Preparation of sample

The collected samples of stem, leaves and fruit were dried under the shade at the room temperature. The processed samples were pulverized using electric grinder. The samples were extracted independently using 100gm of selected solvents like methyl alcohol in the order of highest

extraction yield. The residues were recovered after the extracting with the solvents from a rotary evaporator, after that obtained extracts were suspended in the appropriate solvent for investigation.

2.3 Production of extracts

In method of solvent extraction 30 g of dried powder of stem, leaves and fruit from *Tinospora cordifolia*, were separately extracted for 48 hours with methanol using a Soxhlet device. The extracts were filtered using Whatman filter paper No.1 and utilized to determine antibacterial activity.

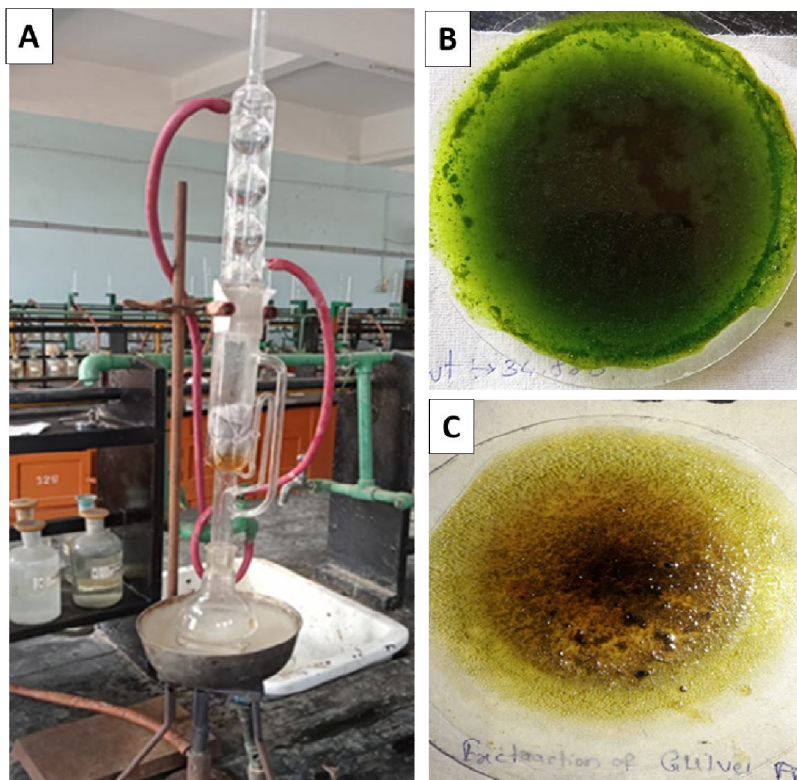


Fig. 2 A. Soxlet extraction, B. Stem extract, C. Fruit extract

2.4 Antibacterial activity

Table 1: Bacterial cultures used in study (Department of Microbiology, D. B. F. Dayanand College of Arts and Science, Solapur, Maharashtra)

Sr No.	Bacterial Pathogens	MTCC Number
1	<i>Escherichia coli</i>	ATCC 8739
2	<i>Staphylococcus aureus</i>	ATCC 6538

2.4.1 Preparation of nutrient broth slants and sub - culturing of microorganisms

Agar 1gm, beef extract 500 mg, peptone 500 mg, and NaCl 250 mg were used to make nutrient agar medium and is dissolved in 50ml distilled water, boiled and then placed inside the test tubes, which was then closed with cotton plug and autoclaved at 15 pounds pressure for fifteen minutes. The test tubes which were containing the agar nutritional medium were placed in an inclined position for 30 minutes following sterilization. Thereafter, in an aseptic setting, pure cultures of *E. coli* (ATCC 8739) and *S. aureus* (ATCC 6538), were streaked over the surface of slants and the petridishes were incubated at 37^o C for 24 hours.

2.4.2 Production of growth medium for antibacterial sensitivity test:

20 g Agar, beef extract 10 g, peptone 10 g, and NaCl 5 g were mixed together and in 1000 ml boiling distilled water to create nutrient agar medium (pH 7.2). After that it was autoclaved in an autoclave at 15 pounds of pressure (121 degrees Celsius) for exact 15 minutes. Following sterilization, the medium was allowed to cool to 45°C before being placed into sterile Petri plates in a sterile manner, an amount of 20 - 25 ml of media poured into each petri plate. Medium from the petriplate was then kept aside to solidify at room temperature.

2.4.3 Inoculation of suspension of microbes on agar medium:

Sterilized, cotton plugs were dipped in to each standardized isolates (turbidity is adjusted so as to get consistent growth on the Petri plates) accompanied by whole petriplate surfaces were streaked with the swab three times exactly, the plates were rotated at 60° angle during streaking. After that the inoculums were dried for 1 - 5 min while covering during entire process. Then bore was punched on the prepared plates by using sterile well (8mm). The 100 l dose of standard medicine Ciprofloxacin was loaded in each bore accordingly in sterile conditions using a sterile micropipette. Plates were kept at an ambient temperature for at least 30 min and then cultured at 37°C for at least 24 hours. The diameters of the zones of inhibition were calculated with scale in millimeters.

3. RESULTS AND DISCUSSION

The medicinal herb Giloy, has been used historically for the treatment of inflammatory conditions, healing of the wounds, to prevent spread of the infection, inflammation of stomach and treatment of Diabetes mellitus. The antimicrobial actions are being attributed to several active components in the extracts. *Tinospora cordifolia* showed strong antimicrobial actions against tested microbes. The whole climber has been evaluated for scientific tests exhaustively and a variety of biomolecules that belong to various families have been confirmed from plant including lignans, flavones, terpenoids, alkaloids, cardiac glycosides etc. (Bansal *et al.*, 2012) that may be responsible for the antimicrobial property of this drug

Methanolic extract of leaves, stems and fruits of *Tinospora cordifolia* had shown antimicrobial activity against *E. coli* (with zone of inhibition 15 mm, 22 mm, 25 mm respectively). Maximum antimicrobial activity against *E.coli* was exhibited by fruit extract of *Tinospora cordifolia* (with zone of inhibition-25 mm).

Methanolic extract of leaves, stems and fruits of *Tinospora cordifolia* had shown antimicrobial activity against *Staphylococcus aureus* (with zone of inhibition 13 mm, 18 mm, 14 mm respectively). Maximum antimicrobial activity against *Staphylococcus aureus* was exhibited by stem extract of *Tinospora cordifolia* (with zone of inhibition-18 mm)



Fig. 3 Zones of inhibition

Table 2 Zone of inhibition for *Escherichia coli* and *Staphylococcus aureus*

Sr. No.	Name of Microorganism	Diameter of zone of inhibition (mm)		
		Stem	Leaves	Fruit
1	<i>Escherichia coli</i>	22	15	25
2	<i>Staphylococcus aureus</i>	18	13	14
3	Negative Control (Methanol)	—	—	—
4	Positive Control	<i>E. coli</i>	21	
	Ciprofloxacin	<i>S. aureus</i>	18	

4. CONCLUSION

Methanol has stronger extraction capacity which could be helpful in extracting greater no of active constituents responsible for antibacterial activity. Our findings prove that, the leaves, stem and fruits of *Tinospora cordifolia* plant have medicinal antimicrobial activities and can use against microorganism under study.

The results obtained in this study are promising which can be employed in complicated diabetic wound healing where single drug often prone to resistance. As per findings obtained in this research, it advised that the historical medicinal use of *Tinospora cordifolia*, be carried on and more scientific studies to be carried out. Such more focused studies on molecular entities found in the plant may help to create more efficacious synthetic as well as semisynthetic medicines.

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