ISSN : 2249-5746

International Journal of Ayurvedic and Herbal Medicine 6:3 (2016) 2220 –2228

Journal homepage: http://www.interscience.org.uk

Antibacterial activity of *pratisaraniya kshara* (*Achyranthes aspera*) in the management of the fistula in ano

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ABSTRACT

Shalya Tantra, the integral part of *Ayurvedic* system contains detail description of *Shastra Karma* along with certain Para surgical procedures such as *Kshara Karma, Agni Karma*, and *Jaloukavacharana.* Among these Para surgical measures, *Kshara Karma* is having supreme place due to its tremendous properties in curing diseases.

There are different methods of treatment are described in *Ayurveda* for the management of fistula in ano based on its severity. Among them *Achyranthes aspera* (*Pratisaraneeya Kshara)* is one, which replaces the surgical intervention even and cures the condition without the help of surgical instruments. *Kshara* is the main ingredient in *ksharasutra.*

Kshara is a kind of medication described in *Ayurveda* Texts for the management of various disorders. The word *Kshara* is derived from the root *Kshara*, means to melt away or to perish. *Acharya Sushruta* defines as the material which destroys or cleans the excessive morbid *doshas* (*Kshyaranat Kshyananat va Kshara).* The drug which has the characteristics of *Kshanan or Ksharan* literally means that which destroys fleshy mass either healthy or unhealthy is *Kshara. Charaka* says *Kshara* is one which scrapes the abnormal tissue from its location and destroys it after dissolving it, because of its corrosive nature.

Despite having so many activities no reports found on the antibacterial activity of *Achyranthes aspera kshara*, present study was under taken to study antibacterial activity of

Achyranthes aspera Kshara. for both type cultures as well as clinical cultures.

The *Achyranthes aspera* was used for the preparation of *ksharasutra* tested its antibacterial activity. According to the Susrutha samhitha, the *Achyranthes aspera* was collected, identified, prepared *kshara* and tested antibacterial activities against bacteria isolated from fistula in ano.

Extract were prepared by cold extraction method in which 25g of *kshara* powders were separately soaked in equal volume of dichloromethane and methanol (125ml*)* for 24 hrs at room temperature and shaken

occasionally. The extract were filtered and then concentrated by evaporating the solvent at room temperature. The residue (50g) was stored in the airtight glass bottle in a refrigerator. Different concentrations of extract (100mg/ml) were prepared in DMS0 (Dimethyle sulofxide) for checking the antibacterial activity.

The test bacteria were *Staphylococcus aureus, Streptococcus pyogens*, *Escherichia coli,* and *Pseudomonas aeuruginosa*, isolated from fistula specimens, and standard strains (American Type Culture Collection (ATCC)], *S. aureus* (ATCC 25923), *S. pyogens* (ATCC 19615), *E. coli* (ATCC 25922)and *P. aeuruginosa* (27853) all the type cultures were obtained from stored samples at Faculty of Medicine, University of Colombo.

The test organisms were grown in Muller Hinton agar medium. Twenty-four hour old pure cultured bacteria were used to prepare a density of 10^8 cells mL^{-1} of 0.5 McFarland standards during each test. Muller-Hinton agar was prepared according to the manufacturer's instruction, autoclaved and dispensed at sterile plate.

The aliquot was spread evenly onto Muller Hinton agar by sterile cotton swab. Then, the plated medium was allowed to dry at room temperature for 30 minutes. On each plate, equidistant wells were made with a 6 mm diameter sterilized, cork borer, 2 mm from the edge of the plate. Fifty micro liter of kshara extract (100 mg/ml) was aseptically introduced into a respective agar well. Amoxicillin (100 mg/ml) were used as positive controls and the distilled water were included as negative controls. This was followed by allowing the agar plate on the bench for 40 minutes pre-diffusion followed by incubation at 37 °C for 24-48 h. The formation of clear inhibition zone of ≥7 mm diameters around the wells was regarded as significant susceptibility of the organisms to the extract. The *Achyranthes aspera pratiksaraniya kshara* showed significant level of antibacterial activities against *Staphylococcus aureus, Streptococcus pyogens, Escherichia coli* .But no inhibition was observed for *P. aeuruginosa.* The zone of inhibition range from 19mm to 23mm was observed for the *Achyranthes aspera kshara*

Key Words : *kshara,* fistula in ano, antibacterial activity

INTRODUCTION

A medicinal plant is factually any plant which in one or more of its parts contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of direct therapeutic agents. Approximately 25% of drugs in modern pharmacopoeia were derived from plants and many others were synthetic analogues built on prototype compounds isolated from plants. Infectious diseases are the world's leading cause of premature deaths3. Therefore, there is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action. Medicinal

plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs (Elumalai et al., 2009).

The antimicrobial properties of plants have been investigated by a number of researchers worldwide. In recent years the interest to evaluate plants possessing antibacterial activity for various diseases is growing and it has also been proved that various plants extracts possess bacterio-static and bactericidal effects 6 and most of the plants contain many bioactive compounds. Plants are potent biochemical factories and have been components of phyto medicine. Plants based natural constituents can be derived from any part of plant like bark, leaves, flowers, roots, fruits, seeds, etc (Makari et al., 2008).

According to *Susruta Samhita*; *Kshara* or caustics are superior to *Shastra* (Sharp instrument) and *Anu Shastra* (Accessory instruments) because of their capability to perform excision (Removal) incision (Cutting) and Scraping, alleviating three Doshas and being used for various special procedures. (*Su: Su*: 11.3)

According to *Ashtanga Hridaya Samhita*; of all the sharp instruments and accessories, instruments *Kshara* (Caustic Alkali) are the best. It performs many functions such as incising; excising and it can be used even in inaccessible pleases. Success can be obtained by its use even in diseases which are very difficult to cure and also because it can be used even in the form of a drink. (*Ah: su*: 30.1 – 2.).

Kshara or caustic substances is considered as one of the most important means of parasurgical means because *Kshara* can produce excision, incision, scrapping and can

Pacify all three *doshas. .Kshara* sourced from different plants are described in Ayurveda to be used in different ways to manage various diseases, which includes many of the ano-rectal conditions like *bhagandara* (fistula-in-ano),*Ksharasutra* in ano-rectal

Diseases has become a common practice in Ayurvedic surgery. *Ksharasutra* is a novel drug delivery system in which a thread smeared with *kshara* (caustic substance) is applied to induce both mechanical and chemical cutting and healing. Exhaustive list of *Kshara* source plants is described in the *Sushruta*, mostly the *Kshara* of

The caustics for external application have been advised to be used against *Kustha* (Dermatoses), *Kitibha (*hyperkeratosis), patch of ring warm, *Kilasa* (Vitiligo), fistula in Ano, tumors, piles, dirty wounds, sinus, warts, moles, birth marks, The effects include (a) correction of the unhealthy tissues (b) Enhancement of the healthy granulation tissue formation (c) enhancement of fibrolysis (d) separation of debris through the fistulous track (e) removal of debris and cleansing wound.

METHODOLOGY

Antibacterial activity of *Kshara* extract

The incidence of fistula in ano and the origin of the predominant microorganism present in ano rectal fistula have been investigated using 100 pus samples obtained from the 30 patients. Total 240 Isolates were identified. Among isolates *Staphyloccus aureus* and *streptococus pyogen* were identified as skin derived organisms. *Escherichia coli, p. aerogunosa* were considered as gastro intestinal tract derived organisms. The *Achyranthes aspera kshara* tested against the above four organisms both clinical isolates as well as type cultures.

Kshara considered as the main components and active component according to Ayurveda tests, so the *Apamarga kshara* (*Achyranthes aspera*) is practically used for the *kshara sutra* preparation, tested for the antimicrobial activity, the whole plant materials was collected separately, washed well to remove dust, mud etc., and dried under shade. Once completely dried, the plants were burnt in a clean place. Burning was carried out that all the twigs completely got burnt to a grayish ash. On an average 5kgof dried raw plant material, yielded about 500 gm of ash. Once cool, the ash was stored in clean containers. To this ash 6 parts of distilled water was added. This was stirred well and allowed to stay overnight. Next day the suspension was filtered 21 times through double Wart man's filter paper and the collected clear filtrated was carefully boiled over mild heat to evaporate water and make the dry powder. The powder store in container and keep humidity controller at 40°C until it used.

Preparation of aqueous extraction of *kshara*

Kshara (fine powder) was dissolved in distills water, making the concentration of 100mg/ml. The extract were contained in brown screw capped bottles and shook at 200 rpm on 30 minutes vortex mixer. Then the extracts filtered through Whatman No.01 filter paper and supernatants were collected for further use. The *Kshara* prepared concentration of 100mg/ml.

Agar well diffusion

Antibacterial activity tests measure the ability of an antibacterial agent to inhibit bacterial growth in vitro .Bacterial broth culture was prepared to a density of 10^8 cells ml^{-1} according to 0.5 McFarland standards. The aliquot was spread evenly onto Muller Hinton agar by sterile cotton swab. Then, the plated medium was allowed to dry at room temperature for 30 minutes on each plate, equidistant wells were made with a 6 mm diameter sterilized, cork borer, 2 mm from the edge of the plate.

Fifty micro liter of each *ksharasutra* extract (100 mg/ml) was aseptically introduced into a respective agar well. Amoxicillin (100 mg/ml) were used as positive controls and the distilled water was included as negative controls. This was followed by allowing the agar plate on the bench for 40 minutes pre-diffusion followed by incubation at 37 °C for 24-48 h. The formation of clear inhibition zone of ≥7 mm diameters

around the wells were regarded as significant susceptibility of the organisms to the extract. The experiment was performed in duplicate. Experiments that gave contradicting results were done for the third time for an easy decision

Observation and results

The ZOI for the *Achyranthes aspera kshara* for the *S. aureus (*ATCC 25923), *E .coli (*ATCC 25922) and *S. pyogens*ATCC (19615) however *there* were no ZOI for either *kshara* or amoxicillin for *aeruginosa* ATCC (27853) *Achyranthes aspera kshara* showed highest mean zone inhibition for the *S.aureus (*ATCC 25923)23.23±0.33 while for the *E. coli (*ATCC 25922) 19.66±0.35 and *S. pyogen* (ATCC 19615) 19.34±0.36 were observed. Amoxicillin responsible for the highest ZOI for the tested bacteria respectively *S. aureus (*ATCC 25923)29.51±0.57, *E. coli (*ATCC 25922) 20.53±0.57 and *S. pyogen* (ATCC 19615) 21.32±0.57.There were significant difference were observed in ZOI for Amoxicillin in comparison to the *Achyranthes aspera kshara* in responded three types of bacteria. This difference was statistically significant (Table 1, Fig 1).

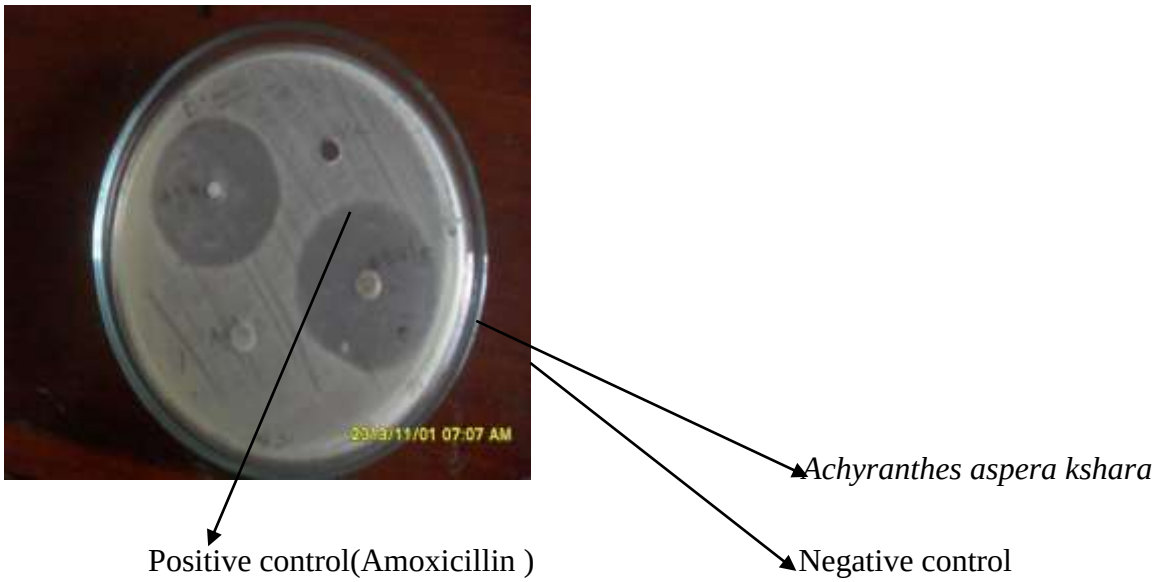

Fig 1 : Antibacterial activity of *Achyranthes aspera kshara for E. Coli (Agar well diffusion methods)*

Table 1: ZOI for Gram negative and Gram positive bacteria for *Achyranthes aspera kshara* for the type cultures of bacteria.

Type of bacteria	Number of replicates	Mean±SE for Amoxicillin	Mean±SE for *Kshara*	t- value	Probability value
S. aureus (ATCC 25923)	3	29.51±0.57	23.23±0.33	11	P<0.01 SIG

E.coli (ATCC 25922)	3	20.53±0.57	19.66±0.35	5.5	P<0.05 SIG
S.pyogen ATCC(19615)	3	21.32±0.57	19.34±0.36	7.67	P<0.01 SIG
P.aeruginosa ATCC (27853)	3	6±0	6±0		

Table 2 : ZOI of Gram negative and Gram positive of bacteria for *Achyranthes aspera kshara* for clinical isolates.

Type of bacteria	Number of replicates	Mean±SE for Amoxicillin	Mean±SE for *Kshara*	t- value	Probability value
S. aureus	3	19.23±0.32	23.23±0.34	6.36	P<0.01 SIG
E.coli	3	19.33±0.81	20.66±0.33	2.41	P<0.05 SIG
S.pyogen	3	17±0.57	19.32±0.31	3.5	P<0.05 SIG
P.aeruginosa	3	6±0	6±0		

For the clinical isolates *Achyranthes aspera kshara* showed highest ZOI for the *S. aureus*23.23±0.34while for the *E.coli* ZOI, 20.66±0.33 and *S.agalactiae*19.32±0.31 inhibition. In contrast Amoxicillin responsible for the lowest ZOI for the tested bacteria respectively *S. aureus*19.23±0.32, *E .coli* 19.33±0.8 and *S.agalactiae*17±0.57 There were significantly difference was observed in ZOI for *Achyranthes aspera kshara* in comparison to the amoxicillin. *Achyranthes aspera kshara* in responded three types of bacteria. This difference was statistically significant (Table 2 Fig 2).

Table 3 Differences of ZOI between the in type cultures and clinical cultures for amoxicillin

Type of bacteria	Number of replicates	Mean±SE for type	Mean±SE for clinical	t- value	Probability value
S. aureus	3	29.53±0.57	19.23±0.3	17	P<0.001 SIG
E.coli	3	20.51±0.57	19.33±0.88	3.48	P<0.05 SIG
S.pyogen	3	21.33±0.66	19.01±0.57	5.12	P<0.01 SIG
P.aeruginosa	3	6±0	6±0		

Table 4 Comparison of ZOI between the type cultures and clinical cultures of *Achyranthes aspera kshara*

Type of bacteria	Number of replicates	Mean±SE for type culture	Mean±SE for clinical culture	t- value	Probability value
S. aureus	3	24.66±0.33	23.66±0.3	2.12	P>0.05 NS
E.coli	3	19.33±0.33	20.66±0.3	0.71	P>0.05 NS
S.pyogen	3	18.66±0.66	19.3±0.3	0.89	P>0.05 NS
P.aeruginosa	3	6±0	6±0		

It was observed that there were some significant difference in ZOI for type cultures and clinical cultures for the standard antibiotic (amoxicillin) responsible for the higher ZOI for the tested bacteria respectively type cultures *S. aureus* (ATCC 25923) 29.53±0.57, *E. coli* (ATCC 25922) 20.51±0.57 and *S. pyogen*(ATCC 19615) 21.33±0.66 but amoxicillin had shown for the lower ZOI for the respective clinical cultures of *S. aureus*19.23±0.3, *E. coli* 19.33±0.8 and *S.pyogen* 19.01±0.57.

So there were significant difference was observed in comparison of ZOI type cultures and clinical cultures. This difference was statistically significant<0.05(Table 3, Table 4) (Fig 2)the clinical cultures reported low zone of inhibition in contrast to the type cultures, because fistula patient developed resistant due to the use of antibiotic but for *kshara* there were no such resistance was developed.

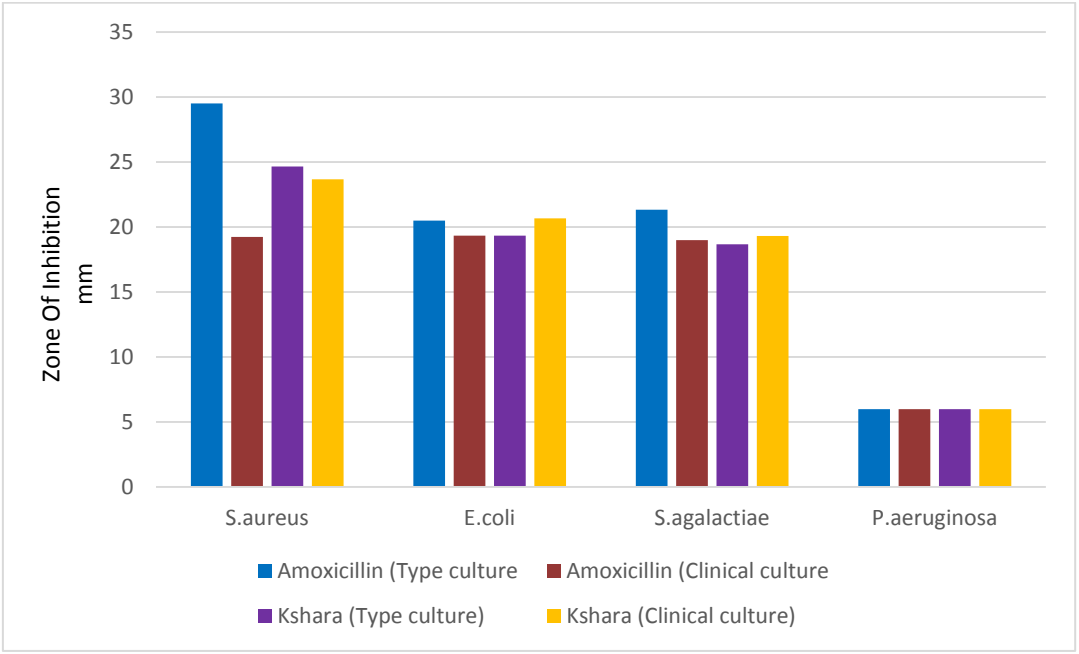

Fig 2: ZOI of type cultures and clinical cultures for *Achyranthes aspera Kshara* vs amoxicillin

DISCUSSION

Kshara possess only inorganic compounds and have considerable antimicrobial activity, this activity responsible by Cu, Zn, Fe and Ca. This elements are available in higher concentration in comparison to the other elements in *Achyranthes aspera*. (Aparna *et al.,* 2013) Inorganic metal oxides may serve as effective antiseptics, due to their relatively nontoxic profile, chemical stability, and efficient antibacterial activity (Gordon *et al.,* 2011). The microbial species of clinical interest, often involved in biofilm-associated diseases that are belonging to a very large spectrum, from the Gram-positive to the Gram negative pathogens *(P. aeruginosa, E. Coli*) (Lazăr *et al*., 2010). Among the metal oxide nanomaterials, ZnO showed greatest antimicrobial activity against both Gram-positive and Gram-negative bacteria. Excess of Zn has been proved to have an inhibitory effect on microbial process, since it binds to the membrane of micororganisms, (Atmaca *et al.*, 1998) prolonging the lag phase of growth cycle and increasing the growth cycle and increasing the generation time of the organisms(Radke *et al*., 1994). In addition to when Zinc binds to the membranes, it uptake and change the fluidity of the membrane. Furthermore, Soderberg *et al.,* 1990 proposed that Gram-positive bacteria were more susceptible to Zinc ion than Gram-negative ones. This has been attributed to the difference in the protein constituents of their cell wall (Sugarman *et al.,* 1983). It was observed that ZnO nanoparticles have excellent bactericidal potential. Moreover iron oxide nano particle attribute antibacterial activity because of their smaller size could penetrate the cell membrane of bacteria and leading to oxidative stress and cause the disruption of the cell membrane (Lee *et al*., 2008,Ameer Azam *et al.,* 2012). Zn is available in *kshara* of *Acyranthes aspera* root 43.52mg/kg, leaf 13.24 mg/kg and stem15.34mg/kg, respectively Aparna *et al.,* 2013). According to this study and that effectively eliminate both Gr^{+ve} and Gr^{-ve} bacteria associated with fistula in ano. It is currently widely accepted that the mechanism of contact killing involves in the following key steps; damage of the outer and or inner bacterial membrane, accumulation of Cu ions in the cell, Cartoon of the tentative events in contact killing. Cu dissolves from the copper surface and causes cell damage. The cell membrane ruptures because of Cu and other stress phenomena, leading to loss of membrane potential and cytoplasmic degradation of the bacterial DNA (Rensing *et al.,* 2003). Calcium reacts with magnesium ions on the surface of the wound to form chelates which have antibiotic properties (Lansdown *et al.,* 1999) hihgerst concentration of calcium avialble in. *Acyranthes aspera kshara.* root 849.43mg/kg, leaf 1363.74 mg/kg and stem588.34mg/kg, respectively Aparna *et al.,* 2013). So these trace elements were responsible for the antibacterial activity of the *Acyranthes aspera kshara.*

CONCLUSION

Kshara posses significant antibacterial activity against *S. aureus, E.coli, S.pyogen* while it showed resistant to the *P.aeruginosa.* So it's antibacterial activity against the pathologically importance bacteria proved *kshara* potential on clinical efficacy fistula in ano..

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