



ANTIBACTERIAL ACTIVITY OF MURCHITA AND AMURCHITA ARKA TAILA PREPARED WITH DIFFERENT RATIOS OF KALKA: AN *IN VITRO* STUDY

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ABSTRACT

Appearance carries a lot in modern world. Human beings are very much aware about the way they present before others. Vicharchika (eczema) is one of the most common skin problems and bacterial infection is one of the causes for this. *Staphylococcus aureus* is one of the bacteria found as a cause in experimental studies. Arka taila is a sneha kalpana preparation explained in Sharangadhara samhita that has been taken for this study with the aim of evaluating the effect of Arka taila prepared with a special concept named as murchana (addition of some other drugs to main formulation according to the base of taila used). All drugs used in this preparation are having anti-bacterial activity separately. Hence two methods (with and without murchana) of Arka taila were prepared with different ratios of kalka. The experimental study shows that on *Staphylococcus aureus*, both the samples of murchita arka taila prepared with 1/8th and 1/4th kalka has shown zone of inhibition at concentrations 25µl, 50µl, 75µl, 100µl. This study has proved that Arka taila prepared with murchana has anti-bacterial effect on *Staphylococcus aureus*.

KEY WORDS: Vicharchika, Eczema, Arka taila, Murchana, *Staphylococcus aureus*, Sneha Kalpana

INTRODUCTION

In the present era, scientific validation of prepared medicine is to be followed to ensure the safety and efficacy of the drugs. It is to be carried out with the techniques available in the contemporary science. With advanced instruments and experimental models, it is possible to identify the microorganism that causes diseased condition and also has made it simple to evaluate the efficacy of the drug for that particular organism. Classical references have mentioned the knowledge of invisible organisms which causes the disease. Arka Taila is a classical and potent taila indicated for vicharchika, kandu and pama¹. The drugs used for Arka taila are Arka (*Calotropis procera*)², Haridra (*Curcuma longa*)³, Sarshapa (*Brassica campstris*)⁴ and the drugs used for Murchana are Haridra (*Curcuma longa*)⁵, Musta (*Cyperus rotundus*)⁶, Bilva (*Aegle marmelos*)⁷, Dadima (*Punica granatum*)⁸, Nagakesara (*Mesua ferrea*)⁹, Krishna jeeraka (*Carum carvi*)¹⁰, Hrivera (*Pavonia odorata*)¹¹, Nalika (*Cinnamomum tamala*)¹², Vibhitaki (*Terminalia belerica*)¹³ and Manjistha (*Rubia cardifolia*)¹⁴.

By a literary search on the anti-bacterial action of the drugs, it can be said that the drugs have anti-bacterial activity. A study has proved that *C. gigantea* (Arka patra) represents a rich source of valuable medicinal compounds and leaves of *C. gigantea* contain high anti-bacterial property and can be further be explored for the isolation of its bioactive compound¹⁵. Another study highlights that Arkataila is effective in management of Vicharchika. It reduces various symptoms of Vicharchika¹⁶ and likewise it has been said in a study that the Kushthaghna and Kandughna properties of Arka can be used in treatment of Vicharchika¹⁷.

In this present study in-vitro evaluation of anti-bacterial activity of four Samples of Arka taila was conducted.

METHODOLOGY

Anti-microbial Test Types¹⁸: Diffusion Test and Dilution Test

Diffusion Test: Diffusion consists of two method i.e., Agar well diffusion and Agar disc diffusion

Agar Well Diffusion

The Agar diffusion assay is one method for quantifying the ability of antibiotics to inhibit microbial growth against the test drug. A known quantity of Micro-organism is grown on Agar plate. The well is bored with the help of Borer, Standard drug and test drug of desired concentration is poured in well. If the organism is susceptible to a particular antibiotic or a test drug, an area of clearing zone where organism is not capable of growing will be noticed i.e., called as zone of inhibition. In general, larger zones correlate with smaller minimum inhibitory concentration (MIC) of antibiotic for that organism. Inhibition produced by the test is compared with that produced by known concentration of a reference compared.

Agar Disc Diffusion

It is similar as the Agar well diffusion method instead of wells, the disc should not be placed closer than 24mm in the agar plate. Not more than 12 discs should be placed on a 150 mm plate. The disc must be pressed own with forceps to ensure complete contact with the Agar surface.

Dilution Method

In this serial dilution of the drug is prepared and inoculated with the test microbe. In the tube dilution method, serial dilutions of the drug in broth are taken in tubes and a standardised suspension of the test microbe inoculated. After overnight incubation, the minimum inhibitory concentration (MIC) is read by noting the lowest concentration of the drug that inhibits growth.

Aim and Objective of the Study

In this study Anti-bacterial susceptibility assessment of Arka taila was done against *Staphylococcus aureus* with four different samples. Sample A (Murchita Arka taila prepared with 1/8th kalka), Sample B (Amurchita Arka taila prepared with 1/8th kalka), Sample C (Murchita Arka taila prepared with 1/4th kalka), Sample D (Amurchita Arka taila prepared with 1/4th kalka).

MATERIAL AND METHODS

Materials: The four samples were named as Sample A, Sample B, Sample C, Sample D and the micro-organism the study conducted on was *Staphylococcus aureus*. The equipments used were Incubator, Laminar air flow (with flame), Autoclave (vertical), Digital colony counter, Water bath, pH meter, Inoculation loop, Borer, Hot air oven and Pipette. The Glass wares used were Petri dish, Beaker, Conical flask, Test tube and Stirrer.

Methods

Anti microbial activity was done by Agar – well diffusion method. Assessment through Disc diffusion study was measured by following measures: Sensitive zone, Intermediate zone and resistant zone

Anti- microbial susceptibility test against *Staphylococcus aureus*
Culture media for the organism

Preparation of Nutrient Broth

The beef extract (1 g), yeast extract (2 g), peptone (5 g), Sodium Chloride (5 g) was dissolved in 990 ml of distilled water. The pH was adjusted to 7.2 and made up the volume to 1000 ml and autoclaved at 121°C for 20 minutes.

Preparation of Nutrient Agar Media

The beef extract (1 g), yeast extract (2 g), peptone (5 g), Sodium Chloride (5 g) was dissolved in 990 ml of distilled water. The pH was adjusted to 7.2 and the volume was made up to 1000 ml. Finally (15 g) agar was added to the media and autoclaved at 121°C for 20 minutes.

Standard Drug¹⁹

In this study the Ampicillin was used as a Standard drug.

Agar Well Diffusion Method

The work place was cleaned in laminar air flow using 70 % ethyl alcohol and switched on the UV for 20 minutes. One loop of *Staphylococcus aureus* was inoculated from the culture into 10 ml of nutrient broth and mixed well. 15 ml of the agar medium was poured uniformly over the sterile petridish. One ml of nutrient broth was added containing the organism uniformly over petridish, mixed well and allowed for the media to solidify. Five equidistant wells on the plate were made. Different volumes of test samples were added of 25 µl, 50 µl, 75 µl and 100 µl. 100 µl of standard (Ampicillin) was taken separately into different wells. All the petridish were incubated at 37°C for 24 hrs. After the incubation period, the zone of inhibition was measured. Experiment was carried out in duplicate.

In Plate 1 **Sample A:** *Murchita arka taila* with 1/8th *kalka* (25 µl, 50 µl, 75 µl and 100 µl) were added to different wells.

In Plate 2 **Sample B:** *Amurchita arka taila* with 1/8th *kalka* (25 µl, 50 µl, 75 µl and 100 µl)

In Plate 3 **Sample C:** *Murchita arka taila* with 1/4th *kalka* (25 µl, 50 µl, 75 µl and 100 µl) to different wells.

In Plate 4 **Sample D:** *Amurchita arka taila* with 1/4th *kalka* (25 µl, 50 µl, 75 µl and 100 µl) to different wells.



AGAR MEDIA



BROTH



STAPHYLOCOCCUS AUREUS



MEDIA

Figure 1: *In vitro* Anti- bacterial activity

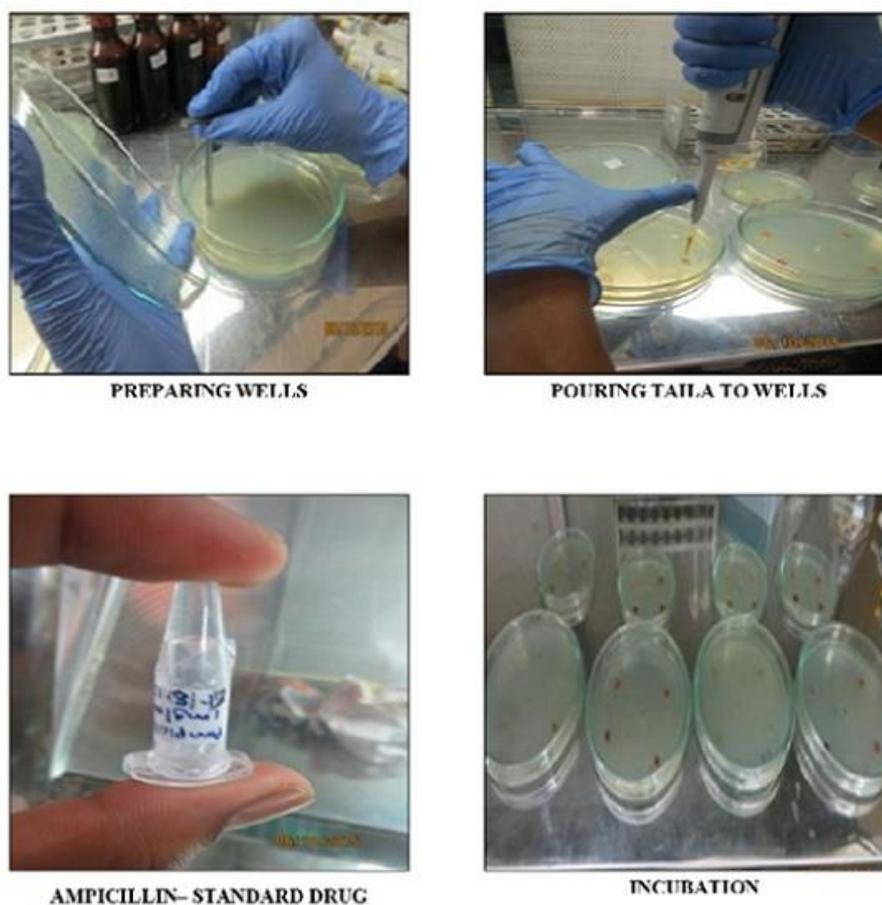


Figure 2: *In vitro* Anti- bacterial activity

RESULTS & DISCUSSION

It is observed that the oil sample A and C at volume 25 μ l, 50 μ l, 75 μ l, 100 μ l showed mild anti- bacterial activity against *Staphylococcus aureus*.
Sample B and D did not show any anti- bacterial activity against *Staphylococcus aureus*.

Table 1: In- vitro Anti- bacterial activity test for oil Sample A, Sample B, Sample C, Sample D against *Staphylococcus aureus*

Volumes (μ l)	Zone of Inhibition (mm) Murchita Arka Taila with 1/8 th kalka (Sample – A)	Zone of Inhibition (mm) Amurchita Arka Taila with 1/8 th kalka (Sample – B)	Zone of Inhibition (mm) Murchita Arka Taila with 1/4 th kalka (Sample – C)	Zone of Inhibition (mm) Amurchita Arka Taila with 1/4 th kalka (Sample – D)
25 μ l	05	0	05	0
50 μ l	05	0	05	0
75 μ l	05	0	05	0
100 μ l	06	0	05	0

Zone of Inhibition of Test Standard Drug in *Staphylococcus aureus* Organism

Average zone of inhibition for Standard drug Ampicillin was found to be 15 mm at 1 mg/ml concentration for 4 Samples (Sample A, B, C, D)

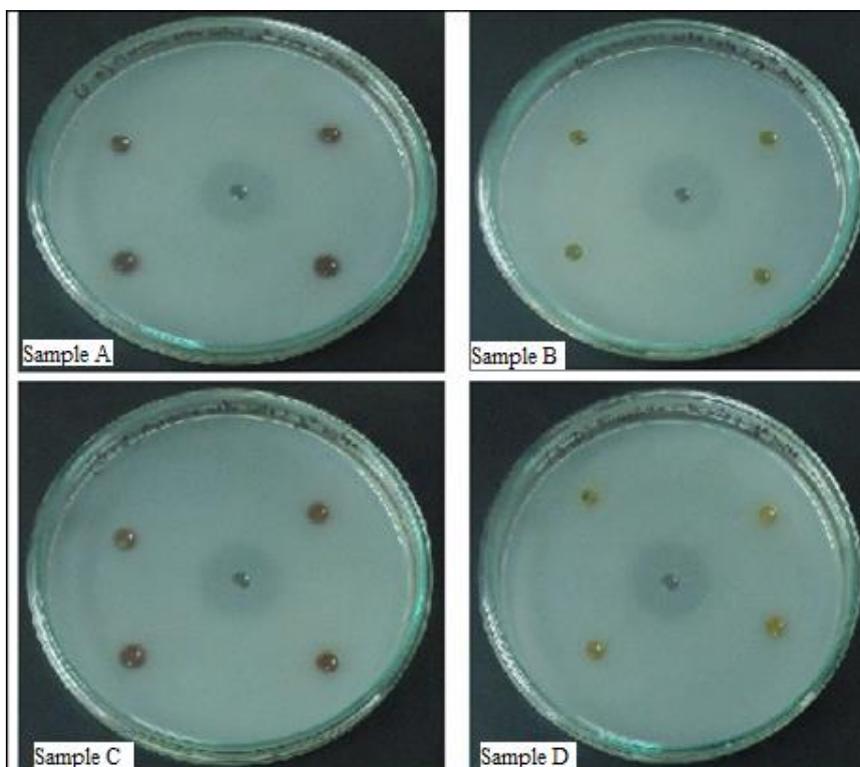


Figure 3: *Staphylococcus aureus* Zone of Inhibition of Sample A, B, C, D

Antibacterial activity against *Staphylococcus aureus* at concentrations of 25µl, 50 µl, 75 µl, 100 µl was observed in the Arka taila samples that were subjected to Murchana. Both the Murchita Arka taila samples with both 1/8th and 1/4th *kalka* have proved effective. This clearly indicates that the process of Murchana is important to extract the chemical constituents of the drugs used in Sneha kalpana. The drugs used for the murchana process may also contribute in adding more therapeutic properties to the formulation.

CONCLUSION

Murchita Arka Taila prepared with both 1/8th and 1/4th *kalka* has shown antibacterial activity against the *Staphylococcus aureus* at concentrations of 25µl, 50 µl, 75 µl, 100 µl. External application of this taila does not lead to growth of bacteria by its anti bacterial activity.

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