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 pharmacutics 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 waterextractable 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 (1.5-2ʺ), 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.

Results:
For antimicrobial evaluation. Test samples were prepared by adopting classical guidelines. Qualitative and microbial contamination analysis was also conducted. 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.
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. 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 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 antibiotic 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, tembotarine, magnoflorine, choline, tinosporin, columbin, isocolumbin, and tetrahydropalmatine have been isolated.

**Fig. 1: Samples of Guduchi satva and Guduchi ghana subjected for antimicrobial screening**
Table 1: Effect of various concentrations of GS on microorganisms

<table>
<thead>
<tr>
<th>Well No.</th>
<th>Sample concentration (µg)</th>
<th>Bacterial strains (zone of inhibition in mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Escherichia coli MTCC 443</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>13</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>15</td>
</tr>
<tr>
<td>4</td>
<td>100</td>
<td>18</td>
</tr>
<tr>
<td>5</td>
<td>250</td>
<td>21</td>
</tr>
</tbody>
</table>

GS: Guduchi satva

Table 2: Effect of various concentrations of GG on microorganisms

<table>
<thead>
<tr>
<th>Well No.</th>
<th>Sample concentration (µg)</th>
<th>Bacterial strains (zone of inhibition in mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Escherichia coli MTCC 443</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>17</td>
</tr>
<tr>
<td>4</td>
<td>100</td>
<td>20</td>
</tr>
<tr>
<td>5</td>
<td>250</td>
<td>22</td>
</tr>
</tbody>
</table>

GG: Guduchi ghana

Table 3: MIC of GS and GG on various microorganisms

<table>
<thead>
<tr>
<th>MIC</th>
<th>Bacterial strains Code no</th>
<th>Bacterial strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIC in µg/ml</td>
<td>GS</td>
<td>62.5</td>
</tr>
<tr>
<td>MIC in µg/ml</td>
<td>GG</td>
<td>100</td>
</tr>
</tbody>
</table>

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

Table 4: MIC of standard antibacterial drugs

<table>
<thead>
<tr>
<th>Drug (µg/ml)</th>
<th>Escherichia coli MTCC 443</th>
<th>Pseudomonas aeruginosa MTCC 1688</th>
<th>Staphylococcus aureus MTCC 96</th>
<th>Salmonella typhi MTCC 98</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gentamycin</td>
<td>0.05</td>
<td>1</td>
<td>0.25</td>
<td>5</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>100</td>
<td>-</td>
<td>250</td>
<td>100</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

MIC: Minimal inhibitory concentration

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

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

*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 times 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 Gaduchi satva, Guduchi ghana and standard antibacterial drugs on various microorganisms

Table 6: Microbial overload values of GS and GG

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Test</th>
<th>Result (cfu/ml)</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total bacterial count</td>
<td>30</td>
<td>10^4 cfu/g</td>
</tr>
<tr>
<td></td>
<td>Yeast and mold count</td>
<td>00</td>
<td>10^4 cfu/g</td>
</tr>
<tr>
<td>3</td>
<td>Escherichia coli</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>4</td>
<td>Salmonella</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>5</td>
<td>Pseudomonas aeruginosa</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>6</td>
<td>Staphylococcus aureus</td>
<td>Absent</td>
<td>Absent</td>
</tr>
</tbody>
</table>

GS: Gaduchi satva, GG: Guduchi ghana

REFERENCES