



Research Article

IN VITRO ANTIMICROBIAL ACTIVITY OF STEM EXTRACTS OF FRESH AND DRY *TINOSPORA CORDIFOLIA* (WILLD) HOOK. F & THOMS

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ABSTRACT

Tinospora cordifolia (Guduchi) is a widely used plant in folk and Ayurvedic systems of medicine. It is widely used as rasayana to improve body resistance against infections and is known to possess anti oxidant, antimicrobial, anti-hyperglycemic, anti allergic and anti inflammatory activities. Guduchi is one among the drugs which are advised to use only in fresh form for medicine preparation according to Sharngadhara Samhitha, an Ayurvedic textbook. The changes that can occur in the pharmacological efficacy of the drug according to the basic state are unknown. The antibacterial activity of the alcohol, kashaya extracts of the fresh and dry stems of *Tinospora cordifolia* and Guduchi sattva were studied using well diffusion method against *Pseudomonas*, *Klebsiella*, *Staphylococcus aureus*, Coagulase negative staphylococci (CNS) and *Escherichia coli*. The Kashaya prepared using fresh Guduchi stem and Guduchi sattva showed significant bactericidal action which indicates the high antimicrobial potential of the drug when used in the fresh form.

Keywords: Fresh and dry Guduchi, Guduchi sattva, Antimicrobial activity

INTRODUCTION

Tinospora cordifolia (Willd.) Hook. f. and Thoms. (Guduchi) is a large, glabrous, deciduous climbing shrub belonging to the family Menispermaceae. The synonym amrutha meaning the 'elixir of life' clearly fits this plant due to its versatile activity. Guduchi is highlighted for its use as a medhya rasayana¹ (learning and memory enhancer) and is also attributed with Rasayana, Sangrahi, Balya, Agnideepana, Tridoshshamaka, Dahanashaka, Meahnashaka, Kasa-swasahara, Pandunashaka, Kamla-Kushta-Vataraktanashaka, Jwarhara, Krimihara, properties. Acharya Vaghbata mentioned it as the agryoushadha for vatarakta.² Guduchi sattva, the aqueous extractable solid substance collected from fresh Guduchi is also used in practice.^{3,4} The plant is scientifically proved anti-diabetic, anti-oxidant, anti-arthritic, anti-allergic, anti-stress, hepatoprotective and immunomodulator.⁵ The chemical constituents reported from this plant belong to different classes, such as alkaloids, diterpenoid lactones, glycosides, steroids, sesquiterpenoid, phenolics, aliphatic compounds and polysaccharides.^{6,7}

In Ayurveda texts the preliminary procedures, from the very collection of drugs, has been clearly mentioned to ensure the quality and purity of the drug and effectiveness of the treatment. There are recommendations regarding the collection of drugs, storage and use of drugs. Specific time, place, methods and forms are explained for many of the medicinal plants to use as an ingredient in different formulations. In Sharngadhara samhitha the drugs are grouped as those which have to be used in dry form and drugs which should always be used as fresh-Sadaivaardram.⁸ While explaining the Kashaya (decoction) preparation, the samhitha advices to use all the fresh drugs in

double the quantity as said in the formulation, but the drugs which are advised to use in the fresh form only should be taken in the same quantity. The logical reasoning behind this statement is least explored. The changes which can occur in the quality and efficacy of these drugs according to the fresh and dry forms are unknown. Guduchi is one amongst these drugs which are recommended to be used in fresh form only. But no studies have yet been reported on the form of the drug that has to be used, whether fresh or dry form. As many of the ayurveda medicine manufacturing units use Guduchi in dry form for preparing medicines, the changes that may occur in phytochemical properties and pharmacological actions of the drug in its different forms should be analysed.

MATERIALS AND METHODS

Collection Of Plant Material

The plant material was collected from Nalanchira, Thiruvananthapuram whenever required. The specimen was authenticated by Research Officer, Pharmacognosy Unit-Poojappura, Government Ayurveda College, Thiruvananthapuram. The stem was cut into small pieces, dried in the shade and powdered. It was kept in an air tight container. The fresh sample of the Guduchi stem was collected whenever required.

Preparation Of Samples

The alcohol extract of the drug is prepared by continuous extraction procedure in Soxhlet apparatus. 250 grams each of dry and fresh stem of *Tinospora cordifolia* (Willd.) Miers. ex. F

& Thoms. is taken in a Soxhlet apparatus and extracted with Ethyl alcohol. The extract obtained is distilled and evaporated to dryness.

To prepare the kashaya extract of fresh and dry stem, 48 gram of coarsely powdered drug was mixed with 768 ml water and reduced to 96 ml according to the kashaya preparation procedure mentioned in Sharnghadhara Samhitha.⁸ The kashaya was filtered through a four folded cotton cloth and the filtrate was evaporated to dryness to get the kashaya extract.

The Guduchi sattva was prepared as per the procedure given in Ayurveda formulary of India.⁹ The fresh stem was cut in to pieces and subjected to pounding in a mixer grinder. This mass was kept for soaking overnight with 21 times water in a stainless steel vessel. When the solid particles were found sedimented, the supernatant liquid portion was decanted carefully. After decantation the starch obtained was again mixed with little quantity of water and allowed again for sedimentation and the liquid portion was decanted carefully. The white starchy sediment which was settled at the bottom was collected into a tray, air dried under running fan and stored in airtight glass jars.

Culture Media And Microorganisms

The agar well diffusion method was used for screening the antimicrobial action of plant extracts. Media used for this method was Mueller Hinton Agar (MHA). The microbial strains of *Escherichia coli*, *Pseudomonas*, *Klebsiella*, *Staphylococcus aureus* and CNS were collected from Public Health Laboratory, Thiruvananthapuram. Agar slants were used to carry microbes from Public Health lab to microbiology lab at Drug Standardisation Unit, Thiruvananthapuram.

Determination Of Zone Of Inhibition

The zone of inhibition by the samples was determined by well diffusion method. A lawn culture is prepared on MHA plates by inoculating with test organisms. DMSO was used as vehicle for diluting the samples. 50 µL of DMSO dissolved samples were pipetted into the well made in agar plates. Disk of standard antimicrobial drug are placed in the agar plate as positive control. DMSO is used as negative control. Petri dishes are incubated at 37^o C for one night. The incubated culture plates are checked for the zone that inhibited the growth of bacteria around the antibiotic disc and the extract. The diameter of zone of inhibition was measured using a geometric scale.

Table 1: Action of alcohol extract of fresh guduchi stem in microbes

No:	Name of the Micro organism	Zone of inhibition (mm)			
		Sample drug	Ciprofloxacin (5 mcg)	Amoxiclav (30 mcg)	DMSO
1	<i>Pseudomonas</i>	Resistant (R)	14	-	R
2	<i>Klebsiella</i>	R	19	-	R
3	<i>E. coli</i>	R	24	-	R
4	<i>Staphylococcus aureus</i>	R	-	24	R
5	CNS	R	-	20	R

Table 2: Action of alcohol extract of dry guduchi stem in microbes

No:	Name of the Micro organism	Zone of inhibition(mm)			
		Sample drug	Ciprofloxacin (5 mcg)	Amoxiclav (30 mcg)	DMSO
1	<i>Pseudomonas</i>	R	14	-	R
2	<i>Klebsiella</i>	R	19	-	R
3	<i>E. coli</i>	R	24	-	R
4	<i>Staphylococcus aureus</i>	R	-	24	R
5	CNS	R	-	20	R

Table 3: Action of kashaya of fresh guduchi in microbes

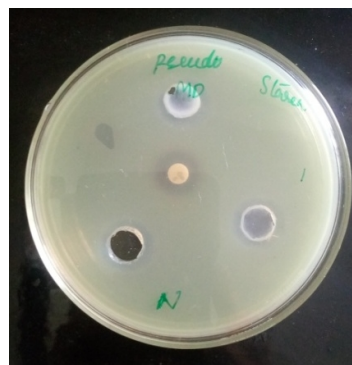
No:	Name of the Micro organism	Zone of inhibition (mm)			
		Sample drug	Ciprofloxacin (5 mcg)	Amoxiclav (30 mcg)	DMSO
1	<i>Pseudomonas</i>	18	14	-	R
2	<i>Klebsiella</i>	R	19	-	R
3	<i>E. coli</i>	R	24	-	R
4	<i>Staphylococcus aureus</i>	R	-	24	R
5	CNS	R	-	20	R

Table 4: Action of kashaya of dry guduchi in microbes

No:	Name of the Micro organism	Zone of inhibition (mm)			
		Sample drug	Ciprofloxacin (5 mcg)	Amoxiclav (30 mcg)	DMSO
1	<i>Pseudomonas</i>	R	14	-	R
2	<i>Klebsiella</i>	R	19	-	R
3	<i>E. coli</i>	R	24	-	R
4	<i>Staphylococcus aureus</i>	R	-	24	R
5	CNS	R	-	20	R

Table 5: Action of guduchi sattva on microbes

No:	Name of the Micro organism	Zone of inhibition (mm)			
		Sample drug	Ciprofloxacin (5 mcg)	Amoxiclav (30 mcg)	DMSO
1	Pseudomonas	16	14	-	R
2	Klebsiella	R	19	-	R
3	E. coli	R	24	-	R
4	Staphylococcus aureus	R	-	24	R
5	CNS	R	-	20	R

Figure 1: *Tinospora cordifolia* (Willd.) Hook. f. and Thoms.Figure 2: Zone of inhibition when *Pseudomonas* tested with Kashaya extract of fresh GuduchiFigure 3: Zone of inhibition when *Pseudomonas* tested with Guduchi sattva

RESULT & DISCUSSION

The microorganisms- *Klebsiella*, *Staphylococcus*, *E.coli* and CNS showed marked resistance against all the 5 samples taken for the study. The kashaya extract of fresh stem of Guduchi and Guduchi sattva showed antimicrobial activity against *Pseudomonas* species with a zone of inhibition 18 mm and 16 mm respectively. All the tested microorganisms showed resistance to the kashaya and alcohol extracts of dry Guduchi and alcohol extract of fresh Guduchi.

The tables 3 & 5 show the antimicrobial activity of kashaya of fresh stem of Guduchi and Guduchi sattva respectively. *Pseudomonas* species is found sensitive to these samples. Both these samples were water extracts of fresh Guduchi prepared by different methods. This shows the presence of water soluble active constituent against *Pseudomonas* in fresh form of Guduchi which was declined during the drying process. The antimicrobial potential of the drug is found to be more when used in the fresh form. Guduchi sattva which was a unique formulation in Ayurveda has shown significant antimicrobial activity. This provides a support for the use of fresh Guduchi

stem in formulations rather than dry stem which corroborates the classical knowledge of the usage of this plant only in its fresh form. The variations in the collection, processing or storage of herbal drugs could impact its efficacy profile. Since prior knowledge regarding collection and usage of most medicinal plants exists in classical text books, it can be used as a guide to quality evaluation.

CONCLUSION

T.cordifolia (Guduchi) is a medicinal plant which has been described to be used in various disease conditions according to different Ayurvedic text. This plant is an ingredient of different ayurvedic pharmaceutical preparations like Amruthothara kwatha, Guduchyadi kashaya and Rasnasaphthakam Kashayam. Bhavaprakasha nigantu attributes krimighna property to Guduchi. The present antimicrobial study gives a proof for this claim. The results of the study also corroborates with the advice of Shargadhara samhitha to use Guduchi only in fresh form. The antimicrobial study is done on limited microorganisms. The study should be repeated using other microorganisms and using different methods.

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