International Journal of Applied Ayurved Research ISSN: 2347- 6362

ANTIBACTERIAL ACTIVITY DECOCTION OF *COSCINIUM FENESTRATUM* ON STAPHYLOCOCCUS AUREUS:AN EXPERIMENTAL STUDY

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ABSTRACT

Coscinium fenestratum is one of the most important plants used in traditional systems of medicine. Pharmacological properties of the plant have been mentioned as *Vranajit* and *Vrananut* in Ayurveda classics especially including wound cleansing property. Unawareness of this, the plant has escaped utilization of *C.fenestratum* as anti bacterium. The current study focused on the bacterial wounds, by *Staphylococcus aureus* and the efficacy was evaluated using decoction of *C.fenestratum* as a washing agent. For antibacterial susceptibility test, testing drug was prepared in six different concentrations homogenously. Prepared decoctions of stem were tested for its potent antibacterial activity against proto type of *S.aureus* from laboratory specimens. Followed the Antibacterial Sensitivity Test, in duplicate discs of modified Agar well diffusion method on bacterial strains and assessed the efficacy comparing with positive control as Amoxicillin. The mean inhibitory zones in samples were measured as 15.5mm, 14.75mm, 20.25mm, 16.5mm, 12.5mm & 18.75mm respectively. All of the testing samples indicated significant antibacterial potential on laboratory specimens of *S.aureus*. The current experimental study has revealed that the stems of *C. fenestratum* have remarkable antibacterial effective on the *S. aureus*.

Keywords: Antibacterial activity, *Coscinium fenestratum, S.aureus,* stem,decoction

INTRODUCTION: There are total of some 250,000 species of higher plants in the world, much less than the species of animals (5-10 million). *Coscinium fenestratum* (Gaertn) Colebr is one of the most important plants used in traditional systems of medicine. *C. fenestratum* is belonging to family Menispermaceae. The vernacular name of the plant is Venivel in Sinhala, False calumba or Tree Turmeric in English and Atturam or Kadari in Tamil [1]. This species observe in medium climatic conditions and occurs in the hills of Malabar region, particularly Western Ghats and in the jungles of South India, Malacca, Singapore Sumatra and Sri Lanka. It is common in the moist low-country forests in Sri Lanka[2]. Morphologically , leaves are heart shape and there are 6 inches length. Outer surface of the leaves are yellow colour and there are yellow colour flowers. Fruits are small and brown colour. Stem and wood are used for medicinal preparations. This parts dried and taken as a medicine. *Vanuval* and *banval* are the synonyms for the *C. fenestratum* among the Sri Lankan people.[3]In SriLanka, people use *C. fenestratum* on behalf of Daru haridra plant (*Berberis aristata*) that Indian people use. *C.fenestratum* is the substitute for Daru haridra plant. The main chemical active compound of both plants is berberine[4]. Berberine is one of the major alkaloid constituent in *C. fenestratum*. Berberine has broad spectrum of

pharmacological activities. Their *Pancha padartha* are similar and we can see when we following literature knowledge that both plants have the same activity[5]. *C. fenestratum* is used for cleaning of wounds, for beauty culture, for eye diseases for skin diseases. Also *C. fenestratum* is used for making bath the small children. *C.fenestratum* is the energetic drink in fever conditions and general weakness of the body.

Also It has the *Raktha shodaka* (blood purifying) quality[6]. Such clinical indications show us that *C. fenestratum* is a valuable drug in the preparation of medicines.When there is a wound condition, our people used to the *C. fenestratum* as a drink. The experiments have been revealed that this plant has the quality to reduce the tetanus due to *berberine* chemical compound. Therefore it has been shown that our ancient people concept was very reasonable. Activities of this plant has been mentioned as *Vranajit* and *Vrananut* in the *Danwanthari nigandu* and *raaja nigandu*[7,8]. *Vruddatraya* also mentioned that *C. fenestratum* has the ability of *Vrana shodana*[9].

According to the Ayurveda definitions, *Vrana* is known as the *"Dosas samdgathaya"* (aggravated *dosas*) that originated in the one place of the human body. The term *"Vrana*" is derived from the verbal root "*vran*" meaning "splitting/ tearing of the body".(causing discontinuity of the skin and other tissues under it); since it causes discontinuity of the body it is called *vrana*. Dalhana gives the meaning of the verb *"vran"* is causing discolouration of the body. There are eight *Vruna vasthu* alias origin places of the *vrana* as *Tvak. mamsa, shira, snayu , Asthi, sandhi, koshta, marma*[10.] Epidermis is firstly invaded even the *Vrana* is originated due to any reason. Small holes in the *Vranas* get the relations with the external sensations. So that *Jivanu* (krimi) alias fungus, bacteria and virus insert to the body through the *Vrana* according to the Ayurveda concept. Following are the *Jivanu* that can be seen with the relations of the *Vrana*. *Staphylococcus aureus, Streptococcus pyogenes, Enterococci, Pseudomonas aeraginosa*[11]. Among this organisms, *Staphylococcus aureus* was identified as the main organism that survive in the open wounds[12]. *Staphylococcus is* one of the five most common causes of infections after injury or surgery. It affects around 500,000 patients in American hospitals annually. It is abbreviated to "*S. aureus*" or "Staph aureus" in medical literature.

In my research work, I attention for wounds which affected by bacteria like *Staphylococcus aureus* and aim of the present study was to evaluate the effect of decoction of *C. Fenestratum* as a washing agent on wound. It is expected by the cleaning a wound with the attacked by organisms such as bacterial, fungal and viral to remove or control their population. According to the Ayurveda text, method of treatment of wounds are sixty in number, such as *kashaya* (washing with astringent liquids), *varti* (keeping wicks of drug), *avachurana* (dusting powder of drug on wound), *ropana* (promoting healing), krimigna(destroying worms/antibacterial) ect. Out of these; *kashaya, varti, kalka, sarpi, taila, rasakriya, avachurana* are meant for *sodhana* (purifying) and *ropana* (healing)[13].Ayurvedic text explain mode of action washing agent should be mainly *shodana, ropana and krumighna*. *C. fenestratum* is such a valuable plant medicine in Ayurveda. When we refer the Pancha padartha of the *C. fenestratum*, We

can see that there is the quality of *Krimi nashaka*[14].According to the Ayurvedic definitions, the living matters that deactivate the human body functions were described under the *Krimi*. The basic principles of treatment methods for *Krimi* (microorganisms) are *krimi Apakarshana, prakurthi Vigathaya and Nidana parivarjana*[15]. it is mentioned under the *prakrthi vigathaya chikistha* as Materials which have *katu, ushna, tiktha* and *kashaya rasas* are very useful[16]. *C.fenestratum* also have the above qualities which are *katu, tiktha, kashaya* and *ushna* and therefore it is identified as a *Krimi nashaka* drug[17].The following books such as *Thalpathe Peliyama, Deshiya chikithsa samgrahaya* which are the traditional books in Ayurveda are presented Specific yogas of *Kriminashaka* ability of *C. fenestratum*[18,19].

Even the Uses of these plants have mentioned in *vruna chikithsa* and *krimi chikithsa* and both of chikithsa common factor is to sterilize the organisms. According to the *Ashtangahrdaya samhitha*, *C. fenestratum* is used as *Daruharidra* for purifying the *Siviya*[20]. *C. fenestratum* also mentioned as "*daruhelidda"* under the *vrana prakshalana kashaya* in the *Dvi vruniya chikithsa adyaya* in *charaka samhitha*[21].In *susrutha misrakadyaya* mentioned *C. fenestratum* was used for *Daruharidra* under the *vrana shodana kashaya* in sri lanka[22]. The *Shaka samgraha* grantha that is used abundantly in Ayurveda field also mentioned about the *C. fenestratum* for cleaning of wounds[23].it is also included in the Sharangadara samhitha uththara kanda under the *Vrana shodana yoga*[24].*Vrana shothadikara* of the *Bavaprakasha* also mentioned it's action against the wound[25]. it also mentioned in the lot of prepared drugs in Ayurveda such as *Kasisadi grithaya and gauradi grithaya* etc[26]. The qualities of this drugs are equal to *Kaha* (*vacha*) according to the *Drvyaguna geethaya*[27].

There are many information about *C. fenestratum* on the *Vrana shodana* effect. So that It is very important to find out the information of the *C. fenestratum* in the research manner. *Staphylococcus aureus* is the abundant bacteria that survive with the relation of the wounds. So that in this experiment, that bacteria is used to determine the objectives with the chemical stream. According to the *Qwatha paribasha* of the *Sharangadhara samhitha*, yellow colour stem (node parts) of the *C. fenestratum* which is the main part is used in the drug preparation. Prepared the six samples in different decoction and named A,B,C,D,E and F. That decoction are put into the bacterial medium and observe the growth of the bacteria. The well diffusion method is used under the ABST (Anti-Bacterial Sensitivity Test). After allowing to grow the bacteria in the appropriate medias and finally measured the inhibitory diameters.

Methodology

Collection of Plant Material

The node parts of venivel (*C. fenistratum*) were collected from Gampaha Wickramaratchi Sidhayurvedha Ausheda Co(PVT) LTD, Yakkala who are supply from Emiththirigala division, Sabaragamuwa province.

Identification Of Medicinal Plant Material

The medicinal plant material was identified and authenticated by Prof. Dr. A.H.M. Tisera from the department of Dravya Guna ,Gampahawickramarachchi Ayurveda Institute, University of Kelaniya, Yakkala, Sri Lanka.

INCLUSION CRITERIA

Laboratory specimens of *Staphylococcus aureus* bacteria.

EXCLUSION CRITERIA

Other bacteria colonies in wounds.

Drug Preparation

According to the "*Qwatha paribasha*" of Sharangadhara samhita[1]

One *pala* (60g) of coarsely powdered drugs is boiled with 16 parts of water in an earthen pot, over a mild fire till the liquid is reduced to 1/8 of the original quantity. this liquid is known as *shrta, qwatha, kashaya or niryuha* (decoction).

Instruments

Measuring scale - Knife
Measuring cylinder - Clay pot
Piece of clot - Measuring stick
Burner

Main Ingredients: *Darvi* (*Coscinium fenistratum*) ***Assessory Material*** - Water

Drug Preparation Method

First all the ingredients were identified. Measured the required amounts of *C. fenistratum*. Then cleaned well.After that cut them into small pieces. Prepared six samples in different decoction.

Sample A – *C. fenistratum* 60g, Water 120ml boiled under moderate heat to obtain 15ml of decoction.
Sample B - *C. fenistratum* 60g, Water 240ml boiled under moderate heat to obtain 30ml of decoction.
Sample C- *C. fenistratum* 60g, Water 480ml boiled under moderate heat to obtain 60ml of decoction.
Sample D- *C. fenistratum*60g, Water 960ml boiled under moderate heat to obtain 120ml of decoction.
Sample E- *C. fenistratum*60g, Water 1920ml boiled under moderate heat to obtain 240ml of decoction.
Sample F- Put unknown grams of *C. fenistratum* (hand measurement) , Water two tea cups boiled under moderate heat for ½ hrs.
positive controller - dilute the 10mg of Amoxicillin,1ml of distil water.
Negative controller - distil water

Antibacterial Assay Of Samples

Followed the Antibacterial Sensitivity Test (ABST) (Kirby-Bauer method) The effect of various plant decoctions on the bacterial strains were assayed by Agar well diffusion method.

Instruments

Auto clave - Hot air oven - Incubator Petri dishes - Glass tube - Glass Flask Glass beaker - Measuring cylinders Glass bottles - Micro pipette - Dry swabs - Foils - Cotton buds - Ruler Gloves- Mask -well cutter

Main Ingredients

Nutrient agar - Distill water -Peptone water - Muller hinton agar- Staphylococcus aureus (ATCC 25923) bacteria

Nutrent Broth Preparation

One litter of nutrient broth was prepared by dissolving 13 g of commercially available nutrient medium in 1000ml distilled water. The medium was dispensed as desired and sterilized by autoclaving at 15 lbs pressure (121ºC) for 15 minutes. After that keep the broth to cool in room temperature. Staphylococcus aureus (ATCC 25923) bacteria inoculated into the broth to growth and incubated at 37ºC for 18hrs.

MullerHinton Agar Medium Preparation

The medium was prepared by dissolving 38.16 g of the commercially available Muller Hinton Agar Medium (HiMedia) in 1000ml of distilled water and boiled to

[1] SARNGADHARA-SAMHITHA(TEXT WITH ENGLISH TRANSLATION), MADHYAMA KHANDA,QUATHA KALPANA

dissolve the medium completely. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mmsterile Petri plates (25-30ml/plate) while still molten and allowed to solidify at room temperature

Spread the Inoculum

Prepared the dilution series using the broth which are the maximum growth of Staphylococcus aureus (ATCC 25923) bacteria.

-1 dilution series - put the 1ml broth in to the 9ml peptone water

-2 dilution series - put the 1ml -1 dilution series in to the 9ml peptone water

-3 dilution series - put the 1ml -2 dilution series in to the 9ml peptone water.

According to the turbidity standard method (0.5 McFarland standard) select the -1 dilution series.

Get 100µl of -1 dilution series and put into the Muller Hinton agar plates.

Using a sterile cotton swab spread cultures of the test organism was made on the Muller Hinton agar plates.

Follow the Well Diffusion Method

Four wells of 8 mm diameter were punched into the agar on each plate using a sterile well cutter. Into two wells in each plate 40 µl of the various plant decoction was added. other wells in each plate positive and negative controller was added. Amoxicillin was used as a positive control and Distil water used as a negative control .The plates were incubated at 37ºC for 24 hrs.The antibacterial activity was evaluated by measuring the zone of inhibition around the well.

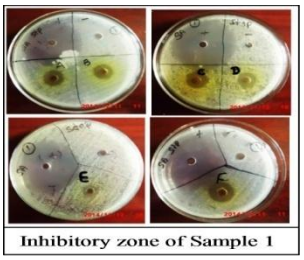
Used 4 samples in each and repeat the method

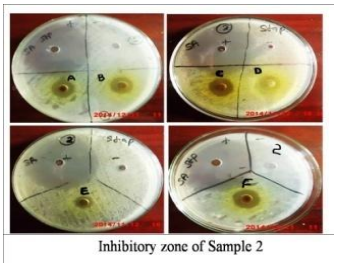
Table I -Zone Diameter / mm

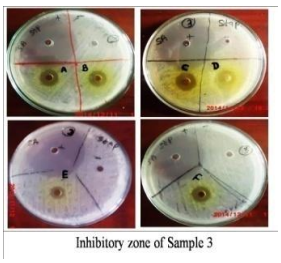
Sample	A	B	C	D	E	F	controller	
							+ ve	-ve
1	16	15	21	16	12	19	40	00
2	15	15	20	17	12	18	40	00
3	15	15	20	16	13	19	40	00
4	16	14	20	17	13	19	40	00

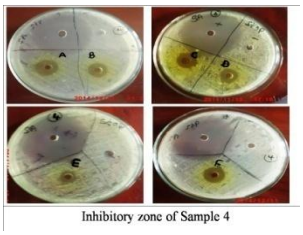
OBSERVATION & RESULT

Fig I : Zone Diameter of sample


Inhibitory zone of Sample 1


Inhibitory zone of Sample 2


Inhibitory zone of Sample 3


Inhibitory zone of Sample 4

In the present study, whole decoctions showed zone of inhibition around the well. Sample A mean inhibitory zone diameter was 15.5mm.Sample B mean inhibitory zone diameter was 14.75mm.Sample C mean inhibitory zone diameter was 20.25mm.Sample D mean inhibitory zone diameter was 16.5mm.Sample E mean inhibitory zone diameter was 12.5mm.Sample F mean inhibitory zone diameter was 18.75mm.Thereof Sample C which are the put 8 times water showed maximum activity against Staphylococcus aureus. Secondary Sample F showed best activity and Sample D, A, B showed their activity respectively. Sample E showed lowest activity against Staphylococcus aureus.

According to Comparative data analyzing, the significant antibacterial activity of the C. Fenistratum decoctions were compared with the standard antibiotic, Amoxicillin.

Standard Amoxicillin Sensitivity chart [2]

Antibiotic (Antimicrobial Agent)	Disc code	Resistant	Intermediate	Susceptible
		≤ or − mm	mm	− or > mm
Amoxicillin (other)	AMC	<13	14-17	>18
Amoxicillin (Staph)	AMC	19		20

Table II - Standard Amoxicillin Sensitivity chart According to T-test Test of μ =< 19 vs > 19

Variable	N(sample size)	Mean	Standard Deviation	SE Mean	95% Lower Bound	T –test value	P value
A	4	15.500	0.577	0.289	14.821	-12.12	0.999
B	4	14.750	0.500	0.250	14.162	-17.00	1.000
C	4	20.250	0.500	0.250	19.662	5.00	0.008
D	4	16.500	0.577	0.289	15.821	-8.66	0.998
E	4	12.500	0.577	0.289	11.821	-22.52	1.000
F	4	18.750	0.500	0.250	18.162	-1.00	0.804

[2] Fall 2011 – Jackie Reynolds, Richland College, BIOL 2421

Table III – T-test chart

Hypothesis

$H_0 : \mu \leq 19$ Vs $H_1 : \mu > 19$

By considering the above table, A,B,D,E and F samples mean less than or equal 19 under 5% significant level. But H_0 is rejected under 5% significant level for sample C. So sample C mean greater than 19.These results indicate that the sample C was in standard sensitivity value of Amoxicillin (= 20 or>20mm).

CONCLUSION

All of *Coscinium fenistratum* decoctions have antibacterial effect against laboratory specimens of *Staphylococcus aureus.*

Sample C which are the put 8 times water showed maximum activity against *Staphylococcus aureus.*Sample C was in standard sensitivity value of Amoxicillin (= 20 or>20mm).

Amoxicillin is normally used as an oral antibiotic for bacterial infections. *Coscinium fenistratum* decoctions are external application. But Sample C was in standard sensitivity value of Amoxicillin (= 20 or>20mm). So it is better to use a local antibacterial application for comparing the efficacy of *Coscinium fenistratum.*

Acknowledgement

I would like to express my utmost gratitude to my supervisor, Senior lecturer Dr. H. S. Sakunthala for her guidance, perpetual energy, enthusiasm in research and educating me during my research. I could not imagine the success of this research without her supervising. Also thanks to staff of the Laboratory and Pharmaceutical factory of the Gampaha Wickramarachchi Ayurveda Institute, University of Kelaniya, Sri lanka.

My deepest gratitude goes to my mother, brothers and sister and relatives for their love, care and support throughout my life.

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Source of support: Nil
Conflict of interest: None Declared

Cite this Article as : *[Aberathna K.A.S et al;Antibacterial activity Decoction of Coscinium Fenestratum on Staphylococcus Aureus ;An Experimental Study] www.ijaar.in : IJAAR VOLUME III ISSUE II SEP-OCT 2017 Page No:873-880*