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Research Article

COMPARATIVE ANTIMICROBIAL SCREENING OF *SATVA* (SEDIMENTED STARCHY AQUEOUS EXTRACT) AND *GHANA* (SOLIDIFIED AQUEOUS EXTRACT) OF *GUDUCHI (TINOSPORA CORDIFOLIA* (WILLD.) MIERS)

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ABSTRACT

Objective: *Guduchi satva* (GS) and *Ghana* are reputed Ayurvedic formulations having huge therapeutic credentials. However, no published reports on comparative antimicrobial profile of GS and *Ghana* are available. This study was, therefore, attempted to evaluate antimicrobial efficacies of these two dosage forms.

Methods: Recommended microbial strain - such as *Salmonella typhi, Escherichia coli, Pseudomonas aeruginosa,* and *Staphylococcus aureus* - was used for antimicrobial evaluation. Test samples were prepared by adopting classical guidelines. Qualitative and microbial contamination analysis was also conducted.

Results: *Satva* required less concentration for inhibition of *E. coli*, while *Ghana* showed better inhibition against *S. aureus* and *S. typhi* at lower concentrations. For *E. coli* and *S. aureus* strains, both samples showed promising results on comparison to Ampicillin. Qualitative analysis revealed the presence of glycosides, alkaloids, tannins, phenols, starch and sterols in *Ghana*, while the presence of alkaloids and starch in *Satva*. No microbial load was detected within both samples.

Conclusion: Both *Ghana* and *Satva* showed significant antibacterial activity and possess great potential against microorganisms. The results also validate the traditional uses of *Guduchi* in various skin ailments and infectious disorders.

Keywords: Antimicrobial activity, Antibacterial, *Guduchi*, *Ghana*, Satva, Phytochemical, *Tinospora cordifolia.*

INTRODUCTION

It is the need of hour to show the effectiveness of the drug in a disease by laboratory findings. Antimicrobial study is an easy tool for assessing the potential of Ayurvedic drugs on various pathological organisms. Therapy of bacterial infections is a frequent problem due to the emergence of bacterial strains resistant to numerous antibiotics. The search for natural products to cure disease represents an area of great interest in which plants have been the most important sources.

Tinospora cordifolia (Willd.) Miers locally known as *Guduchi, Amrita or Giloy*, possess wide range of therapeutic attributes, thus is of great interest for several researchers [1-4]. In traditional and folklore use, 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 systems of the body [5]. Ghana Kalpana (preparation of solidified aqueous extract), a concentrated dosage form, is mentioned in Ayurvedic pharmaceutics as an *Upakalpa* (secondary derivative preparation) of Kwatha Kalpana (decoction). *Guduchi ghana* (GG) is appreciated for its valuable role as febrifuge and in skin disorders [6,7]. *Satva* or *Sara* of an herb is the essence or active part and here it refers to the water extractable solid substance collected from herbal drug [8]. It can be considered as a secondary derivative of Hima Kalpana (cold infusion) because a part of pharmaceutical process involved in it is analogous to Hima Kalpana. Among all herbal satvas, *Guduchi satva* (GS) (aqueous extract of *T. cordifolia*) is a widely used formulation in Indian system of medicine as febrifuge and a general tonic. The standard manufacturing procedures and quality control profiles of *Satva* and *Ghana* are well documented [9-14].

Several recent reports explored the potent antimicrobial roles of *Guduchi* and its various extracts [15-21]. However, no published reports are available so far on comparative antimicrobial profile of GS and *Ghana*. Considering this, the present study was undertaken to evaluate their comparative antimicrobial efficacies.

METHODS

Plant collection and authentication

Fresh *Guduchi* stem spreading over *Nimba* (*Azadirachta indica*) was collected from the campus of Gujarat Ayurved University, Jamnagar, Gujarat, India (Fig. 1) and authenticated at the pharmacognosy laboratory from same institute.

Fresh *Guduchi* stem was collected as per classical guidelines - "*Sdaiva Adra Prayojyeta.*" [22] *Guduchi* plant which grows on *Nimba* is said to be the best as the synergy between these plants enhance its efficacy [23]. Matured stem was separated from other parts of the plant such as roots, leaves, flowers, fruits, and other physical impurities and washed thoroughly with potable water for three times.

Samples preparation

GS and GG was prepared by adopting classical guidelines [11,12].

Preparation of GS

Guduchi stem was collected and washed with water. Stems were chopped (1.5-2"), pounded to get homogeneous bolus and mixed with six parts of potable water in a SS vessel and kept undisturbed for soaking (12 hrs). The mass was vigorously macerated manually (1 hr) and filtered slowly through a clean four-folded cotton cloth. The liquid was kept undisturbed for 4 hrs. The supernatant liquid was decanted carefully and heavy starchy, sticky layer of sediment settled at the bottom was removed, air dried and stored in airtight glass jars.

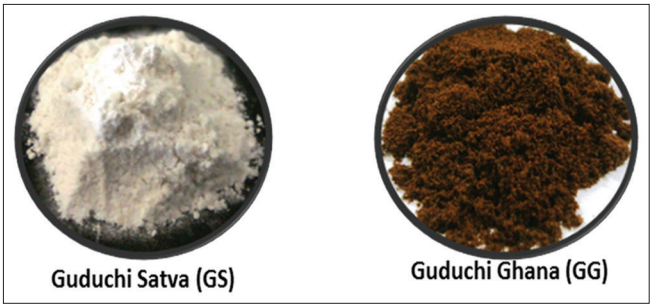

Fig. 1: Samples of *Guduchi satva* and *Guduchi ghana* subjected for antimicrobial screening

Preparation of GG

The physical impurities and papery bark of *Guduchi* were removed and washed thoroughly with water. Stem was made into pieces of 1-2" having 1.6-2.1 cm diameter and crushed thoroughly, added with four times of potable water in a SS vessel and kept for soaking overnight (12 hrs). Next morning, the contents were subjected to heat with continuous stirring. Water was evaporated slowly till its reduction to 1/4th and galenical was filtered through four-fold cotton cloth to obtain *Guduchi Kwatha*. The *Guduchi Kwatha* was subjected to heat with constant stirring till the entire mass converted into semi solid state. The mass was shifted into a glass tray and placed in oven at 45°C-50°C for complete drying. After complete drying it was collected, made into fine powder through mixer grinder, passed through 80 number sieve and packed in airtight container.

The final samples of GS and GG, prepared by following above-mentioned classical Ayurvedic methods are demonstrated in Fig. 1.

Bacterial strains and culture conditions

In this study, the test microorganisms used (bacteria: *Escherichia coli* (MTCC No. 443), *Pseudomonas aeruginosa* (MTCC No. 1688), *Staphylococcus aureus* (MTCC No. 96), and *Salmonella typhi* (MTCC No. 98), were procured from MTCC Chandigarh. Antimicrobial study was carried out in AccuPrec Research Labs PVT. LTD., Gandhinagar, Gujarat.

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.

The test is performed by making the wells of specific diameter (generally 6 mm) on to the surface of the presterilized agar plates over which culture of the microorganism is inoculated. After 18-24 hrs of incubation, the size of a clear zone of inhibition around the well is determined; this is related to the antimicrobial activity of the drug against the test strain.

Determination of minimum inhibitory concentration (MIC)

MIC of drug was determined by broth dilution method. It is one of the nonautomated *in vitro* bacterial susceptibility tests. This classic method yields a quantitative result for the amount of antimicrobial agents that is needed to inhibit growth of specific microorganisms. It is carried out in tubes.

Procedure

Well diffusion assay

Muller-Hinton agar media was prepared and sterilized by autoclaving at 121°C, 15 lbs. pressure for 15 minutes. Then medium was cooled to 45-50°C in water bath and poured in presterilized Petri-plate and allowed to solidify. 01 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 then loaded with 5 µg, 25 µg, 50 µg, 100 µg, and 250 µg of the sample and incubated for 18 hrs at 37°C. After incubation, each plate was observed for Zone of inhibition and diameter of zones was measured in mm.

Broth dilution method for determination of MIC

Primary screening

In primary screening serial dilutions of sample were prepared as 1000 µg/ml, 500 µg/ml, and 250 µg/ml in Muller-Hinton broth by double dilution in tubes from stock solution of 2000 µg/ml. To each tube 0.1 ml of inoculums is added and incubated at 37°C for 24 hrs. The MIC is recorded by noting the lowest concentration of the drug at which there is no visible growth as demonstrated by lack of turbidity in the tube.

Secondary screening

Secondary screening is done by following the procedure mention in primary screening with sample concentrations as 200 µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, and 6.25 µg/ml.

Qualitative and microbial contamination analysis

Both GS and GG were also analyzed to screen the microbial contamination and qualitative differences for various functional groups, if any.

RESULTS AND DISCUSSION

In recent years, antimicrobial properties of Indian medicinal plants have been increasingly reported [24-26]. Over the years there have been several studies documenting the antibacterial properties of plants from various parts of India [27-34]. *Guduchi* is a well reported antimicrobial herb and its various extracts are found effective against enteric bacteria, respiratory tract pathogens, peritonitis infection, dental pathogens, and bacteremia [20,35]. The crude extracts of *Guduchi* stem have well reported activity against several bacterial and fungal strains [36]. *Satva* and *Ghana* are widely used two dosage forms of this botanical; hence, this study is conscientious attempt to find out their antimicrobial potentials against selected microbial strains. The results obtained in the study are depicted in Tables 1 and 2 which show the growth inhibition produced by GS and GG on four species of bacteria at various concentrations. The activities can be referred as either less, moderate or highly active based on the zone of inhibition that ranges from 9 to 12 mm, 12 to 16 mm or >16 mm, respectively.

It is evident from Tables 1 and 2 that GS and GG were found to be highly active against *E. coli*, *S. aureus*, *P. aeruginosa*, and *S. typhi* at concentration of 250 µg/ml. On analysis of Table 3, it is found that, comparatively, GS required less concentration for inhibition of *E. coli*, while GG showed better inhibition against *S. aureus* and *S. typhi* at lower concentrations.

Results on Tables 3 and 4 revealed that, for *E. coli* and *S. aureus* strains, both GS and GG showed promising results on comparison to Ampicillin. For *E. coli*, GG showed similar MIC as that of Ampicillin, while GS demonstrated comparatively better results than GG. For *S. aureus*, both samples showed better MIC in comparison to Ampicillin, where GG demonstrated comparatively better results than GS. Comparative MIC of GS, GG and standard antibacterial drugs on various microorganisms has been illustrated in Fig. 2. Qualitative analysis for various functional groups revealed the presence of glycosides, alkaloids, tannins, phenols, starch, and sterols in GG, while the presence of only alkaloids and starch in GS. Although all aforesaid functional groups are well reported and pharmacologically active antimicrobial phytochemicals in the plant, the alkaloidal constituents which are commonly found in both *Satva* and *Ghana* suggests that the alkaloidal might be accountable for their major antimicrobial potential of the plant (Table 5). Alkaloids such as berberine, palmatine, tembetarine, magnoflorine, choline, tinosporin, columbin, isocolumbin, and tetrahydropalmatine have been isolated

Table 1: Effect of various concentrations of GS on microorganisms

Well No.	Sample concentration (µg)	Bacterial strains (zone of inhibition in mm)			
		Escherichia coli **MTCC 443**	***Pseudomonas aeruginosa*** **MTCC1688**	***Staphylococcus aureus*** **MTCC 96**	***Salmonella typhi*** **MTCC 98**
1	5	-	-	-	-
2	25	13	13	17	14
3	50	15	15	19	15
4	100	18	18	21	17
5	250	21	20	22	20

GS: *Guduchi satva*

Table 2: Effect of various concentrations of GG on microorganisms

Well No.	Sample concentration (µg)	Bacterial strains (zone of inhibition in mm)			
		Escherichia coli **MTCC 443**	***Pseudomonas aeruginosa*** **MTCC1688**	***Staphylococcus aureus*** **MTCC 96**	***Salmonella typhi*** **MTCC 98**
1	5	-	-	10	6
2	25	15	14	15	12
3	50	17	16	17	18
4	100	20	17	19	19
5	250	22	21	23	21

GG: *Guduchi ghana*

Table 3: MIC of GS and GG on various microorganisms

MIC					
Bacterial strains	**Code no**	**Bacterial strains**			
		Escherichia coli **MTCC 443**	***Pseudomonas aeruginosa*** **MTCC1688**	***Staphylococcus aureus*** **MTCC 96**	***Salmonella typhi*** **MTCC 98**
MIC in µg/ml	GS	62.5	200	125	200
MIC in µg/ml	GG	100	200	100	125

MIC: Minimal inhibitory concentration, GS: *Guduchi satva*, GG: *Guduchi ghana*

Table 4: MIC of standard antibacterial drugs

Drug (µg/ml)	***Escherichia coli*** **MTCC 443**	***Pseudomonas aeruginosa*** **MTCC 1688**	***Staphylococcus aureus*** **MTCC 96**	***Salmonella typhi*** **MTCC 98**
Gentamycin	0.05	1	0.25	5
Ampicillin	100	-	250	100
Chloramphenicol	50	50	50	50
Ciprofloxacin	25	25	50	25
Norfloxacin	10	10	10	10

MIC: Minimal inhibitory concentration

Table 5: Results of qualitative test for various functional groups of GS and GG

S. No.	Functional group	GS	GG
1	Glycosides	−ve	+ve
2	Alkaloids	+ve	+ve
3	Tannin	−ve	+ve
4	Saponin	−ve	−ve
5	Flavonoids	−ve	−ve
6	Phenols	−ve	+ve
7	Proteins	−ve	−ve
8	Carbohydrates	+ve	+ve
9	Starch	+ve	+ve
10	Sterol/Steroid	−ve	+ve

+ve: Present, −ve: Absent. GS: *Guduchi satva*, GG: *Guduchi ghana*

from the extracts of stem and roots of the plant [20]. In microbiological study, in both the samples, pathogens viz. *E. coli, S. typhi, S. aureus,* and *P. aeruginosa* were absent, while total bacterial count of GG and GS were 20 cfu/g and 30 cfu/g, respectively. The yeast and mold count was nil in GG, and in GS, it was found 10 cfu/g which is within permissible limits (Table 6). This study provides leads for future studies to ascertain its curative role through pharmacological and clinical studies.

CONCLUSION

The results obtained in this study suggest that selected GS and *Ghana* showed significant antibacterial activity and possess great potential against microorganisms. The obtained results validate the classical guidelines that *Guduchi Kwatha* for GG should be prepared by adding 4 time water and ¼ reduction of the same after heating. Phytochemical analysis revealed few differences in various functional groups among the samples and suggests that the alkaloidal contents might be accountable for their antimicrobial potential. The results also validate the traditional/folklore uses of *Guduchi* in various skin ailments and infectious disorders. Further investigations and isolation of compound are necessary to establish the exact constituent responsible for their antimicrobial activity.

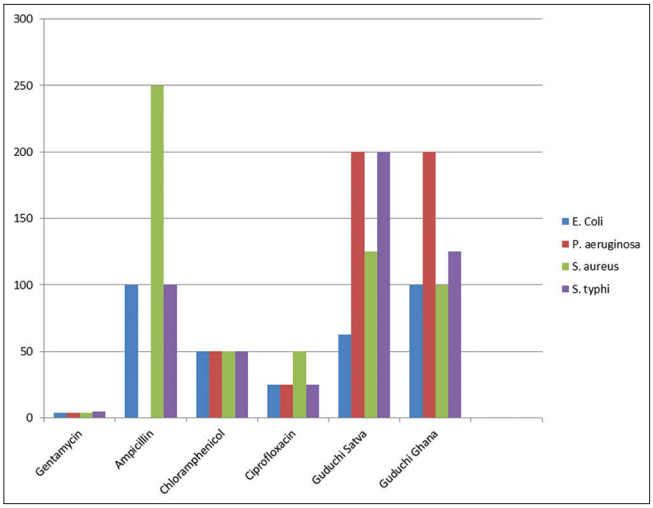

Fig. 2: Comparative minimal inhibitory concentration of *Guduchi satva, Guduchi ghana* and standard antibacterial drugs on various microorganisms

Table 6: Microbial overload values of GS and GG

S. No.	Test	Result (cfu/ml)		Specification
		GS	GG	
1	Total bacterial count	30	20	10^5 cfu/g
2	Yeast and mold count	00	10	10^3 cfu/g
3	*Escherichia coli*	Absent	Absent	Absent
4	*Salmonella*	Absent	Absent	Absent
5	*Pseudomonas aeruginosa*	Absent	Absent	Absent
6	*Staphylococcus aureus*	Absent	Absent	Absent

GS: *Guduchi satva*, GG: *Guduchi ghana*

REFERENCES

1 Sharma R, Amin H, Galib R, Prajapati PK. Therapeutic vistas of Guduchi (*Tinospora cordifolia* (Willd.) Miers): A medico-historical memoir. J Res Educ Indian Med 2014;XX(2):121-35.
2 Sharma R, Amin H, Galib R, Prajapati PK. Antidiabetic claims of *Tinospora cordifolia* (Willd.) Miers: Critical appraisal and role in therapy. Asian Pac J Trop Biomed 2015;5(1):68-78.
3 Sharma R, Kumar V, Ashok BK, Galib R, Prajapati PK, Ravishankar B. Evaluation of hypoglycaemic and anti-hyperglycaemic activities of Guduchi Ghana in Swiss albino mice. Int J Green Pharm 2013;7:145-8.
4 Sharma R, Kumar V, Ashok BK, Galib R, Prajapati PK, Ravishankar B. Hypoglycemic and anti-hyperglycemic activity of Guduchi Satva in experimental animals. Ayu 2014;4:217-20.
5 Sinha K, Mishra NP, Singh J, Khanuja SP. *Tinospora cordifolia* (Guduchi), a reservoir plant for therapeutic applications: A review. Indian J Tradit Knowl 2004;3:257-70.
6 Acharya YT. Siddha Yoga Sangraha, Jwaradhikar1/6:4. 13th ed. Nagpur: Baidyanath Ayurveda Bhavan Ltd.; 2008.
7 Sharangadhara. Sharangadhara Samhita, Madhyama Khanda. 6th ed. Ch. 8/1. Varanasi: Chaukhamba Orientalia; 2005.
8 Anonymous. The Ayurvedic Formulary of India. Part 1. 2nd ed. New Delhi: Government of India, Ministry of Health and Family Welfare; 2003.
9 Sharma R, Amin H, Galib R, Prajapati PK. Quality control evaluation of Guduchi Ghana (dried aqueous extract of *Tinospora cordifolia* (Willd) Miers)-an herbal formulation. SLJIM 2013;3(1):174-9.
10 Sharma R, Amin H, Shukla VJ, Kartar D, Galib R, Prajapati PK. Quality control evaluation of Guduchi Satva (solid aqueous extract of *Tinospora cordifolia* (Willd.) Miers): An herbal formulation. Int J Green Pharm 2013;7:258-63.
11 Sharma R, Amin HG, Prajapati PK. Validation of standard manufacturing procedure of Gud?c? Sattva (aqueous extract of *Tinospora cordifolia* (Willd.) Miers) and its tablets. Anc Sci Life 2013;33:27-34.
12 Sharma R, Galib R, Prajapati PK. Validation of standard manufacturing procedure of Guduchi Ghana (dried aqueous extract of *Tinospora cordifolia* (Willd) Miers) and its tablets. Ayurpharm Int J Ayurveda Allied Sci 2013;2(7):224-32.
13 Sharma R, Harisha CR, Galib R, Patgiri BJ, Prajapati PK. Quantitative estimation of Satva extracted from different stem sizes of Guduchi (*Tinospora cordifolia* (Willd.) Miers. J Pharm Sci Innov 2012;1(1):38-40.
14 Sharma R, Amin H, Galib R, Prajapati PK. Seasonal variations in physicochemical profiles of Guduchi Satva (starchy substance from *Tinospora cordifolia* [Willd.] Miers). J Ayurveda Integr Med 2013;4(4):193-7.
15 Amane H, Kaore S, Kaore N. *In vitro* study of antimicrobial properties of *Tinospora cordifolia* (Guduchi). Int J Pharm Bio Sci 2014;5(1):747-53.
16 Rose M, Noorulla KM, Asma M, Kalaichelvi R, Vadivel K, Thangabalan B, *et al*. *In vitro* antibacterial activity of methanolic root extract of *Tinospora cordifolia* (Willd). Int J Pharm Res Dev 2010;2(5):1-5.
17 Islam MK, Ashakin K. Antimicrobial screening and brine shrimp lethality bioassay of *Tinospora cordifolia* (Fam: Menispermaceae). Int J Pharm Sci Res 2011;2(11):3091-5.
18 Duraipandiyan V, Ignacimuthu S, Balakrishna K, Al-Harbi NA. Antimicrobial activity of *Tinospora cordifolia*: An ethnomedicinal plant. Asian J Tradit Med 2012;7(2):59-65.
19 Jeyachandran R, Xavier TF, Anand SP. Antibacterial activity of stem extracts of *Tinospora cordifolia* (Willd) Hook. f & Thomson. Anc Sci Life 2003;23:40-3.
20 Vermani A, Navneet, Gautam SS. Screening of antibacterial activity of *Tinospora cordifolia* Miers extracts against dental pathogens. J Pharmacol Toxicol 2013;8(1):28-34.
21 Mishra P, Jamdar P, Desai S, Patel D, Meshram D. Phytochemical analysis and assessment of *in vitro* antibacterial activity of *Tinospora cordifolia*. Int J Curr Microbiol Appl Sci 2014;3(3):224-34.
22 Sharangadhara. Sharangadhara Samhita. Prathama Khanda. 6th ed. Ch. 1/45. Varanasi: Chaukhamba Orientalia; 2005.
23 Anonymous. Quality Standards of Indian Medicinal Plants. Vol. 1. New Delhi: Indian Council of Medical Research; 2003. p. 212.
24 Aswal BS, Goel AK, Kulshreshtha DK, Mehtrota BN, Patnaik GK. Screening of Indian medicinal plants for biological activity. Indian J Exp Biol 1996;34:444-67.
25 Ahmad UV, Ali MS, Usmanghani K. Bodenolide, a new diterpenoid from the seeds of caesalpinia bonduc. Z Naturforsch 1997;52b:410-2.
26 Ahmad I, Beg AZ. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. J Ethnopharmacol 2001;74(2):113-23.
27 Valsaraj R, Pushpangadan P, Smitt UW, Adsersen A, Nyman U. Antimicrobial screening of selected medicinal plants from India. J Ethnopharmacol 1997;58(2):75-83.
28 Ahmad I, Mehmood Z, Mohammad F. Screening of some Indian medicinal plants for their antimicrobial properties. J Ethnopharmacol 1998;62(2):183-93.
29 Perumal Samy R, Ignacimuthu S, Sen A. Screening of 34 Indian medicinal plants for antibacterial properties. J Ethnopharmacol 1998;62(2):173-82.
30 Samy RP, Ignacimuthu S. Antibacterial activity of some folklore medicinal plants used by tribals in Western Ghats of India. J Ethnopharmacol 2000;69(1):63-71.
31 Srinivasan D, Nathan S, Suresh T, Lakshmana Perumalsamy P. Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. J Ethnopharmacol 2001;74(3):217-20.
32 Dabur R, Singh H, Chhillar AK, Ali M, Sharma GL. Antifungal potential of Indian medicinal plants. Fitoterapia 2004;75(3-4):389-91.
33 Vonshak A, Barazani O, Sathiyamoorthy P, Shalev R, Vardy D, Golan-Goldhirsh A. Screening South Indian medicinal plants for antifungal activity against cutaneous pathogens. Phytother Res 2003;17(9):1123-5.
34 Vaijayanthimala J, Anandi C, Udhaya V, Pugalendi KV. Anticandidal activity of certain South Indian medicinal plants. Phytother Res 2000;14(3):207-9.
35 Thatte UM, Kulkarni MR, Dahanukar SA. Immunotherapeutic modification of *Escherichia coli* peritonitis and bacteremia by *Tinospora cordifolia*. J Postgrad Med 1992;38(1):13-5.
36 Jeyachandran R, Xavier TF, Anand SP. Antibacterial activity of stem extracts of *Tinospora cordifolia* (Willd) Hook. f & Thomson. Anc Sci Life 2003;23(1):40-3.