

## EVALUATION OF ANTI MICROBIAL POTENTIAL OF APAMARGA KSHARA SAMPLES PREPARED BY JALA AND GOMUTRA

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### ABSTRACT

**Introduction:** *Kshara* (alkali derived from ash of selected plants) is known to possess properties like *Shodhana* (wound cleansing action), *Ropana* (wound healing action), *Darana* (slough removing action), *Kusthaghna* (acts on skin ailments) and *Krimighna* (anti-microbial). *Kshara* is widely utilised in the management of *Nadi Vrana* (fistula) and *Dusta Vrana* (infected wound) indicating that it may have some anti-microbial properties. At the same instance we do get the reference of preparing *Kshara* by using different menstrum. With keeping these facts in mind present study was planned.

**Aim:** The aim of the study was to evaluate the anti-microbial action of *Apamarga Kshara* with *Jala* (AKJ) and *Apamarga Kshara* with *Gomutra* (AKG). **Materials and Methods:** The study was carried out at S.D.M. Centre for Research in Ayurveda and Allied Sciences, Udupi by opting well diffusion method on *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Microsporium canis*. **Results:** The results showed that both samples rendered anti-bacterial action at different concentrations on selected pathogens while they have not shown any action on the selected fungus at any concentration. **Conclusion:** *Kshara* possess *Krimighna* action as mentioned in classics if bacteria are taken into consideration.

**Key words:** *Apamarga*, *Kshara*, *Jala*, *Gomutra*, Anti-microbial action.

### INTRODUCTION

We get an elaborate description of the drugs and formulations which are indicated in condi-

tions like *Arsha* (haemorrhoids), *Bhagandara* (fistula-in-ano) etc. *Kshara* is one among

those medicaments which have proved their efficacy in effectively managing these clinical entities. When the opinion of the contemporary science is taken into consideration, these conditions are often attributed to infection caused by different pathogens. As far as the mode of action of *Kshara* in such conditions is concerned, it can easily be explained on the basis of the status of various *Dosha*, *Dhatu* and *Mala*. The *Kshara* helps in bringing these factors in a state of equilibrium with the help of its properties like *Dahana*, *Darana*, *Pachana* (digestion), *Shodhana*, *Ropana*, *Krimighna*<sup>1</sup> etc. and thereby managing the signs and symptoms of the disease. It also indicates that the *Kshara* may have anti-microbial potential which help in combating these infectious conditions. Furthermore, *Kshara* is said to be *Kusthaghna*<sup>2</sup> in nature. *Kustha* is an elaborate term under the umbrella of which various skin diseases are described. With the recent studies it has been proved that many pathogens as known to cause various skin ailments. So the *Kusthaghna* nature of *Kshara* again intimates about the anti-microbial potential of *Kshara*.

Thus, to understand and interpret the *Kshara Dravya's* 'mode of action' and to establish its anti-microbial potential on the selected pathogens, this study was carried out.

### OBJECTIVES OF THE STUDY

To evaluate the anti-microbial potential of two samples of *Apamarga Kshara* prepared by *Jala* and *Gomutra* and to understand the role of different menstrum on the efficacy of the medicament.

### MATERIALS AND METHODS

**Sample Preparation:** The preparation of *Apamarga Kshara with Jala* (AKJ) and *Apamarga Kshara with Gomutra* (AKG) samples was carried out at Practical Hall, Department of Rasashastra and Bhaishajya Kalpana, Shri Dharmasthala Manjunatheshwara College of Ayurveda, Kuthpady, Udupi, Karnataka. The methodology opted was as mentioned in Sushruta Samhitha<sup>3</sup>.

**Source of Data:** The study was carried out at S.D.M. Centre for Research in Ayurveda and Allied Sciences, Kuthpady, Udupi, Karnataka.

**Pathogens used for the study:** The following micro-organisms were selected for the study-

#### Bacteria-

- *Escherichia coli*
- *Klebsiella pneumoniae*
- *Staphylococcus aureus*
- *Pseudomonas aeruginosa*.

#### Fungus-

- *Microsporium canis*

### SELECTION OF MICRO-ORGANISMS-

As told in the classics, *Kshara* is the drug of choice in the management of the clinical conditions like *Arsha*, *Bhagnadara* etc. If we look upon the contemporary point of view these conditions are correlated with haemorrhoids, ano-rectal abscess and fistula-in-ano. These conditions are often found associated with bacterial infections. Varied microbes are held responsible for their incidence<sup>4</sup>. Four of such bacteria were selected for the present study based upon their availability and the feasibility to carry out the study.

*Kshara* application is also indicated in condition of *Kustha*. *Kustha* can be understood as a broader term under the umbrella of which the diseases manifested on skin are categorised

and described in *Ayurveda*. *Microspoum canis* is one of the fungi which is found responsible in skin disorders like ring worm infestation etc. Keeping this fact in mind it was selected for the study.<sup>5,6</sup>

**Materials required:**

- Test strains
- Distilled water, saline
- Test tube, Incubator and Laminar air flow
- Hot air oven and graduated micropipettes- 20, 50, 100  $\mu$ l, sterile tips
- Growth medium: Nutrient agar
- Samples of AKJ and AKG.

**Method for Anti-bacterial Study:** The whole procedure can be sub-divided in following steps-

- Preparation of different dilutions of AKJ and AKG.
- Preparation of nutrient medium.
- Preparation of inoculum.
- Well diffusion method for testing the anti-microbial action and reading of Zone of Inhibition (ZOI).
- **Preparation of different dilutions of AKJ and AKG:**

500 mg of the sample was taken and made to a volume of 0.9 ml with distilled water. As a result thick slurry was formed. From this slurry volumes approximating to 10 mg and 40 mg of the drug were taken for the study. Along with these diluted samples, 15 mg of the sample was loaded directly for the well diffusion method. The same methodology was opted for both the samples of AKJ and AKG.

- **Preparation Of Nutrient Medium:**

**a) Preparation of Nutrient agar media:<sup>7</sup>**

Beef extract (1 g), yeast extract (2 g), peptone (5 g) and Sodium Chloride (5 g) were 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.

**b) Sterilization:**

Sterilization of Nutrient Agar, spatula, pipettes and flasks was done by autoclaving at 121°C for 20 minutes.

- **Preparation of the inoculum:**

**Bacterial cultures:**

Work place was cleaned in laminar air flow using 70% ethyl alcohol and exposed to UV for 20 minutes.

The bacterial cultures of the selected bacteria were grown in Nutrient Agar media at 37°C for 24 hours. Growth on the slant was used for preparation of inoculum in saline.

- **Well diffusion method:<sup>8</sup>**

The work was done in aseptic conditions in laminar air flow unit. The sterilised nutrient agar medium was allowed to cool. When the temperature reached around 45-55°C, approximately 20 ml of this medium was poured in the petridish. To this 1 ml of the inoculum of selected bacteria were added and mixed uniformly. This was then allowed to solidify. After solidification seven wells were bored in the agar medium with the help of borer.

In first well 40  $\mu$ g Ampicillin was loaded as the positive control.

In second well 15mg of AKJ was loaded directly. In third and fourth wells already diluted sample of AKJ were loaded in the volumes corresponding to 10 mg and 40 mg respectively.

In the fifth well 15 mg AKG was loaded directly. In sixth and seventh wells already diluted sample of AKG were loaded in the vol-

umes corresponding to 10 mg and 40 mg respectively.

The petridish was incubated at 37°C for 24 hours.

The test was performed in duplicate and the presence of zone of inhibition in petridish was observed on next day. The average value was considered as the result.

**Method for Anti-fungal Study:** The whole procedure can be sub-divided in following steps-

- Preparation of different dilutions of AKJ and AKG.
- Preparation of Nutrient Medium.
- Preparation of inoculum.
- Well diffusion method for testing the anti-microbial action and reading of Zone of Inhibition (ZOI).
- **Preparation of different dilutions of AKJ and AKG:**

500 mg of the sample was taken and made to a volume of 0.9 ml with distilled water. As a result thick slurry was formed. From this slurry volumes approximating to 10 mg and 40 mg of the drug were taken for the study. Along with these diluted samples, 15 mg of the sample was loaded directly for the well diffusion method. The same methodology was opted for both the samples of AKJ and AKG.

- **Preparation Of Nutrient Medium:**

**a) Preparation of Sabouraud's agar media:<sup>8</sup>**

Glucose (40 g) and peptone (10 g) were dissolved in 990 ml of distilled water. The pH was adjusted to 5.5 and the volume was made up to 1000 ml. Finally add 20 g of agar to the media and autoclave at 121°C for 20 minutes.

**b) Sterilization:**

Sterilization of Sabouraud's agar, spatula, pipettes and flasks was done by autoclaving at 121°C for 20 minutes.

- **Preparation of the inoculum:**

**Fungal cultures:**

Work place was cleaned in laminar air flow using 70% ethyl alcohol and exposed to UV for 20 minutes.

The fungal culture of *Microsporum canis* was grown in Sabouraud's agar medium at 25°C for 10 days. Saline was added to the slant, tube was swirled and the resultant hazy suspension was used as inoculum.

- **Well diffusion method:<sup>8</sup>**

The work was done in aseptic conditions in laminar air flow unit. The sterilised Sabouraud's agar medium was allowed to cool. When the temperature reached around 45-55°C, then approximately 20 ml of this medium was poured in the petridish. To this 1 ml of the inoculum of *Microsporum canis* was added and mixed uniformly. This was then allowed to solidify. After solidification seven wells were bored in the agar medium with the help of borer.

In first well Clotrimazole 1µg was loaded as the positive control.

In second well 15mg of AKJ was loaded directly. In third and fourth wells already diluted sample of AKJ were loaded in the volumes corresponding to 10 mg and 40 mg respectively. In the fifth well 15 mg AKG was loaded directly. In sixth and seventh wells already diluted sample of AKG were loaded in the volumes corresponding to 10 mg and 40 mg respectively.

The petridish was incubated at 25°C for 10 days.

The test was performed in duplicate and the presence of zone of inhibition on petridish was

observed on eleventh day. The average value was considered as the result.

## RESULTS AND DISCUSSION

**Action on *E. coli*-** *Escherichia coli* is a gram negative, straight, rod measuring 1-3 x 0.4-0.7  $\mu\text{m}$  arranged singly or in pairs. It is motile by peritrichate flagella, though some strains may be non-motile. Capsules and fimbriae are found in some strains. Spores are not formed.<sup>9</sup> Both the samples of AKJ and AKG have shown anti-bacterial action on *Escherichia coli*. Maximum zone of inhibition was observed in the wells in which the samples were loaded directly in the amount of 15 mg. Zone of inhibition was also observed when the samples were diluted and loaded in the wells. The results are shown in Table 1.

**Action on *Klebsiella pneumonia*-** *Klebsiella pneumonia* is a non-motile, capsulated rod that grows well in ordinary media forming large, dome shaped, mucoid colonies of varying degree of stickiness. They are short, plump straight rods, about 1-2 x 0.5-0.8 mm in size. The capsule is often prominent and can be made out even in gram stained smears as haloes around the bacilli.<sup>10</sup>

Both the samples of AKJ and AKG have shown anti-bacterial action on *Klebsiella pneumoniae*. The zones of inhibition obtained at different dilutions are as depicted in Table 2.

**Action on *Staphylococcus aureus*-** They are spherical cocci, approximately 1  $\mu\text{m}$  in diameter, arranged characteristically in grape like clusters. They are non-motile and non-sporing. A few strains possess microscopically visible capsules, particularly in young cultures. They stain readily with aniline dyes and are uniformly gram positive.<sup>11</sup>

AKJ and AKG have shown their anti-bacterial potential on the selected bacteria. The zones of inhibition obtained at different dilutions are tabulated in Table 3.

**Action on *Pseudomonas aeruginosa*-** It is a slender gram negative bacillus, 1.5-3 x 0.5  $\mu\text{m}$ , actively motile by a polar flagellum. Occasional strains have two or three flagella. Clinical isolates are often piliated. It is non capsulated but many strains have a mucoid slime layer.<sup>12</sup>

Both the samples of AKJ and AKG have shown anti-bacterial action on *Pseudomonas aeruginosa*. Maximum zone of inhibition was observed in the wells in which the samples were loaded directly in the amount of 15 mg. Zone of inhibition was also observed when the samples were diluted and loaded in the wells. The AKG sample did not show any action in 10 mg diluted concentration while it shows activity in other two wells loaded with 15 mg and 40 mg diluted concentration. The results are as shown in the Table 4.

**Action on *Microsporium canis*-** *Microsporium canis* is a pathogenic, asexual fungus in the phylum Ascomycota that infects the upper, dead layers of the skin of humans. It forms a white, coarsely fluffy spreading colony with a distinctive “hairy” or “feathery” texture. On the underside of the growth medium, a characteristic deep yellow pigment develops due to the metabolites secreted by the fungus. The intensity of this yellow pigmentation is on peak on the 6<sup>th</sup> day of colony growth and fades gradually making the identification of older colonies difficult. Some of the strains fails to produce yellow pigment altogether, exhibit

abnormally slow colony growth and form undeveloped macroconidia.<sup>13</sup>

When the results were evaluated then it was found that both the samples did not have any action on *Microsporium canis*. This shows that *Kshara* has action on bacteria but it does not act on fungus. The results are as shown in Table 5.

When the results are analysed it was found that both the samples have shown anti-bacterial action but they have not shown any activity against selected fungus. The reason behind anti-bacterial action can be attributed to the constituents of *Kshara*. It basically contains the alkaline substances like hydroxides of sodium, potassium and magnesium. It has been proven by recent studies that bacteria are not able to survive in high concentrations of alkaline medium. Moreover it also contains carbonates of calcium, sodium and potassium. It is also shown by recent studies that the carbonate ions have bactericidal effect on *E.coli*. This may be the probable reason behind the observation of greater ZOI in case of *E.coli* as compared to other specimens.

By the present study it was affirmed that *Kshara* is having *Krimighna* properties as far as anti-bacterial action is concerned. *Kshara* has the properties of *Lekhana* (scrapping), *Ksharana* and *Kshadana* (removal of vitiated tissues). This action of *Kshara* may be attributed to these properties. It is also considered to be *Ushna* and *Teekshna* in nature which may also help in the attainment of the desired *Krimighna* action.

*Kshara* is known to have scrapping action where it removes the excess *Snighdhta* from the tissues. The excess *Snighdhta* can be compared to the vitiated *Kapha Dosha*. When there is bacterial infestation in the body tis-

sues, formation of slough can be seen. This slough is comparable to the excess *Snighdhta* caused by the vitiation of *Kapha Dosha*. *Kshara* acts on this vitiated *Dosha* removing the slough which ultimately denotes the anti-bacterial action of the *Kshara*.

*Acharya Sushruta* has described the *Ksharana* and *Kshadana* properties of *Kshara*. *Acharya Dalhana* has elaborated these terms stating that it removes *Dushta Twaka* and *Mamsaadi Dhatus*. In the case of bacterial infection the skin tissues along with flesh of the affected area get degraded. This further hampers the process of wound healing and provides suitable site for bacterial growth. The removal of these *Dushta Twaka Mamsaadi Dhatus* may probably help in destroying the abode of the bacteria and hence preventing its further growth.

*Kshara* is having the specific properties of *Vilayana*, *Shodhana* and *Ropana* which are responsible for the efficient action of *Kshara*. *Shodhana* denotes the removal of the slough collected in the pus pockets formed due to the infection. Moreover it further helps in wound healing by the property of *Ropana*. These properties might also be helpful in the anti-bacterial action of *Kshara*. It also possesses the properties of *Dahana*, *Pachana* and *Soshana*. *Dahana* and *Pachana* actions might act in bringing out the bactericidal or bacteriostatic action of *Kshara* while *Shoshana* property may again help in the absorption and ultimately removal of the extra amount of fluid seen in the vicinity of the wound. These properties of *Kshara* are collectively helpful in contributing towards the anti-bacterial action of the *Kshara*.

## CONCLUSION

Both the samples have shown promising results in the anti-bacterial studies on the selected pathogens. The results obtained affirm the *Krimighna* action of *Kshara* as mentioned in the classical texts. The study helps in the affirmation of the principles of *Ayurveda* on the recent scientific basis.

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**Table 1.** Action of AKJ and AKG on *E. coli*

S.No.	Sample Amount	Radius of Zone of Inhibition of AKJ (in mm)	Radius of Zone of Inhibition of AKG (in mm)
01.	15 mg (direct)	16	15
02.	10 mg	11	12
03.	40 mg	13	14

**Table 2:** Action of AKJ and AKG on *K. pneumoniae*

S.No.	Sample Amount	Radius of Zone of Inhibition of AKJ (in mm)	Radius of Zone of Inhibition of AKG (in mm)
01.	15 mg (direct)	10	10
02.	10 mg	09	09
03.	40 mg	10	09

**Table 3:** Action of AKJ and AKG on *S.aureus*

S.No.	Sample Amount	Radius of Zone of Inhibition of AKJ (in mm)	Radius of Zone of Inhibition of AKG (in mm)
01.	15 mg (direct)	15	15
02.	10 mg	12	12
03.	40 mg	14	14

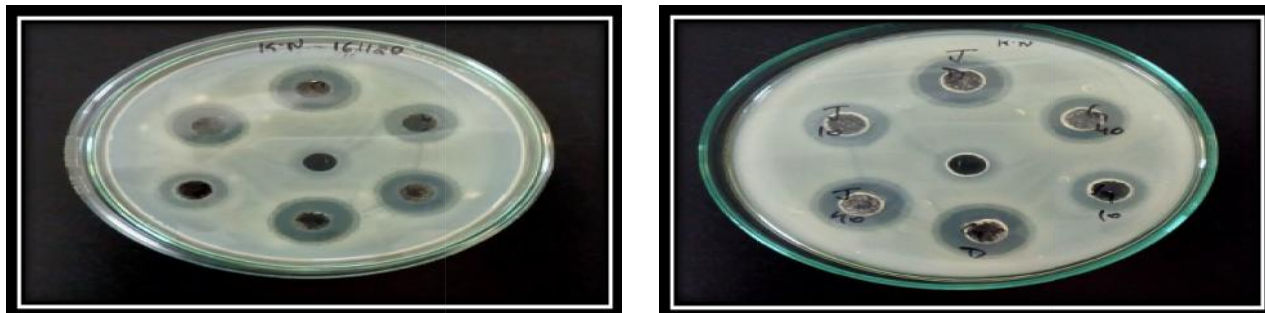
**Table 4:** Action of AKJ and AKG on *P.aeruginosa*

S.No.	Sample Amount	Radius of Zone of Inhibition of AKJ(in mm)	Radius of Zone of Inhibition of AKG (in mm)
01.	15 mg (direct)	10	08
02.	10 mg	06	00
03.	40 mg	10	08

**Table 5:** Action of AKJ and AKG on *M. canis*

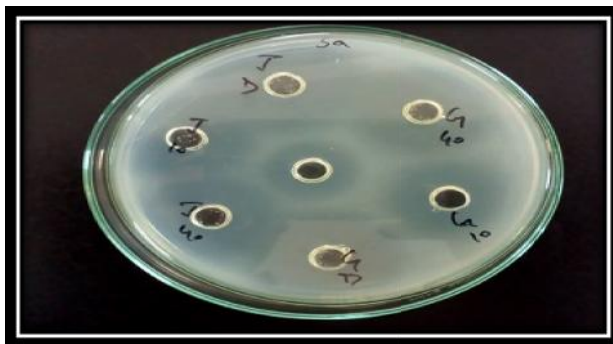
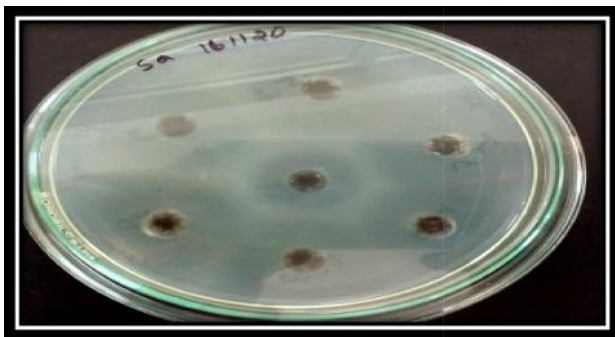
S.No.	Sample Amount	Radius of Zone of Inhibition of AKJ (in mm)	Radius of Zone of Inhibition of AKG(in mm)
01.	15 mg (direct)	00	00
02.	10 mg	00	00
03.	40 mg	00	00

**Figure 1:** Zone of Inhibition on different pathogens

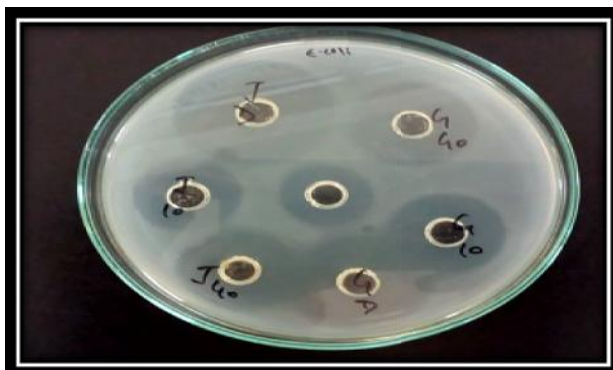
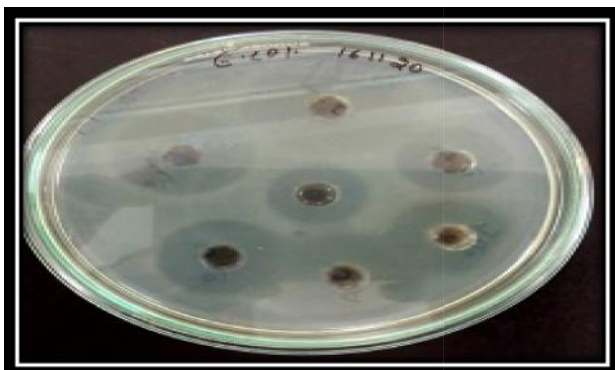




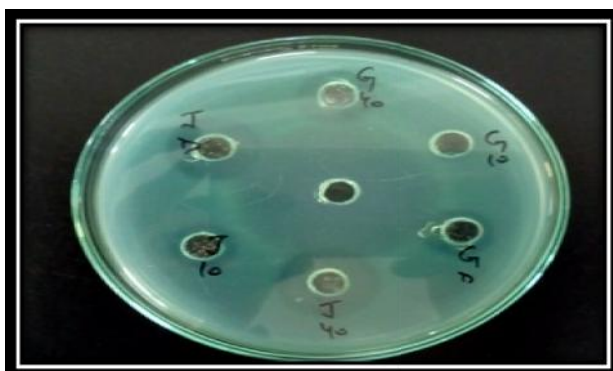
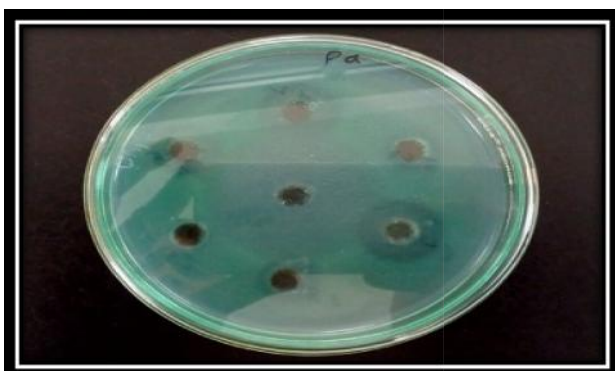
**Figure 1.1. Action On *K.pneumonia***



**Figure 1.2. Action On *S. aureus***



**Figure 1.3. Action on *E. coli***



**Figure 1.3. Action on *P. aeruginosa***

**Source of Support: Nil**

**Conflict Of Interest: None Declared**

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