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Screening of Antimicrobial activity of *Guduchi Ghana* (dried aqueous extract of *Tinospora cordifolia* (Willd.) Miers)

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ABSTRACT

Guduchi (*Tinospora cordifolia* (Willd.) Miers) is one of the most versatile rejuvenating herbs, possessing numerous therapeutic attributes. Recent reports investigated and ascertained its role as a potent antimicrobial herb. No published reports on antimicrobial profile of its dosage form- *Guduchi Ghana* are available till date. Present study was therefore attempted to evaluate comparative antimicrobial efficacies of Guduchi Ghana prepared by two different methods – (i) classical Ayurvedic method and (ii) modified method. Recommended microbial strain like; *Salmonella typhi*, *Escherichia coli*, *P. aeruginosa* and *Staphylococcus aureus* were used in this study for the same purpose. Both samples showed significant antibacterial activity and possess great potential against microorganisms, where *Guduchi Ghana* prepared by classical method showed better results. Physicochemical analysis showed insignificant difference between samples. Phytochemical analysis for various functional groups revealed the presence of glycosides, alkaloids, tannins, phenols, starch and sterols in both samples, which might be accountable for their antimicrobial potential. No microbial load was detected within both samples. The results also validate the traditional uses of Guduchi in various skin ailments. Present study may prove a torch bearer for future studies to understand its biological activities.

Keywords: Antimicrobial activity, *Guduchi, Guduchi Ghana*, Physicochemical, *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. Nosocomial pneumonia is most common in the surgical intensive units. 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.[1-3] Its safety and non-toxic nature have been reported in experimental and clinical studies on various systems of the body.[4] *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).

Several recent reports explored the potent antimicrobial roles of *Guduchi* and its various extracts.[5-11] However, no published reports are available so far on antimicrobial profile of *Guduchi Ghana*. Considering this, the present study was undertaken. Here, *Guduchi Ghana* was prepared by two different methods (classical and modified) and their comparative antimicrobial efficacies are evaluated.

MATERIALS AND METHODS

Plant collection and authentication

Fresh *Guduchi* stem spreading over *Neem* (*Azadirachta indica*) tree was collected from the campus of Gujarat Ayurved University, Jamnagar and authenticated at Pharmacognosy Laboratory of the institute. *Guduchi* plant, which grows on *Neem* tree is said to be the best as the synergy between these plants enhance its efficacy.[12]

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Samples preparation

The physical impurities were removed and washed thoroughly with water. *Guduchi Ghana* was prepared by following two methods: (i) Modified method (coded as GG 1) (ii) Classical method,[13,14] (coded as GG 2).

Preparation of GG 2 (Classical method):

The physical impurities and papery bark of *Guduchi* were removed and washed thoroughly with water. Stem was made into pieces of 1-2 inches 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 $1/4^{th}$ 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 $45^{o}C$ - $50^{o}C$ for complete drying. After complete drying it was collected, made into fine powder through mixer grinder, passed through 80 number sieve and packed in air tight container.

Preparation of GG 1 (Modified method):

Here the *Kwatha* was prepared by adding eight times potable water and reduction to $1/4^{th}$. Rest of the pharmaceutical processs was same as that of GG 2.

Bacterial strains and culture conditions

In present 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 pre sterilised agar plates over which culture of the microorganism is inoculated. After 18 to 24 hours 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)

Minimum Inhibitory concentration of drug was determined by Broth dilution method. It is one of the non automated *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 $121^{0}C$, 15 lbs. pressure for 15 minutes. Then medium was cooled to 45 – 50 ^{0}C in water bath and poured in pre sterilized 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 5µg, 25 µg, 50 µg, 100 µg, and 250 µg of the sample 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.

Broth dilution method for determination of MIC

Primary Screening

In primary screening serial dilutions of sample were prepared as 1000 µg/ml, 500µg/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 $37^{o}C$ 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 200µg/ml, 100µg/ml, 50µg/ml, 25µg/ml, 12.5µg/ml and 6.25µg/ml.

Physicochemical and Qualitative analysis

Both samples were also analyzed by employing various analytical parameters to screen the physicochemical or qualitative differences, if any. Physicochemical analysis like pH value, loss on drying at 110°C, ash value (%w/w), moisture content, bitter values and various extractive values like water soluble, methanol soluble, chloroform soluble, benzene soluble, diethyl ether soluble extracts were carried out. GG 1 and GG 2 were further also subjected to qualitative tests for various functional groups and microbial contamination.[15,16]

RESULT AND DISCUSSION

The results obtained in the study are depicted in Table 1 to 2 which show the growth inhibition produced by GG 1 and GG 2 on 4 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- 12mm, 12 – 16mm or >16mm respectively.

It is evident from Table 1 and 2 that formulation GG 2 can be considered as comparatively more potent antimicrobial than that of GG 1 at lower concentration on studied strains of *S. typhi, E. coli, P. aeruginosa* and *S. aureus*. Upon analysis of Tables 3 it is found that, GG 2 required less concentration for inhibition of microorganisms of studied strains to grow as compare to GG 1. Hence it is supposed to be comparatively more potent as that of GG 1. Results on Tables 3 and 4 revealed that, for *E. coli* strain, all two samples showed MIC similar to as that of Ampicillin. For *S. aureus*, both samples showed better MIC in comparison to Ampicillin, where GG 2 demonstrated comparatively better results than GG 1. Insignificant differences were found between both samples in physicochemical analysis (Table 5). Qualitative analysis revealed presence of glycosides, alkaloids, tannins, phenols, starch and sterols in both samples (Table 6). No microbial load was detected within both samples (Table 7).

Table 1: Effect of various concentration of GG 1 on microorganisms

Well no.	Sample Concentration (µg)	Bacterial Strains (Zone of inhibition in mm)			
		E. coli MTCC 443	*P. aeruginosa* MTCC1688	*S. aureus* MTCC 96	*S. 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

Table 2: Effect of various concentration of GG 2 on microorganisms

Well no.	Sample Concentration (µg)	Bacterial Strains (Zone of inhibition in mm)			
		E. coli MTCC 443	*P. aeruginosa* MTCC1688	*S. aureus* MTCC 96	*S. typhi* MTCC 98
1	5	-	-	8	6
2	25	14	14	14	10
3	50	17	16	18	14
4	100	18	18	18	18
5	250	22	20	22	21

Table 3: MIC (Minimal Inhibitory Concentration) of GG 1 and GG 2 on various microorganisms

Minimum Inhibitory Concentration					
Bacterial Strains	Code no	Bacterial strains			
		E.coli MTCC 443	*P.aeruginosa* MTCC1688	*S. aureus* MTCC 96	*s.typhi* MTCC 98
MIC in µg/ml	GG 1	100	200	100	125
MIC in µg/ml	GG 2	100	175	75	125

Table 4: Showing MIC of Standard anti bacterial drugs

Drug	*E. coli* MTCC 443	*P. aeruginosa* MTCC 1688	*S. aureus* MTCC 96	*S. Typhi* MTCC 98
	(Microgram/ ml)			
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

Table 5: Physicochemical parameters of GG 1 and GG 2

Parameter	Samples	
	GG 1	GG 2
pH value	5.93	5.90
Loss on drying	7.71	7.46
Ash value (%w/w)	16.50	16.35
Moisture content	47.20	47.56
Water sol. extract (%w/w)	48.79	48.32
Alcohol sol. extract (%w/w)	16.9	17.2
Chloroform sol. extract (%w/w)	0.85	0.83
Benzene sol. extract (%w/w)	0.07	0.07
Diethyl ether sol. extract (%w/w)	0.04	0.06
Bitter values	1.004	1.004

Table 6: Results of qualitative test for various functional groups of GG 1 and GG 2

Sr. No.	Functional group	GG 1	GG 2
1.	Glycosides	**+ve**	**+ve**
2.	Alkaloids	+ve	+ve
3.	Tannin	**+ve**	**+ve**
4.	Saponin	-ve	-ve
5.	Flavanoids	-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

Table 7: Microbial overload values of GG 1 and GG 2

Sr. No.	Test	Result (CFU/ ml)		Specification
		GG 1	GG 2	
01.	Total Bacterial count	20	20	10^5 CFU/ g
02.	Yeast & Mould count	00	10	10^3 CFU/ g
03.	*E. coli*	Absent	Absent	Absent
04.	*Salmonella*	Absent	Absent	Absent
05.	*Psuedomonas aeruginosa*	Absent	Absent	Absent
06.	*Staphylococcus aureus*	Absent	Absent	Absent

CONCLUSION

The results obtained in this study suggest that selected *Guduchi Ghana* showed significant antibacterial activity and possess great potential against microorganisms. Thus its active constituents can be helpful in the therapeutic treatments. The obtained results validate the classical guidelines that *Guduchi Kwatha* for *Guduchi Ghana* should be prepared by adding 4 time water and ¼ reduction of the same after heating. The phytochemical analysis for various functional groups revealed the presence of glycosides, alkaloids, tannins, phenols, starch and sterols in both samples, which might be accountable for their antimicrobial potential. The results also validate the traditional uses of Guduchi 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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