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TO EVALUATE SENSITIVITY OF VARUNA AND GOKSHURA AGAINST β - LACTAMASES RESISTANT AND SENSITIVE ATCC *KLEBSIELLA PNEUMONIA*

Ajantha[1], Nandesh Mohan P[1], Shashirekha K.S[2]

[1]Professor [1]Associate Professor, Microbiologist[2] Department of Roga nidāna and Vikruthi Vijnana, Shree Dharmasthala Manjunatheshwara Ayurveda College and Hospital. Hassan, Karnataka, India

Corresponding Author: shashirekha9887@gmail.com

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ABSTRACT

Currently efficacy of antimicrobial agents found to be restricted and thus the need of hour becomes the alternative source that has antimicrobial activity and easily available. Multi-drug resistant *Klebsiella pneumonia* isolates are major one among health setback, therefore in the current study ATCC 700603 *Klebsiella Pneumonia* strains were collected and cultured and antibiotic bacterial sensitivity pattrens were observed on the strains with spectrum of β-lactamase resistance and sensitivity. Ayurvedic drug possess various medicinal properties and are attributed with the quality of Krimighna (anti-bacterial) but has specific drug for specific causative microorganisms. On further evaluation, therefore present study is undertaken on sensitivity of Gokshura and Varuna against ATCC 700603 *Klebsiella Pneumonia* by well diffusion method. The Study concludes that aqueous extract of Varuna is better acting at lower concentration and alcoholic extract of Varuna act better at higher concentration Unlike to Gokshura where alcoholic extracts of Gokshura act better at all concentrations compared to aqueous extracts though it shows moderate to mild zone of inhibition at all concentration of aqueous extracts against ATCC 700603 *Klebsiella Pneumonia*.

Keywords: Krimighna; *Klebsiella Pneumonia;* Varuna; Gokshura.

INTRODUCTION

Gokshura and Varuna is indicated in mutrakrichra and possess krimighna property. Hence, adoption of new approaches like culture and sensitivity methods would strength existing Ayurveda knowledge and help in achieving improved diagnostic and curative abilities[1]. Therefore, the present study undertaken to study various attributes of the microorganism *Klebsiella pneumonia* its laboratory diagnosis, its culture and evaluate upashaya capability in vitro by sensitivity with Gokshura and Varuna collected. In general, clinical practice Urinary tract infection amounts for 1 to 3% of total consultation and upto 50% women suffer from urinary tract infection[2]. The management of Urinary tract infection is primly by antibiotics in modern system of medicine, though useful at times due to improper prescription or due to prolong usage the organism become resistant also there are considerable amount of side effects that arise. Hence the current situation the demand of the hour to search for aims alternative drug that is from natural sources, that are effective as well as cheap and easily available with less or no adverse effects so that they bestow upashaya[3].

Aims and Objectives: To compare antibacterial activity of Varuna and Gokshura against ATCC 700603 *Klebsiella pneumonia* resistant to β-lactamase antibiotics.

Methodology:

Drug collection : *Varuna* were collected from SDM Ayurveda pharmacy Udupi and *Gokshura*: was collected from SDMCAH Hassan dispensary. It was stored in a clean and airtight container(Fig. 1,2). **Authentication of the drug :** The authentication of the all the raw drugs were done at the Department of Dravyaguna, in Shri Dharmasthala Manjunatheshwara College of Ayurveda, Hassan

Preparation of aqueous and alcoholic extracts of *Varuna* and *Gokshura* (Fig. 3)

The cold maceration method were used here for preparation of both aqueous and alcoholic extracts of *Varuna* and *Gokshura* using 100g each of coarse powdered drug was weighed using a weighing balance. Coarse powder of each drug of 100g was added to 300ml distilled water taken in a 1000ml capacity conical flask and remaining 100g to 300ml ethanol taken in a 1000ml capacity conical flask. The conical flasks were then plugged tightly with cotton and sealed with tape. The conical flasks were shaken manually for 10-15min at an interval of every 3 hours during daytime. The procedure was repeated for 7 days. On the 7th day, the contents of conical flasks were filtered, that yielded 210ml of aqueous filtrate and 190ml of alcoholic filtrate. The filtrates were then kept over water bath in China dish at 60^{0}C (Fig. 4). 7.02 grams of aqueous extract and 7.31 grams of alcoholic extracts of *Varuna* were obtained by this process[4] (Fig. 5,6). ATCC *Klebsiella pneumonia on* MacConkey agar (Fig.7).

Sensitivity test

ATCC *Klebsiella pneumonia* were subjected to antibiotic sensitivity study by Kirby-Bauer disc diffusion method against β-lactamase sensitive and resistant antibiotic like, Merpeneum, Cefodoxime, Cefoperazone/ Sulbactum, Ceftriaxone, Cedoperazone, Amoxicillin/ sulbactum, Azithromycin, Cefuroxime, Piperacillin, Ampicillin, Cephalexin, Cefaclor, Amoxicillin, Cefixime and Ceftazidime, after recording the results were tabulated[5] (Fig. 8). **Antibacterial assay of *Varuna* and *Gokshura* against ATCC 700603 *Klebsiella pneumonia* resistant to β-lactamase antibiotics.**

Cleaned the workplace in laminar air flow using 70% of Ethyl alcohol and switched on to UV for 20 min . Poured around 15 ml Muller Hinton agar media uniformly over the petri dish, mixed well and allowed the media to solidify for 30 minutes. One loop full of ATCC *Klebsiella pneumonia* from 24-48 hours culture was transformed into the Muller Hinton agar plate (one for each extract) with a sterile non-toxic cotton swab and swabbed over the media (Fig.9). Made five equidistant wells on both the plates with sterile corn borer and added different concentrations of aqueous extract into wells on one plate and alcoholic extracts into wells on the other plate (Fig.10).Tests were conducted for five different concentrations of aqueous and alcoholic extracts of *Var-*

una and *Gokshura* (2000µg/ml,1000µg/ml,500µg/ml,250µg/ml, and 125µg/ml) separately[6] (Fig. 11,12,13). Petri plates were Incubated at 37°C for 24-48 hours. After the incubation period, the zone of inhibition was measured in mm with a ruler (Fig. 14.15). If the drug is sensitive a clear circular " halo" (technically known as " plaque" or Zone of Inhibition) will appear around the well, indicating absence of bacteria. If that zone appears, it shows that the particular drug is effective against the ATCC *Klebsiella pneumonia* bacteria. The disc diffusion study was measured as Sensitive (S) zone, Moderate (M)/Intermediate (I) sensitive zone and Resistant (R) zone.

Results:

Collection of Samples: Samples of Varuna and Gokshura were collected in a pre-sterilized container. Aqueous and Alcoholic extracts of collected samples of Varuna and Gokshura were done by cold maceration method. The extracts of Cold macerated aqueous and alcoholic extract are kept on water bath to obtain the dried extract and further are stored in pre sterilized glass container. The ATCC *Klebsiella pneumonia* organism collected were subcultured by lawn culture method in MacConkey plate with all the precautions. The antibiotic sensitivity of the ATCC *Klebsiella pneumonia* was observed with β-lactaase group of antibiotics for sensitive and resistance. The anti-microbial activity of Varuna and Goksura is checked on lawn cultured ATCC *Klebsiella pneumonia* on Muller Hintin Agar and further cork boure in on MHA plate. The alcoholic and aqueous extracts of Varuna and Golskura are serially diluted to various concentrations to check the anti- microbial activity at different concentrations and poured to respective wells created by cork bourer. The observed zone of inhibition at different concentration for both Varuna and Gokshura are noted and evaluated. The zone of Inhibition of aqueous and alcoholic extracts of Varuna and Gokshura on ATCC *Klebsiella pneumonia* is as follows in Table 3 and 4. Maximum zone of inhibition is observed for alcoholic extracts of Varuna at 2000 µg/ml, 1000 µg/ml and 500 µg/ml compared to aqueous extract, where the zone of inhibition of alcoholic extract at 2000 µg/ml, 1000 µg/ml and 500 µg/ml are 22 mm, 18mm and 16 mm respectively and zone of inhibition of aqueous extract of Varuna at 2000 µg/ml, 1000 µg/ml and 500 µg/ml are 08 mm, 10mm and 14 mm respectively. But at 250µg/ml and 125 µg/ml the zone of inhibition of aqueous extract is observed to be maximum (14mm and 16 mm) compared to alcoholic extract at same concentration (Table 1). On comparing the anti-microbial activity of aqueous extracts and alcoholic extracts of Gokshura maximum zone of inhibition is observed in alcoholic extract compared to aqueous extracts at all concentration such as 2000µg/ml,1000 µg/ml,500 µg/ml,250 µg/ml, and 125 µg/ml(14mm,14mm,14mm, 10mm and 12mm), whereas the zone of inhibition of aqueous extracts of Gokshura is observed to be 10mm,10mm,10mm, 08mm and 08mm at 2000µg/ml,1000µg/ml,500 µg/ml,250 µg/ml, and 125µg/ml respectively (Table 2).

On analyzing the antibiotic sensitivity study of ATCC *Klebsiella pneumonia* against β-lactamase sensitive and resistant antibiotic, it is observed that ceftazidime, cephalexin, cefaclor, Amoxicillin, cefixime. Maximum β –lactamase sensitive antibiotics are observed to be merpeneum 38, cefodoxime 30mm, and cefoperazone/ sulbactum 28 mm followed by ceftriaxone 26 mm, cedoperazone 22mm, Amoxicillin/ sulbactum 20 mm, Azithromycin 16 mm, cefuroxime, piperacillin, Ampicillin 12 mm each (Table 3).

Fig 1 Gokshura

Fig 2 Varuna

Fig 3 Alcoholic and aqueous cold maceration of Varuna and Gokshura

Fig 4 Drying of cold macerated aqeous and alcoholic extract of Varuna and Gokshura

Fig 5 Dried extract of Varuna and Gokshura

Fig 6 Alcoholic and aqueous extract of Varuna and Gokshura stored in sterilized bottle.

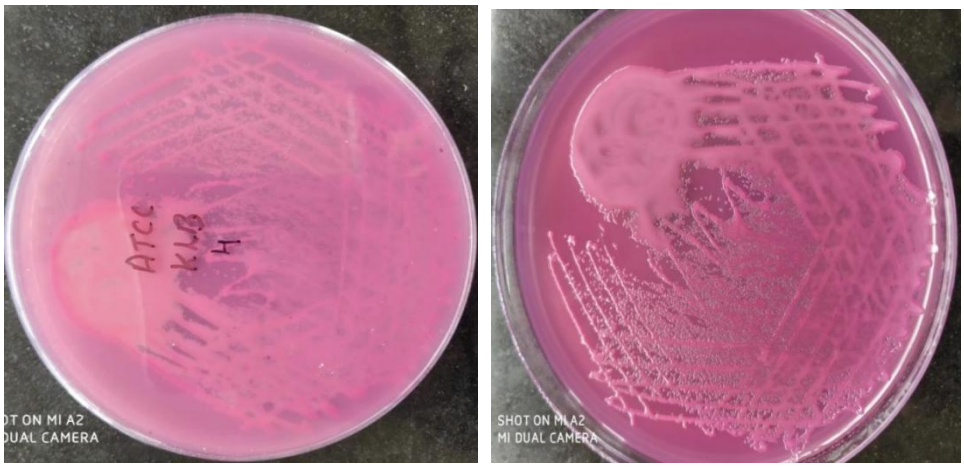

Fig 7 ATCC *Klebsiella pneumoniae* on MecConkey Agar

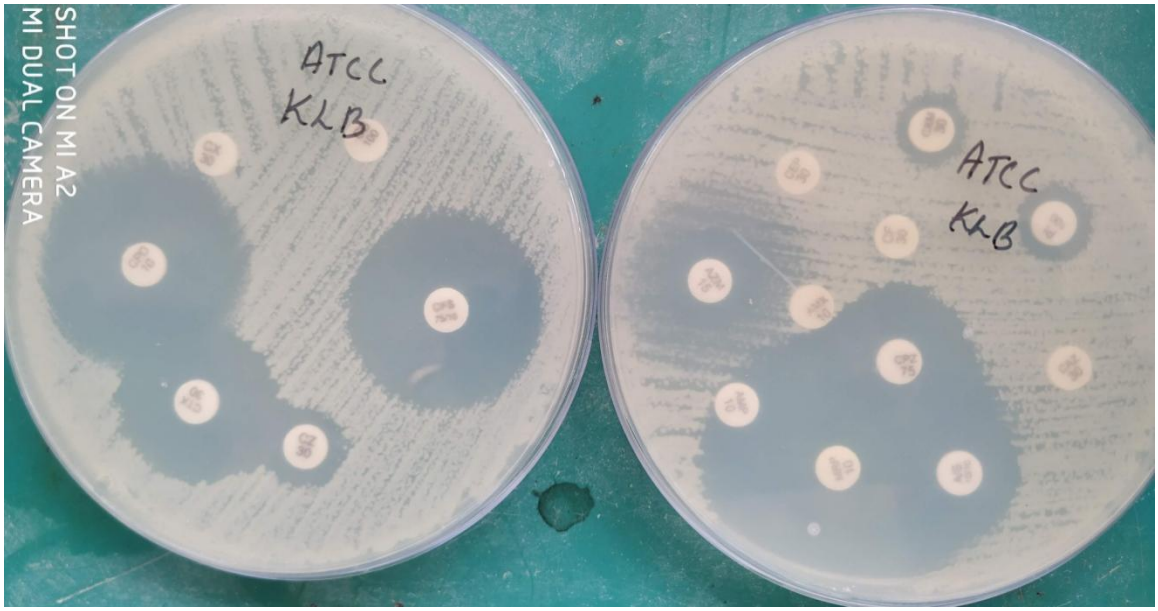

Fig 8 Antibiotic sensitivity study of ATCC *Klebsiella pneumoniae*

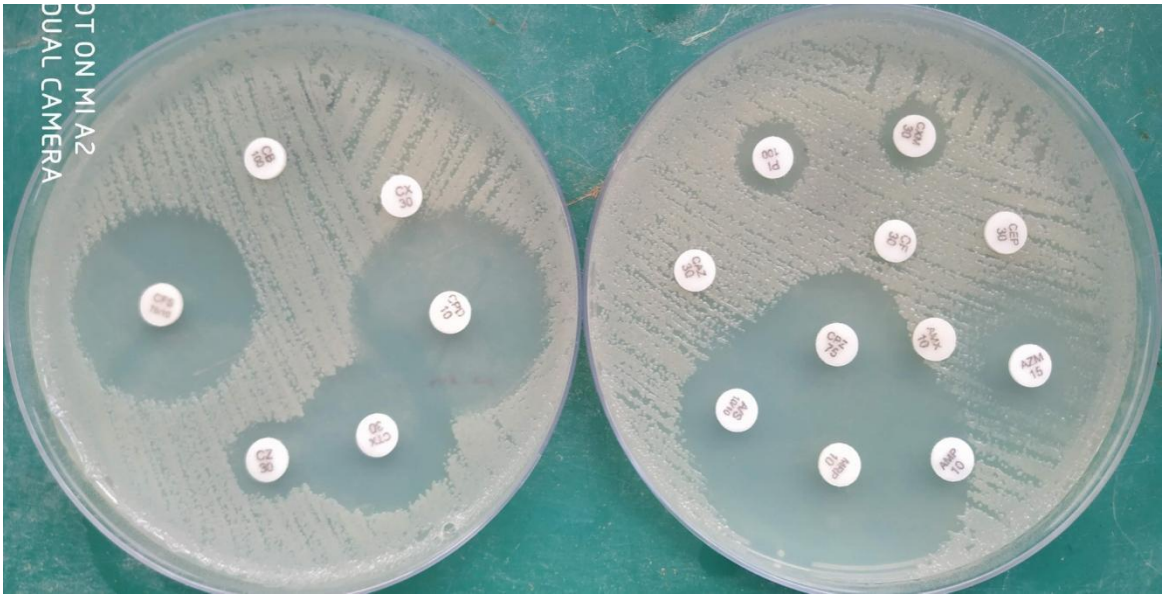

Fig 8(a) β-lactamase antibiotic sensitivity study showing sensitive and resistance to ATCC *Klebsiella pneumoniae.*

Fig 9 Lawn culturing of *K.pneumoniae* on agar plate (MHA)

Fig 10 Cork boureing of MHA plate to check Muller hinton antibacterial of Gokshura and Varuna

Fig 11 Preparation of different dilutions **Fig 12** Labeled plates for antibacterial assay using Varuna and Gokshura of Respective solutions.

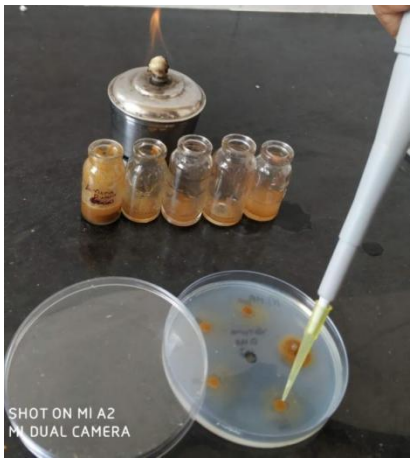

Fig 13 Pouring of different dilutions of Aqueous and alcoholic extracts of Varuna and Gokshura into the respective wells

Fig 14 Before incubation of plates

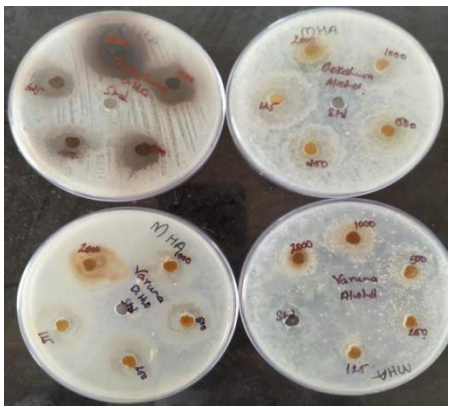

Fig 15 After incubation of plates

Table 1: Antibacterial assay of aqueous and alcoholic extract of Varuna against ATCC *Klebsiella pneumonia*

VARUNA	AQUEOUS	ALCOHOL
2000 µg/ml	08mm	22mm
1000 µg/ml	10mm	18mm
500 µg/ml	14mm	16mm
250 µg/ml	14mm	12mm
125 µg/ml	16mm	12mm

(Note: µg-micro gram mm-mili liter)

Table 2: Antibacterial assay of aqueous and alcoholic extract of Gokshura against ATCC *K. pneumonia*

GOKSHURA	AQUEOUS	ALCOHOL
2000 µg/ml	10mm	14mm
1000 µg/ml	10mm	14mm
500 µg/ml	10mm	12mm
250 µg/ml	08mm	10mm
125 µg/ml	08mm	10mm

(Note; µg-micro gram mm-mili liter)

Table 3: Antibiotic sensitivity study of ATCC *Klebsiella pneumonia* against β-lactamase sensitive and resistant antibiotics.

β-lactamase Resistant	**β –lactamase sensitive**	
Antibiotics	**Antibiotics**	**Zone of inhibition in mile-meters**
Ceftazidime	Cefoperazone/ sulbactum	28mm
Cephalexin	Cefodoxime	30mm
Cefaclor	Ceftriaxone	26mm
Amoxicillin	Cefoperazone	22mm
Cefixime	Amoxicillin/sulbactum	20mm
	Meropeneum	38mm
	Azithromycin	16mm
	Cefuroxime	12mm
	Piperacillin	12mm
	Ampicillin	12mm

DISCUSSION

In the current study aqueous and alcoholic extracts of *Varuna & Gokshura* were prepared using cold maceration method. On analyzing the antibiotic sensitivity of Aqueous and alcoholic extracts of Varuna and Gokshura towards ATCC *Klebsiella pneumonia*, maximum zone of inhibition was observed for alcoholic extracts of Varuna at 2000 µg/ml , 1000 µg/ml and 500 µg/ml compared to aqueous extract, where the zone of inhibition of alcoholic extract at 2000 µg/ml, 1000 µg/ml and 500 µg/ml were 22 mm, 18mm and 16 mm respectively and zone of inhibition of aqueous extract of Varuna at 2000 µg/ml, 1000 µg/ml and 500 µg/ml were 08 mm, 10mm and 14 mm respectively.. But at 250 µg/ml and 125 µg/ml the zone of inhibition of aqueous extract is observed to be maximum(14mm and 16 mm) compared to alcoholic extract at same concentration. Anti-microbial activity of aqueous extracts and alcoholic extracts of Gokshura maximum zone of inhibition is observed in alcoholic extract compared to aqueous extracts at all concentration such as 2000 µg/ml,1000 µg/ml,500 µg/ml,250 µg/ml, and 125 µg/ml(14mm,14mm,14mm, 10mm and 12mm), whereas the zone of inhibition of aqueous extracts of Gokshura is observed to be 10mm,10mm,10mm, 08mm and 08mm at 2000 µg/ml,1000 µg/ml,500 µg/ml,250 µg/ml, and 125 µg/ml respectively. On comparison anti-bacterial assay of alcoholic Varuna and Gokshura against ATCC *Klebsiella pneumonia*, Alcoholic extract of Varuna shows maximum zone of inhibition at all concentration such as 2000 µg/ml,1000 µg/ml,500 µg/ml,250 µg/ml, and 125 µg/ml with zone of inhibition to be 22mm, 18mm, 16mm, 12mm, 12mm respectively in comparison to alcoholic extract of Gokshura at the same concentration the zone inhibition being 14mm, 14mm, 12mm, 10mm, 10mm at the same respective concentration. Analyzing anti-bacterial assay of aqueous Varuna and Gokshura against ATCC *Klebsiella pneumonia*, Aqueous extract of Varuna at lower concentration such as 500 µg/ml,250 µg/ml, and 125 µg/ml showed maximum zone of inhibition that is 14mm, 14mm, and 16 mm respectively compared to aqueous extract of Gokshura. But at 1000 µg/ml both aqueous extract of both Varuna and Gokshura showed same (10mm) zone of inhibition. At 2000 µg/ml, Aqueous extract of Gokshura showed better zone of inhibition of 10mm compared to aqueous extracts of Varuna. On analyzing the antibiotic sensitivity study of ATCC *Klebsiella pneumonia* against β- lactamase sensitive and resistant antibiotic, it was observed that maximum β –lactamase sensitive antibiotics were meropenem 38mm, cefpodoxime 30mm , and cefoperazone/ sulbactum 28 mm followed by ceftriaxone 26 mm, cedoperazone 22mm, Amoxicillin/ sulbactum 20 mm, Azithromycin 16 mm, cefuroxime, piperacillin, Ampicillin 12 mm each. Alcoholic extract of Varuna and Gokshura in higher concentration and lower concentration of aqueous extract of Varuna were showing significant zone of inhibition against the ATCC *K pneumonia* resistant to β –lactamase antibiotics such as Ceftazidime, Cephalexin, Cefaclor, Amoxicillin and Cefixime were resistant to the bacterial strain.

CONCLUSION

Pharmaceutical processing is a technique that converts natural products into therapeutically potent dosage form, which is easily absorbable in the biological system. Processing helps in preparing ideal medicine, which alleviates the sufferings of patients without complications. It also helps increasing potency of drugs and to make acceptable to the patients. In this study aqueous and alcoholic extracts of *Varuna & Gokshura* were prepared using cold maceration method. This method was preferred as it is very easy to perform, cheap and simple without using any complex instruments but yields highly potent extract with many bios' active principles. The active phytochemical constituents of the plants are contained within the cells of the plant. Alcohol provides a particularly effective way of maximizing the bioavailability of the active principles extracted from the plant. Ethanol is a molecule with both a polar and a non-polar end. Many taste molecules are polar whereas most aroma molecules are non-polar, and the good thing is that ethanol can be used to extract both

groups of compounds. While conducting sensitivity, these constituents interact with components of cell membrane of bacteria, causing elimination of flux of protons towards cell exterior which will cause cell death[7]. In other hands, hydrophobic characters of these extracts enable them to react with protein of microbial cell membrane and mitochondria to disturb their cell structures and permeability[8]. Likewise, the antimicrobial effects of drugs involve into inhibition of various cellular processes followed by an increase in plasma membrane permeability and finally ion leakage from the cells[9]. Meantime for different concentrations of the same drug, it may exhibit different zones of inhibition. Because the different components diffuse at different rates may have been responsible for the varying zone of inhibition against the bacteria. In lower concentrations, the molecular size of the active components will be too small via complete dissolution and thereby these components can penetrate easily through cell membrane of bacteria. So, it will show maximum zone of inhibition than other higher concentrations[10]. For higher concentrations, even if the drug content is more, it may not show significant zone of inhibition. Hence based on the current study it can be concluded that alcoholic extracts of Varuna have better antibacterial activity compared to alcoholic extract of Gokshura at all concentration and aqueous extract of Varuna is said have better antibacterial property compared to aqueous extract of Gokshura at lower concentration.

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