


**Research Article**
**IN VITRO ANTIBACTERIAL EFFECT OF AQUEOUS EXTRACT OF *SIDDHARTAKA SNANA* USED IN TREATMENT OF *VICARCHIKA* (ECZEMA)**
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**ABSTRACT**

*Vicarchika* is a type of *Kushta Roga* (skin disease). It can be correlated with eczema. *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* are some of the bacteria which cause skin diseases. *Siddhartaka Snana* is a herbal bath that is described in Charaka Samhita as a treatment for *Kushta Roga*. *Siddhartaka Snana* is a herbal preparation which consists ten herbs namely; *Cyperus rotundus*, *Catunaregum spinosa*, *Phyllanthus emblica*, *Terminalia chebula*, *Terminalia bellarica*, *Cassia fistula*, *Pongamia pinnata*, *Holarrhena antidysentrica*, *Alastonia scholaris* and *Coscinum fenestratum*. Antibacterial effect of *Siddhartaka Snana* against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* that are responsible for skin diseases was studied using disc diffusion method and well diffusion methods. In disc diffusion method, no inhibition zone was observed with any of the tested bacteria. However, an inhibition zone of 9.67mm was observed against *Staphylococcus aureus* in well diffusion method with *Siddhartaka Snana*. Minimum Inhibitory Concentration (MIC) of *Siddhartaka Snana* against *Staphylococcus aureus* was 0.9225 mg/1ml. Ingredients of *Siddhartaka Snana* contain tannins, phenols and flavonoids, all of which possess antibacterial properties. Antibacterial effect of *Siddhartaka Snana* may have been exhibited due to actions of these phytochemicals. It was concluded that *Siddhartaka Snana* has an antibacterial effect against *Staphylococcus aureus*.

**KEYWORDS:** Eczema, *Siddhartaka Snana*, Antibacterial effect, *Kushta Roga*.

**INTRODUCTION**

*Vicarchika* is a type of *Kushta Roga* (skin diseases) described in Ayurveda.<sup>[1]</sup> *Siddhartaka Snana*, a type of herbal bath and a disinfecting and cleansing liquid composed of following medicinal herbs is mentioned in Charaka Samhita. It is prescribed in the treatment of *Kushta* including *Vicarchika* (eczema). Ingredients of *Siddhartaka Snana* are *Cyperus rotundus* (Family: Cyperaceae, Sinhala name: Kalanduru), *Catunaregum spinosa* (Family: Rubiaceae, Sinhala name: Kukuruman), *Phyllanthus emblica* (Family: Euphorbiaceae, Sinhala name: Nelli), *Terminalia chebula* ((Family: Combretaceae, Sinhala name: Aralu), *Terminalia bellarica* (Family: Combretaceae, Sinhala name: Bulu), *Cassia fistula* (Family: Fabaceae, Sinhala name: Ehela), *Pongamia pinnata* (Family: Fabaceae, Sinhala name: Magul Karanda), *Holarrhena antidysentrica* (Family: Apocynaceae, Sinhala name: Kelinda), *Cedrus deodara* Family: Pinaceae, Sinhala name: Devadara) and *Alastonia scholaris* (Family: Apocynaceae, Sinhala name: Gasrukattana). *Coscinum*

*fenestratum* (Family: Menispermaceae, Sinhala name: Weniwegeta) was used in this study instead of *Cedrus deodara*.<sup>[2]</sup> Skin and soft tissue infections are one of the most common infections associated with *Kushta Roga* in patients of all age groups. *Escherichia coli* strains are one of the most frequently isolated microbes from skin and soft tissue infections (SSTI).<sup>[3]</sup> *Staphylococcus aureus*, *Pseudomonas aeruginosa* are some bacteria that are responsible for the infections in the skin. So far, no known scientific study has been carried out to evaluate the antibacterial effect of *Siddhartaka Snana*. Therefore, it was decided to study the antimicrobial effect of *Siddhartaka Snana* on bacterial skin infections such as caused by *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*.

**Materials and Methods**
**Preparation of the extract of *Siddhartaka Snana***

1 gm each of tubers of *C. rotundus*, fruits of *C. spinosa* and *P. emblica*, pericarps of *T. chebula* and *T. bellarica*, stem barks of *C. fistula*, *P. pinnata*, *H.*

*antidysentrica* and *A. scholaris* and vine of *C. fenestratum* were cleaned and powdered separately and mixed together. The mixture refluxed with 50ml of distilled water for one hour and filtered. The filtrate was boiled for another 5 minutes and poured into separate bottles (5ml bottles). Then, the extracts were filtered, freeze dried and weighed to determine the concentration for preparing stock solution which was 36.9 mg/ml.

**Evaluation of Antibacterial Activity**

Antibacterial activity of the water extract of *Siddhartaka Snana* were evaluated using Disc diffusion method and also with Well diffusion method against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*.

**Disc diffusion method**

Disc diffusion assay was carried out as follows. 6mm diameter filter paper discs were sterilized by dry heat sterilization at 160 °C for one hour. 10 microliters of test suspension solution was then in cooperated into the discs. A suspension of each test organism at an inoculum density of 0.5 Macfarland standard was prepared in sterile normal saline.<sup>[4]</sup> Mueller-Hinton Agar (MHA) plates were inoculated with suspensions of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. Discs containing test solution of Siddhartaka Snana were placed on the plates. All tests were performed in triplicate against each organism. A purity plate was put up as the negative control for suspension tested. Plates were kept at room temperature for 2 hours and then incubated at 37 °C degrees overnight. The purity plates were checked for growth. The diameter of the inhibitory zones were measured and recorded.

Conventional antibiotic discs of Ampicillin and Ciprofloxacin were tested against as positive control for the test organisms and zone size was measured and recorded.

**Well diffusion method**

The same procedure used in Disc Diffusion method was followed but instead of discs, 10 microliters of test suspension was added to wells (6mm in diameter) cut in the plate. The diameter of the inhibitory zones were measured and recorded.

**Detection of Minimum Inhibitory Concentration (MIC) of Siddhartaka Snana against selected bacteria**

Minimum Inhibitory Concentration (MIC) of *Siddhartaka Snana* against selected bacteria was detected using Agar dilution method and with a serial dilution of *Siddhartaka Snana* stock solution as follows.

A serial doubling dilutions of the stock solution was prepared in sterile saline avoiding any contamination, making volume of concentration to 10ml. Dilution series was as follows. 1:10 1:20, 1:40, 1:80, 1:160, 1:320 and 1:640.

3.8 gm of Mueller Hinton powder was weighed for 100 ml for each concentration. 100ml of for each concentration was prepared in separate container with 90ml of water added to it and sterilized at 121°C for 15 minutes. 1:10, 1:20, 1:40, 1:80, 1:160, 1:320 and 1:640 diluted extractions, 10ml of each are added to separate cooled (45°C - 50°C) Mueller Hinton Agar base. Those plates were allowed to set and dried at 37°C.

Inoculation was carried out as follows. 18-24 hours incubated actively growing standard cultures were used to prepare inoculation broths. Suspension in saline similar to 0.5 MacFarland standard is diluted 100 times and used as working suspension. Inoculum is applied as a spot circle about 5-8 mm using 10ul calibrated wire loop. On a single plate 5 spots are made and for each concentration and 3 plates are used to test in triplicate. Control plate (Muller Hington Agar without any drug) for each organism was also similarly inoculated.

After the inoculation plates were kept upward on the bench for 4 hours to dry the inoculum and then inverted and incubated at 37°C for 18 hours.

**Results**

No antibacterial activity of Siddhartaka Snana was observed against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* in Disc diffusion method. However in well diffusion method considerable antibacterial effect of *Siddhartaka Snana* was observed against *Staphylococcus aureus* as given in Table 2. Minimum Inhibitory Concentration (MIC) of *Siddhartaka Snana* against *Staphylococcus aureus* was 0.9225 mg/1ml (Table 3.)

**Table 1: Antibacterial activity of Siddhartaka Snana against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* with Disc Diffusion method**

| Microorganisms                | Disc Diffusion Method |         |         |
|-------------------------------|-----------------------|---------|---------|
|                               | Plate A               | Plate B | Plate C |
| <i>Staphylococcus aureus</i>  | NZ                    | NZ      | NZ      |
| <i>Escherichia coli</i>       | NZ                    | NZ      | NZ      |
| <i>Pseudomonas aeruginosa</i> | NZ                    | NZ      | NZ      |
| NZ=No Zone                    |                       |         |         |

**Table2: Antibacterial effect of Siddhartaka Snana against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* observed in the Well diffusion method**

| Compound  | Growth Inhibition on                     |                                     |   |
|---|--|-------------------------------------|---|
|   | <i>Staphylococcus aureus</i> (Zone size) | <i>Escherichia coli</i> (Zone size) | <i>Pseudomonas aeruginosa</i> (Zone size) |
| Water extract of <i>Siddhartaka Snana</i>   | 9.67mm                                   | NZ                                  | NZ  |
| Positive Control  | 30 mm (Ampicillin 10mcg)                 | 40 mm (Ciprofloxacin 5 mcg)         | 28 mm (Ciprofloxacin 5 mcg)               |
| Negative Control (Muller Hinton Agar without any drug). Each organism is similarly inoculated | NZ                                       | NZ                                  | NZ  |

NZ=No Zone

**Table3: Minimum Inhibitory Concentration (MIC) of *Siddhartaka Snana* on *Staphylococcus aureus***

| Bacteria                     | Concentrations of the solution |    |    |                        |    |    |                         |    |    |                         |    |    |                          |    |    |                          |    |    |                          |    |    | MIC             |
|------------------------------|--------------------------------|----|----|------------------------|----|----|-------------------------|----|----|-------------------------|----|----|--------------------------|----|----|--------------------------|----|----|--------------------------|----|----|-----------------|
|                              | 1/10<br>(3.69 mg/1ml)          |    |    | 1/20<br>(1.845 mg/1ml) |    |    | 1/40<br>(0.9225 mg/1ml) |    |    | 1/80<br>(0.4612 mg/1ml) |    |    | 1/160<br>(0.2306m g/1ml) |    |    | 1/320<br>(0.1153 mg/1ml) |    |    | 1/640<br>(0.0577m g/1ml) |    |    |                 |
|                              | P1                             | P2 | P3 | P1                     | P2 | P3 | P1                      | P2 | P3 | P1                      | P2 | P3 | P1                       | P2 | P3 | P1                       | P2 | P3 | P1                       | P2 | P3 |                 |
| <i>Staphylococcus aureus</i> | N                              | N  | N  | N                      | N  | N  | N                       | N  | N  | G                       | G  | G  | G                        | G  | G  | G                        | G  | G  | G                        | G  | G  | 0.9225 mg /1 ml |

P = Plate ; NG= No growth; MIC = Minimum Inhibitory Concentration

## Discussion

In the present study, *Siddhartaka Snana* exhibited antibacterial action against only *Staphylococcus aureus*. However antibacterial activity of the ingredients of *Siddhartaka Snana* singly against *Escherichia coli*, *Streptococci* and *Pseudomonas aeruginosa* were scientifically proven through several researches as presented in Table 4. But the *Siddhartaka Snana* which was prepared in combination of these ingredients exhibited antibacterial action only on *Staphylococcus aureus* among the tested bacteria. It may be due to varying concentration of test samples.

Ingredients of *Siddhartaka Snana* contain flavonoids, phenols and tanins as tabulated in Table 5. *Siddhartaka Snana* may have exhibited its antibacterial action by inhibiting nucleic acid synthesis, cytoplasmic membrane function, attachment and biofilm formation, the porin on the cell membrane and by altering the membrane permeability due to action of flavonoids in the ingredients of *Siddhartaka Snana*.<sup>[5]</sup>

Ingredients of *Siddhartaka Snana* have Phenol and phenolic compounds. *Siddhartaka Snana* may have exhibited its antibacterial activity by the modifying permeability of cell membranes. It may have exhibited its antibacterial activity by causing changes in a multitude of intracellular functions that are induced by hydrogen binding of the phenolic compounds to enzymes or by the alteration of cell wall rigidity with integrity losses due to different interactions with the cell membrane through Phenol and phenolic compounds.<sup>[6]</sup>

*Siddhartaka Snana* may have exhibited its antibacterial action through Tannin which is contained in the ingredients of *Siddhartaka Snana*. Tannin may have exhibited its antibacterial activity through inhibition of extracellular microbial enzymes, deprivation of the substrates required for microbial growth or direct action on microbial metabolism through inhibition of oxidative phosphorylation.<sup>[7,8]</sup>

**Table 4: Antibacterial effect of the ingredients of Siddhartaka Snana described in previous investigations**

| Ingredient                            | <i>Staphylococcus aureus</i> | <i>Pseudomonas aeruginosa</i> | <i>Escherichia coli</i> |
|---------------------------------------|------------------------------|-------------------------------|-------------------------|
| <i>Cyperus rotundus</i> [9,10]        | +                            | +                             | +                       |
| <i>Catunaregum spinosa</i> [11]       | -                            | +                             | +                       |
| <i>Phyllanthus emblica</i> [12]       | -                            | +                             | +                       |
| <i>Terminalia chebula</i> [13]        | +                            | +                             | -                       |
| <i>Terminalia bellarica</i> [14]      | +                            | +                             | +                       |
| <i>Cassia fistula</i> [15]            | +                            | -                             | +                       |
| <i>Pongamia pinnata</i> [16]          | +                            | +                             | +                       |
| <i>Holarrhena antidysentrica</i> [17] | +                            | -                             | +                       |
| <i>Coscinum fenestratum</i> [18]      | +                            | -                             | +                       |
| <i>Alastonia scholaris</i> [19]       | -                            | +                             | -                       |

**Table 5: Phytochemicals of ingredients of Siddhartaka Snana**

| Ingredient                            | Alkaloids | Flavonoids | Saponin | Phenol | Tannins | Terpenoids | Phlobatannins | Anthraquinones |
|---------------------------------------|-----------|------------|---------|--------|---------|------------|---------------|----------------|
| <i>Cyperus rotundus</i> [20]          | -         | +          | -       | +      | +       | -          | -             | -              |
| <i>Catunaregum spinosa</i> [11]       | +         | +          | +       | +      | +       | +          | +             | -              |
| <i>Phyllanthus emblica</i> [21]       | -         | +          | +       | +      | +       | +          | -             | -              |
| <i>Terminalia chebula</i> [13]        | +         | -          | +       | +      | +       | -          | -             | -              |
| <i>Terminalia bellarica</i> [22]      | +         | +          | -       | +      | +       | -          | -             | -              |
| <i>Cassia fistula</i> [15]            | -         | +          | +       | +      | +       | +          | -             | +              |
| <i>Pongamia pinnata</i> [23]          | +         | +          | +       | -      | +       | -          | -             | -              |
| <i>Holarrhena antidysentrica</i> [24] | +         | +          | +       | -      | -       | -          | -             | -              |
| <i>Coscinum fenestratum</i> [25]      | +         | +          | +       | -      | +       | +          | -             | -              |
| <i>Alastonia scholaris</i> [26]       | +         | -          | +       | -      | -       | +          | -             | -              |

## CONCLUSION

As *Siddhartaka Snana* exhibited growth Inhibition on *Staphylococcus aureus*, it can be concluded that it is beneficial in the treatment of *Vicarchika* (eczema) including infection caused by *Staphylococcus aureus*. However, further studies are essential to understand the underlying mechanism responsible for the documented healing effect of *Siddhartaka Snana*.

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