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Research Article

Comparative antimicrobial efficacy of ayurvedic formulations; Satva and Ghana of *Tinospora cordifolia* (Willd.) Miers (*Giloy*) against commensals and opportunistic pathogens

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Abstract

In our classical Ayurvedic texts, it has been described as beneficial in treating various types of diseases. *Tinospora cordifolia* satva (TCS) *and Tinospora cordifolia* ghana (TCG) is one of the unique Ayuvedic classical preparations, prepared from aqueous of extract of *Giloy* (*Tinospora cordifolia* Miers.) stem. These are frequently used medicines for the treatment of Madhumeha, Pandu, Kamala, Amlapitta, Grahani, Kustha, Jirna Jwara and Viswamjwara, Trishna, Shool, Yakritavikara, etc. Therefore, an attempt was made in this study to evaluate antimicrobial efficacy of these two dosage forms i.e. TCS and TCG. *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae* and *Propionibacterium acnes* were used for antimicrobial evaluation and the classical method was used for the testing. The results shows that TCS can be considered as comparatively more potent antimicrobial than that of TCG at lower concentration on studied strains of *E. coli*, *P. acnes.*

Keywords: *Guduchi satva*, *Guduchi ghana*, *Giloy*, *Tinospora cordifolia,* anti-microbial activity, commensal pathogens

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INTRODUCTION

Tinospora cordifolia is a plant also known as Giloy, having heart-shaped leave, a herb of the family Menispermaceae, native of warm regions of the Indian subcontinent. It is the need of hour to show the effectiveness of the drug in a disease by laboratory findings. Antimicrobial studies are the easy tool for assess the potency of Ayurvedic medicines on various pathological organisms. Treatment of bacterial infections is a frequent problem due to the emergence of bacterial strains resistant to many antibiotics [1]. In traditional and folklore, it is commonly used for fever, skin ailments, and infectious disorders. Its safety and nontoxic nature have been reported in experimental and clinical studies on various body systems [4]. Several recent reports explored the potent antimicrobial roles of Guduchi and its various extracts [5-11]. Both S*atva* and *Ghana* of *Giloy* are used in topical herbal preparations for acne management. However, there is no published reports are available so far on comparative antimicrobial profile of *Tinospora cordifolia* satva (TCS) and *Tinospora cordifolia* Ghana (TCG). Considering this, the present study was undertaken to evaluate their comparative antimicrobial efficacy.

Considering this fact here, we have designed this study to analyze the differential yield of each formulation and anti-bacterial activity against some common commensal and opportunistic organisms present in human body.

MATERIAL AND METHODS:

Plan Collection and Authentication

Fresh Giloy or guduchi plant stem spreading over neem tree (*Azadirachta indica*) was collected from herbal garden of the AIIMS, Bhopal. Plant was identified in AYUSH department from same institute and the plant material was deposited in departmental store for future reference *(Reference No.: HERB/AYUSH/AIIMSBPL/2021/01)* Stem was separated from other parts like leaves, roots etc. and cleaned under running water.

Preparation of *Tinospora cordifolia Satva* (TCS) and Ghana (TCG)

The preparation of TCS and TCG was done as per the methods adopted by Sharma R and Prajapati PK [9, 10]. Firstly the stem of the plant was washed and dried under shade. Stem was properly peeled off its outer covering with the help of knife. Further, the small 8 to 10 cm long pieces were cut by knife. Material was divided into two parts and weighted before further processing. These pieces of both the parts were pounded in mortar and pestle to macerate plant material.

Formulation of *Tinospora cordifolia Satva* (TCS)

The above macerated bolus was soaked into six times water soaked stainless steel vessel for whole night (approximately 12 hours). Next morning, the soaked material was manually

crushed vigorously by hand for about one hour and filtered through four fold clean muslin cloth very gently. This liquid was again kept for whole night undisturbed. This supernatant liquid was further decanted cautiously and thick starchy layer of sediment was found settled at the bottom of vessel. This material was kept in shade and allowed to air dried. After about 10 days the dry material was scratched from bottom with the help of knife. Further, it was crushed in mortar and pestle to obtain dry powder. It was weighted and stored in airtight glass jars.

Formulation of *Tinospora cordifolia Ghana* (TCG)

Second part of macerated plant material was boiled with four times water on hot plate with continuous stirring. The concoction was boiled till half of the water evaporated. This concoction was filtered in similar manner and kept for sedimentation overnight. Supernatant liquid was decanted and settled material was allowed to dry for approximately 13 days and dry material was obtained and stored in similar manner. (Fig. 1)

Figure 1: Preparation of Satva and Ghana

Bacterial strains and culture conditions

Test micro-organism used bacteria *Enterococcus faecalis* (MTCC No. 439), *Escherichia coli* (MTCC No.452), *Klebsiella pneumonia* (MTCC No. 618) and *Propionibacterium acnes* (MTCC No.1951) were procured from MTCC Chandigarh. Antimicrobial study was carried out in Scan Research Laboratory, Indrapuri A sector, Piplani, Bhopal, Madhya Pradesh.

Antimicrobial activity using well diffusion assay

Well diffusion assay is the most common method used routinely for determination of antibiotic sensitivity of bacteria isolated from clinical specimens. It provides qualitative or semi qualitative information on the susceptibility of a given microorganism to a given antimicrobial drug.

For well diffusion assay cell count of bacterial suspension were maintained at 1×10^8 CFU/ml. Nutrient agar media (NAM) for *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumonia* and selective Blood Agar Media (BAM) for *Propionibacterium acnes* was prepared and sterilized by autoclaving at 121°C, 15 *psi* pressure for 15 minutes. The medium was cooled to 45-50°C and poured in pre sterilized Petri-plate and allowed to solidify. 0.1 ml of each bacterial suspension was spread over the solidified agar medium with the help of sterilized glass spreader and allowed to dry for few minutes. After inoculation small wells were punched in solidified gel with the help of sterile cork borer. These wells were loaded with the sample in the concentration of 100, 50 and 25 mg per ml and incubated for 18 hours at 37°C. After incubation each plate was observed for zone of inhibition and diameter of zones were measured in mm [11].

RESULTS AND DISCUSSION

Organoleptic characteristics of TCS and TCG were shown in table 1. The results obtained in the study are depicted in Table 3 to 4 which show the growth inhibition produced by TCS and TCG on 4 species of bacteria at various concentrations. Sample was not showed any inhibition against *E. faecalis K. pneumonia*. The activities can be referred as either less, moderate or highly active based on the zone of inhibition that ranges from 8- 11mm, 7-9 mm, against *E. coli* and *P. acnes* respectively. It is evident from Table 3 and 4 that formulation TCS can be considered as comparatively more potent antimicrobial than that of TCG at lower concentration on studied strains of *E. coli*, *P. acnes.* Upon analysis of Tables 4 it is found that, TCS required less concentration for inhibition of microorganisms of studied strains to grow as compare to TCG. Hence it is supposed to be comparatively more potent as that of TCS. The results show the good antimicrobial activity of Ampicillin at low concentration against various pathogens.

Table 1: Organoleptic character of TCS and TCG

S. No.	Parameter	*TCS*	*TCG*
1	**Color**	Off white	Brown
2	**Appearance**	Solid powder	Cheesy soft muddy
3	**Smell/odor**	Non Specific	Pungent
4	**Touch**	Soft+	Soft++
5	**Taste**	Sour+	Sour++

Table 2: Process Validation for TCS and TCG

Parameters	***TCS***	***TCG***
Amount. of Green stem (gram) – 1 part	0.462 gm	0.544 gm
Quantity of Water	6 parts	4 parts
Total time taken	10 days	13 days
Total yield	9.147 gm	7.289 gm
% of Yield	1.98%	1.34%

Table 3: Results of sensitivity of samples

S. No.	**Microbes strains**	**Sample-1**	**Sample-2**
1.	*Enterococcus faecalis*	No	No
2.	*Klebsiella pneumoniae*	No	No
3.	*Escherichia coli*	Yes	Yes
4.	*Propionibacterium acnes*	Yes	Yes

Table 4: Antimicrobial activity of samples against selected microbes

S. No.	**Name of microbes**	**Zone of inhibition (mm)**		
		TCS (Sample 1)		
		25mg/ml	**50 mg/ml**	**100mg/ml**
1.	*Escherichia coli*	8.00±0.47	9.00±0.47	11.00±0.47
2.	*Propionibacterium acnes*	7.00±0.47	8.00±0.47	9.00±0.47
		TCG (Sample 2)		
1.	*Escherichia coli*	7.00±0.47	9.00±0.47	10.00±0.47
2.	*Propionibacterium acnes*	6.00±0.5	7.00±0.5	8.00±0.47

*Average of three determination, Mean ±SD

Table 5: Antimicrobial activity of Ampicillin (Standard Drug) against selected microbes

S. No.	**Name of microbes**	**Zone of inhibition (mm)**
		Ampicillin (10µg/ml)
1.	*Enterococcus faecalis*	15.10±0.543
2.	*Klebsiella pneumoniae*	12.78±0.211
1.	*Escherichia coli*	13.55±0.320
2.	*Propionibacterium acnes*	18.21±0.121

Figure 2: Antimicrobial activity of TCS and TCG against *Enterococcus faecalis*

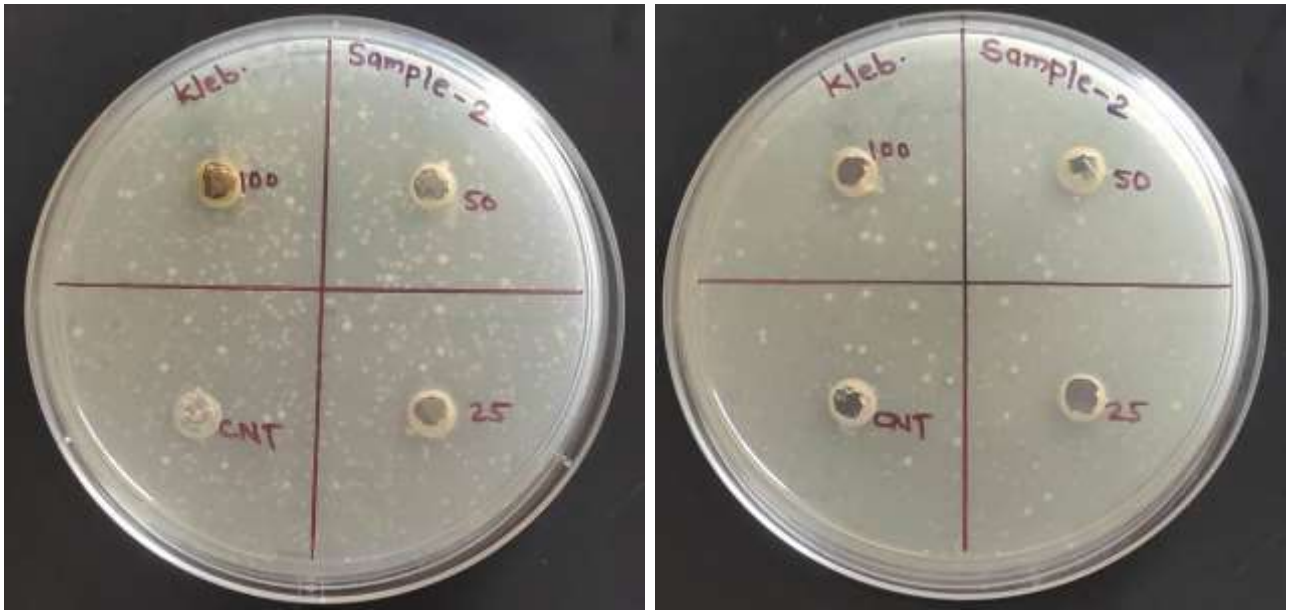


Figure 3: Antimicrobial activity of TCS and TCG against *Klebsiella pneumoniae*

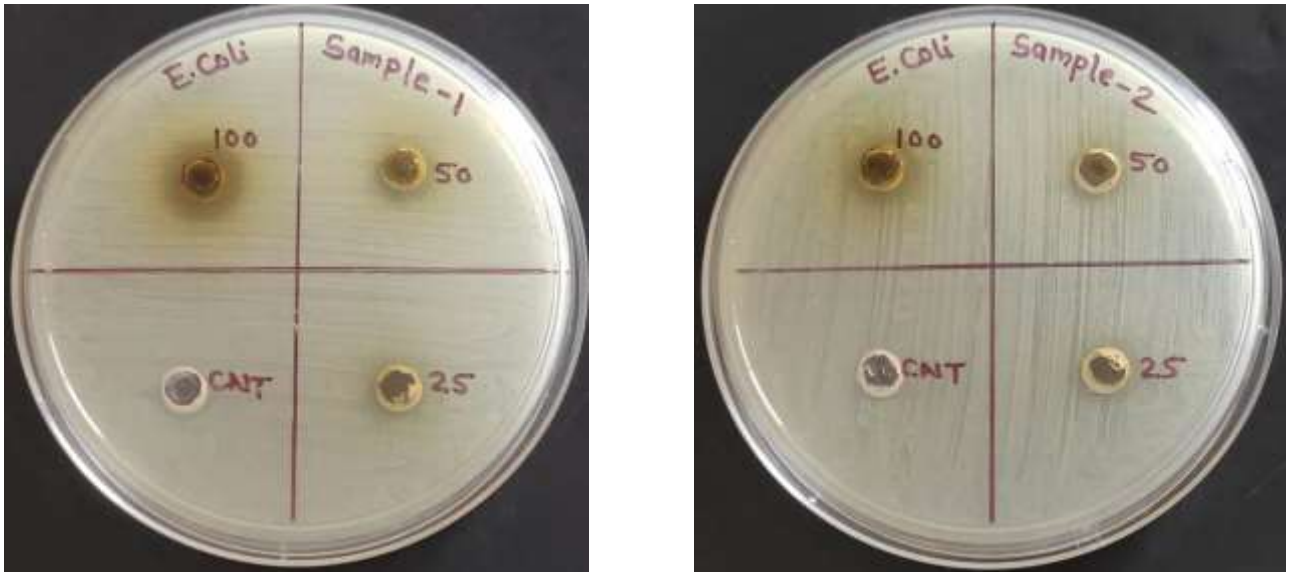


Figure 4: Antimicrobial activity of TCS and TCG against *Escherichia coli*

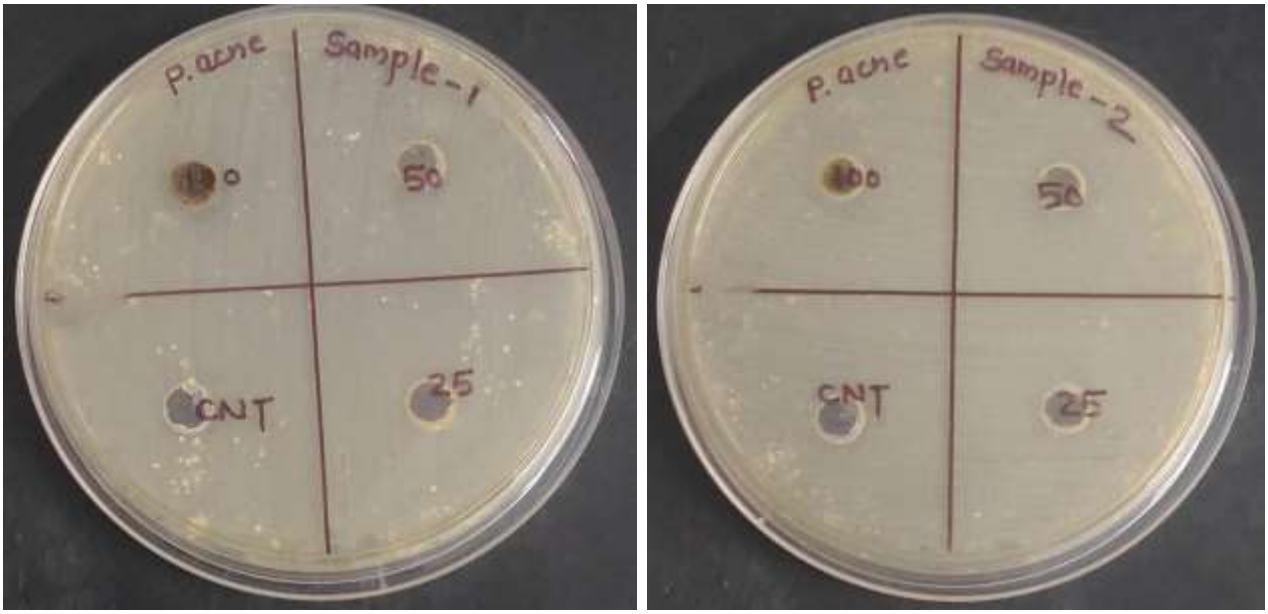


Figure 5: Antimicrobial activity of TCS and TCG against *Propionibacterium acnes*

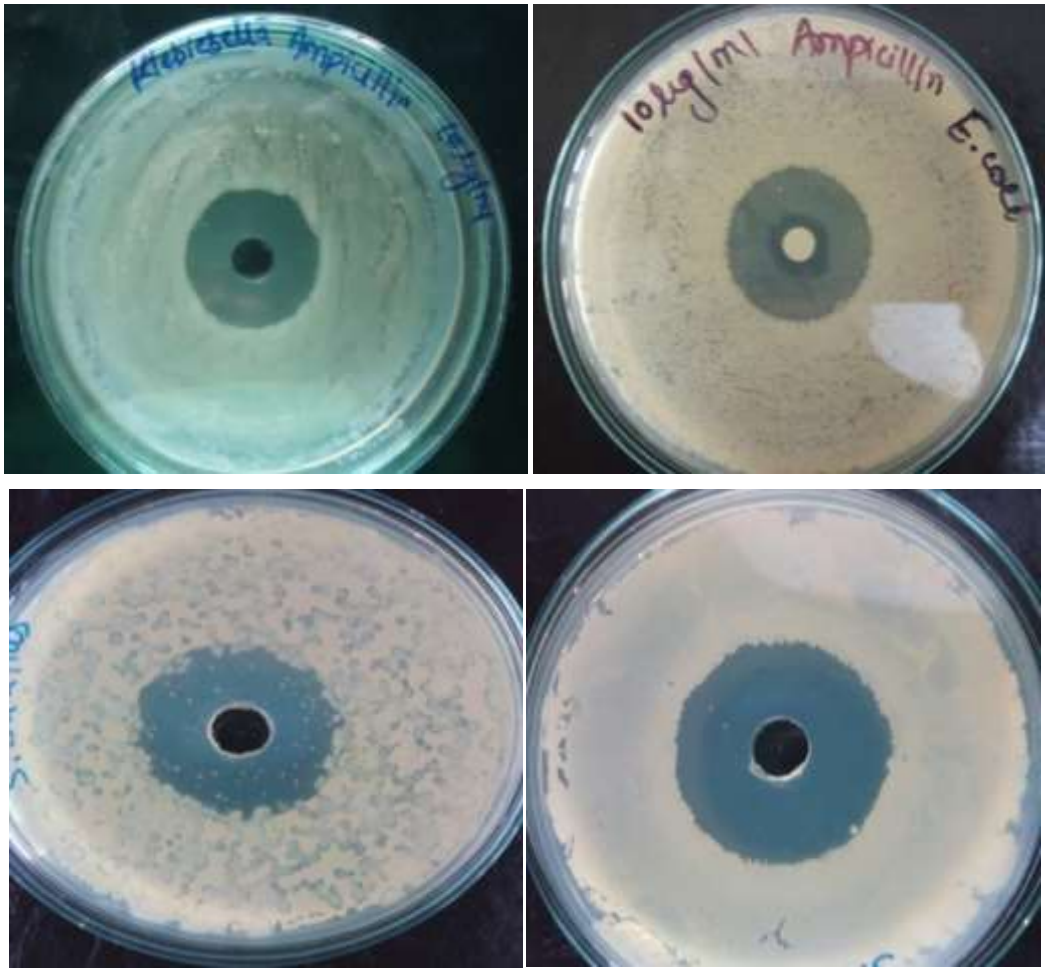
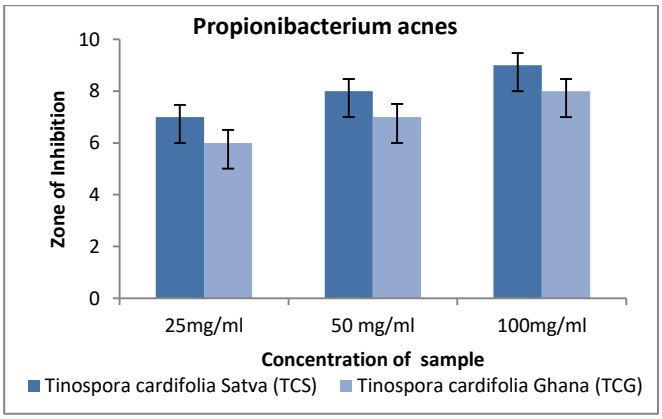
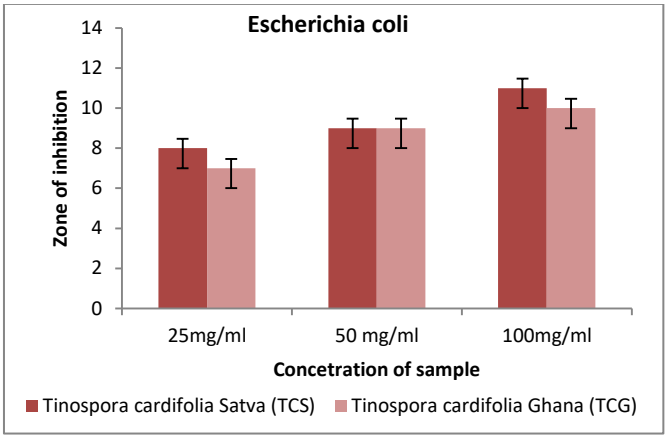


Figure 6: Antimicrobial activity of Ampicillin against organisms



Graph 1: Well diffusion assay of *Propionibacterium acnes*



Graph 2: Well diffusion assay of *Escherichia coli*

CONCLUSION:

The results obtained in this study suggest that selected *Tinospora cordifolia* Satva and *Tinospora cordifolia* Ghana showed significant antibacterial activity and have great potential against microorganisms. Thus its active constituents can be helpful in the therapeutic treatments. The obtained results validate the classical guidelines that satva for Ghana should be prepared by adding 4 time water and 1/4 reductions of the same after heating. The results also validate the traditional uses of *Tinospora cordifolia* in various skin ailments. Present study provides leads for future studies to ascertain its curative role through pharmacological and clinical studies.

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Conflict of Interest

None of the authors have any potential conflict of interest associated with this research.

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