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# Antibacterial Effect of *Gokshura* against *Klebsiella Pneumoniae* by Urine Culture and Sensitivity

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## ABSTRACT

Genus Klebsiella accounts for various nosocomial infections especially urinary tract infections (UTI). Administration of antibiotics in order to manage the condition may help to resist the infection up to borderline. Here, propounding techniques such as *culture and sensitivity to* diagnose UTI and thereby antimicrobial susceptibility testing uplift current updated investigatory tools. Microorganisms are considered under concept of krimi in Ayurveda. Ayurveda drugs possessing 'krimighna' property are effective against microorganism, which is the need of the hour, as drug resistance is increasing towards many allopathic medicines. Therefore, it is necessary to establish antimicrobial activity of such drugs against pathogenic microorganisms before administering in patients. *Gokshura*, the drug one among the krimighna dashaimani is being reported as a good antibacterial drug. It was found that ethanolic extract of *Gokshura*, which is enriched with active phytochemical constituents, is capable of exerting antimicrobial activity. So the present paper is intended to prove the antibacterial action of *Gokshura* against *Klebsiella pneumoniae* by urine culture and sensitivity.

## KEYWORDS

*Urine culture and Sensitivity, Klebsiella pneumoniae, Antibacterial action of Gokshura*

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## INTRODUCTION

*Klebsiella pneumoniae*, a virulent Gram negative bacteria causes nosocomial infections like pneumonia, urinary tract infections and so on. Different strains of *Klebsiella pneumoniae* is said to possess antibiotic resistance and very perplexing to treat[1].The pathological process of UTI caused by *Klebsiella pneumoniae* bacteria may affect any part of urinary system. Ayurveda mentions Krimighna drugs, but specific drug for specific causative microorganism is missing and it shows a lacunae in this regard. So it is necessary to implement microbiology based diagnostic ways like urine culture and sensitivity and identify causative micro-organism, its characteristics and other attributes, further culture these organisms in vitro. Before the drug is used clinically on patients, its activity needs to be evaluated on causative micro-organisms in vitro and confirmed for sensitivity against micro-organisms. Thereby preliminary evidence in-vitro study will be generated scientifically, so that drug can be later used in patients as Upashaya. Sensitivity is done to assess anti-microbial activity of a drug possessing krimighna property against a particular bacteria and to define the anti-microbial property of that particular drug for known concentrations.

*Gokshura* is indicated in *mutrakrichra* and also possess krimighna property[2,3,4]. Here, current study is undertaken to envisage various diagnostic techniques related to microbiology, its laboratory diagnosis, culture and sensitivity of *Klebsiella pneumoniae* against *Gokshura*.

## OBJECTIVE

To study the antibacterial action of *Gokshura* (*Tribulus terrestris Linn*.) against *Klebsiella pneumonia*

## MATERIALS AND METHODS

Plant collection and Authentication was conducted. Fruits of *Gokshura* was collected from a shop and authentication of the raw drug was conducted. Alcoholic extract of *Gokshura* by Hot Extraction was prepared using Soxhlet method**.** 50 gram of coarse powder of *Gokshura* and 500 ml ethanol was taken for extraction. The cycle was repeated for 14 siphons in one day (Figure No.1-7).

**Figure 1** Raw drug Gokshura fruits


**Figure 2** Powdered Gokshura fruits

**Figure 3** Soxhlet extraction of Gokshura

**Figure 4** Soxhlet extraction of Gokshura


**Figure 5** Extract collected after Hot extraction

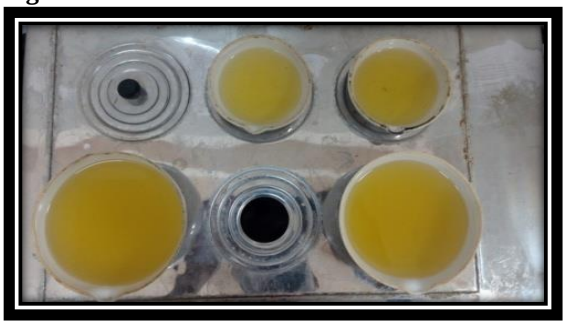


**Figure 6** Extract over water bath

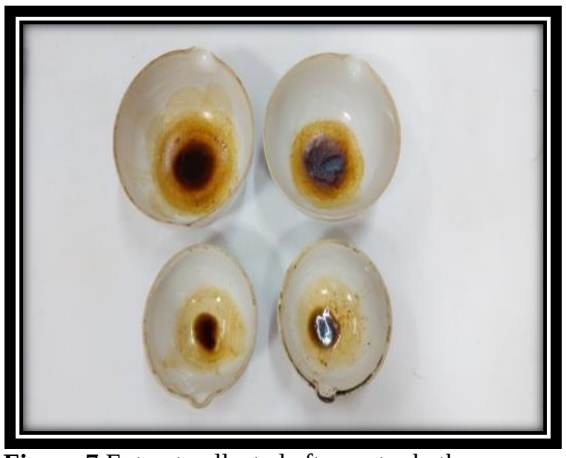
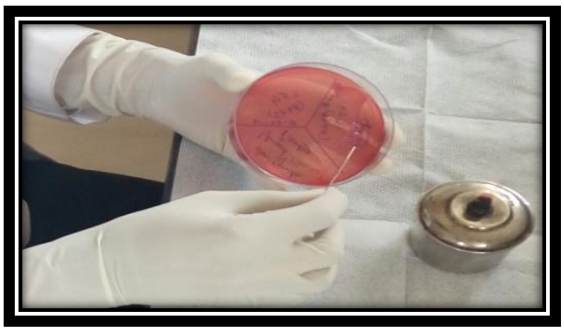


**Figure 7** Extract collected after water bath

Further, a sample of prepared extract has sent for phytochemical screening and HPTLC (Table No.1 & 2/Figure No.13). Culturing, isolation and identification of bacteria *Klebsiella pneumonia* was conducted. The urine sample was subjected to microscopical examination. Culturing was done over Macconkey agar and kept under incubation for 24 hours in culture condition. Microscopical examination was done for cultured organism. Sting test and Gram's staining was carried out for *Klebsiella pnuemoniae* followed with sub-culturing (Figure No.8-9).

**Figure 8** Cultured bacteria *Klebsiella pneumoniae*



Evaluation of sensitivity was conducted in laminar air flow. Agar well diffusion method was chosen to perform sensitivity. One loop full of *Klebsiella pneumoniae* from 24 hours culture was transformed into the Muller Hinton agar media with a sterile non-toxic cotton swab and swabbing done over the media (lawn culture). Six equidistant wells were made on the plate with sterile cork bourer. The wells in the dishes were filled with different concentrations (20, 10, 5, 2.5, 1.25 and 0.625µg/ml) of prepared extract. Plates were kept for incubation at 37◦C for 24 hours. After 24 hours, aassessment was done via identifying presence of "*Halo"* around the wells and the measurement was tabulated (Figure No.10-12).

**Figure 10** Dilutions of different concentrations of Gokshura alcoholic extract

**Figure 11** Sensitivity shown by alcoholic extract of Gokshura at different concentrations

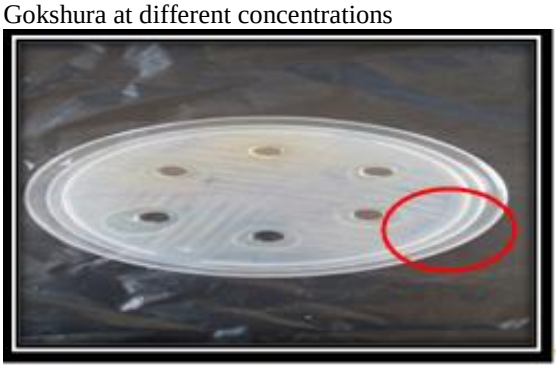


**Figure 12** Sensitivity shown by alcoholic extract of Gokshura at different concentrations

**Results of Preliminary phytochemical tests and HPTLC of Alcoholic extract of *Gokshura***

Zone of inhibition was categorised as sensitive zone, moderately sensitive / intermediate sensitive zone and resistant zone.

**Part A: Particulars of sample submitted**

**Test requested by**: Dr. Anand S, SDM College of Ayurveda Hassan

**Requested on**: 26-09-18

**Investigation to be performed**: HPTLC

**Sample coded as**: 18090604

**Sample details**: Gokshura extract

**Part B: Methodology**

**HPTLC**

100mg of Gokshura exatrct was dissolved in 1.0 ml of *alcohol* . 4, 8, 12µl of the above

extract was applied on a pre-coated silica gel F254 on aluminum plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in Toluene: Ethyl acetate (8.0: 1.0). The developed plates were visualized in under short UV, long UV and then derivatised with vanillin sulphuric acid and scanned under UV 254nm, 366nm. $R_f$, colour of the spots and densitometric scan were recorded.

Following preliminary phytochemical tests have been conducted with alcoholic extract of *Gokshura* to detect presence of the phytochemical components (Table No.1)

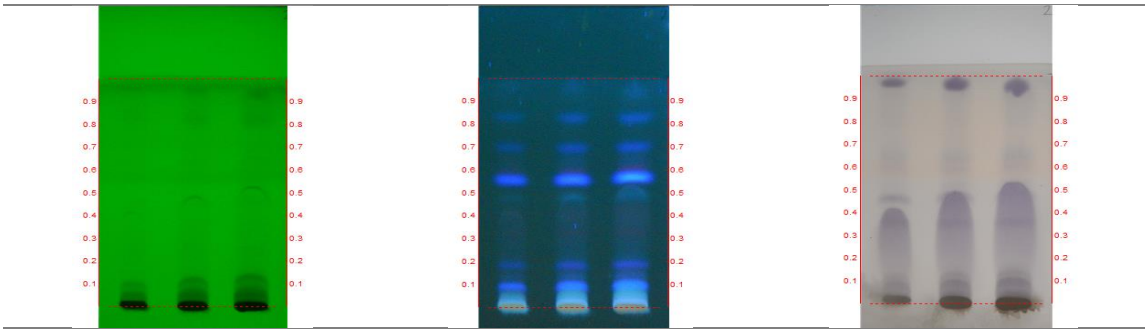
**Table 1** Preliminary phytochemical tests

| **Tests** | **Colour if positive** | Alcoholic extract of Gokshura |
|---|---|---|
| **Alkaloids** | | |
| **Dragendroff's test** | Orange red precipitate | Orange red precipitate |
| **Wagners test** | Reddish brown precipitate | Reddish brown precipitate |
| **Mayers test** | Dull white precipitate | Dull white precipitate |
| **Hagers test** | Yellow precipitate | Yellow precipitate |
| **Steroids** | | |
| **Liebermann- buchard test** | Bluish green colour | Bluish green colour |
| **Salkowski test** | Bluish red to cherry red color in chloroform layer and green fluorescence in acid layer | Bluish red to cherry red color in chloroform layer and green fluorescence in acid layer |
| **Carbohydrate** | | |
| **Molish test** | Violet ring | Violet ring |
| **Fehlings test** | Brick red precipitate | Brick red precipitate |
| **Benedicts test** | Red precipitate | Red precipitate |
| **Tannin** | | |
| **With $FeCl_3$** | Dark blue or green or brown | Green color |
| **Flavanoids** | | |
| **Shinoda's test** | Red or pink | Pink color |
| **Saponins** | | |
| **With $NaHCO_3$** | Stable froth | No stable froth |
| **Triterpenoids** | | |
| **Tin and thionyl chloride test** | Pink | No pink color |
| **Coumarins** | | |
| **With 2 N NaOH** | Yellow | Yellow color |
| **Phenols** | | |
| **With alcoholic ferric chloride** | Blue to blue black | Green color |
| **Carboxylic acid** | | |
| **With water and $NaHCO_3$** | Brisk effervescence | No effervescence |
| **Amino acid** | | |
| **With ninhydrine reagent** | Purple colour | Purple color |
| **Resin** | | |
| **With aqueous acetone** | Turbidity | Turbidity |
| **Quinone** | | |
| **Conc. sulphuric acid** | Pink/purple/red | Yellow color |

Results of Preliminary phytochemical tests are tabulated in the following table (Table No.2)

**Table 2** Part C: Results

| Test | Inference |
|---|---|
| | **Alcoholic extract of Gokshura** |
| Alkaloid | + |
| Steroid | + |
| **Carbohydrate** | + |
| **Tannin** | + |
| **Flavanoids** | + |
| **Saponins** | - |
| **Terpenoid** | - |
| **Coumarins** | + |
| **Phenols** | - |
| **Carboxylic acid** | - |
| **Amino acids** | + |
| **Resin & Quinone** | + |



Short UV | **Long UV** | **After derivatisation**

Observation on Antibacterial activity shown at different concentrations of alcoholic extracts of *Gokshura* against *Klebsiella pneumonia* is mentioned below (Table No.3)

**Table 3** Observation on Antibacterial activity shown at different concentrations of alcoholic extracts of *Gokshura* against *Klebsiella pneumoniae*

| Extract | *ZOI in mm against *Klebsiella pneumoniae* | 20 µg/ml N=30 | | 10 µg/ml N=30 | | 5 µg/ml N=30 | | 2.5 µg/ml N=30 | | 1.25 µg/ml N=30 | | 0.625 µg/ml N=30 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | F | % | F | % | F | % | F | % | F | % | F | % |
| **Alcoholic extract of *Gokshura*** | **0** | 21 | 70 | 22 | 73.3 | 14 | 46.7 | 3 | 10 | 2 | 6.7 | 2 | 6.7 |
| | **10** | 3 | 10 | 2 | 6.7 | 1 | 3.3 | 1 | 3.3 | - | - | 1 | 3.3 |
| | **12** | 1 | 3.3 | 1 | 3.3 | - | - | - | - | - | - | - | - |
| | **14** | - | - | 1 | 3.3 | - | - | - | - | - | - | - | - |
| | **18** | 1 | 3.3 | - | - | - | - | 1 | 3.3 | - | - | 1 | 3.3 |
| | **20** | 3 | 10 | 1 | 3.3 | 6 | 20 | 12 | 40 | 9 | 30 | 8 | 26.7 |
| | **22** | 1 | 3.3 | 2 | 6.7 | 6 | 20 | 5 | 16.7 | 7 | 23.3 | 5 | 16.7 |
| | **24** | - | - | 1 | 3.3 | 3 | 10 | 6 | 20 | 8 | 26.7 | 5 | 16.7 |
| | **26** | - | - | - | - | - | - | - | - | 2 | 6.7 | 2 | 6.7 |
| | **28** | - | - | - | - | - | - | 1 | 3.3 | 1 | 3.3 | 4 | 13.3 |
| | **30** | - | - | - | - | - | - | - | - | - | - | 1 | 3.3 |
| | **32** | - | - | - | - | - | - | - | - | - | - | 1 | 3.3 |
| | **34** | - | - | - | - | - | - | 1 | 3.3 | 1 | 3.3 | - | - |
| | **Total** | 30 | 100 | 30 | 100 | 30 | 100 | 30 | 100 | 30 | 100 | 30 | 100 |

**ZOI = Zone Of Inhibition**

*** F = Frequency of samples showing sensitivity against alcoholic extract of *Gokshura***

*** N = Total no. of samples**

Sensitivity at different concentrations of alcoholic extract of *Gokshura* against *Klebsiella pneumonia* is given below (Table No.4*)*

**Table 4** Sensitivity at different concentrations of alcoholic extract of *Gokshura* against *Klebsiella pneumonia*

| Concentrations | 20µg/ml | | | 10µg/ml | | | 5µg/ml | | | 2.5µg/ml | | | 1.25µg/ml | | | 0.625µg/ml | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | S | M | R | S | M | R | S | M | R | S | M | R | S | M | R | S | M | R |
| **No. of samples** | 4 | 1 | 25 | 4 | 0 | 26 | 16 | 0 | 14 | 25 | 1 | 4 | 28 | 0 | 2 | 26 | 1 | 3 |

Sensitivity at different concentrations of alcoholic extract of *Gokshura* against *Klebsiella pneumoniae* was fixed as follows*:* S (Sensitive) = 22-20 mm, M

(Moderately sensitive) = 18-16 mm, R (Resistant) = 14-12 mm

Mean values of zone of inhibition in millimetre at different concentrations of alcoholic extract of *Gokshura* against *Klebsiella pneumonia* is tabulated below (Table No.5)

**Table.5** Mean values of zone of inhibition in millimetre at different concentrations of alcoholic extract of *Gokshura* against *Klebsiella pneumonia*

| **Different concentrations of alcoholic extract of *Gokshura*** | **20µg/ml** | **10µg/ml** | **5µg/ml** | **2.5µg/ml** | **1.25µg/ml** | **0.625µg/ml** |
|---|---|---|---|---|---|---|
| **N** | 30 | 30 | 30 | 30 | 30 | 30 |
| **Mean of zone of inhibition in mm** | 4.73 | 4.47 | 11.13 | 19.47 | 21.33 | 21.47 |

Explanation on above mentioned tables and results are discussed in discussion below.

## DISCUSSION

Sensitivity of different concentrations of *Gokshura* extract (20,10,5,2.5,1.25 and 0.625µg/ml) against *Klebsiella pneumonia* in 30 samples isolated by urine culture and sensitivity has drawn the following data (Table No.3).

At 20µg/ml *Gokshura* showed maximum zone of inhibition was 22 mm in 1 (3.3%) sample, 20 mm in 3 (10%), 18 mm in 1 sample (3.3%), 12 mm in 1 (3.3%) sample, minimum zone of inhibition 10 mm was observed in 3 (10%) samples.

Similarly, at 10µg/ml, 24 mm in 1 (3.3%) sample, 22 mm in 2 (6.7%) samples, 20 mm in 1 (3.3%) sample, 14 mm in 1 (3.3%) sample, 12 mm in 1 sample (3.3%) , 10 mm in 2 (6.7%) samples.

At 5µg/ml, 24 mm in 3 (10%) samples, 22 mm in 6 (20%) samples, 20 mm in 6 (20%) samples, 10 mm in 1 (3.3%) sample.

At 2.5µg/ml, 34 mm in 1 (3.3%) sample, 28 mm in 1 (3.3%) sample, 24 mm in 6 (20%) samples, 22 mm in 5 (16.7%) samples, 20 mm in 12 (40%) samples, 18 mm in 1 (3.3%) sample, 10 mm in 1 (3.3%) sample.

At 1.25µg/ml, 34 mm in 1 (3.3%) sample, 28 mm in 1 (3.3%) sample, 26 mm in 2 (6.7%) samples, 24 mm in 8 (26.7%) samples, 22 mm in 7 (23.3%) samples, 20 mm in 9 (30%) samples.

At 0.625µg/ml, 32 mm in 1 (3.3%) sample, 30 mm in 1 (3.3%) sample, 28 mm in 4 (13.3%) samples, 26 mm in 2 (6.7%) samples, 24 mm in 5 (16.7%) samples, 22 mm in 5 (16.7%) samples, 20 mm in 8 (26.7%) samples, 18 mm in 1 (3.3%) sample, 10 mm in 1 (3.3%) sample.

Out of 30 samples of *Klebsiella pneumoniae*, at 20µg/ml concentration, 4

samples were sensitive, 1 sample is moderately sensitive and 25 samples are resistant. At 10µg/ml, 4 samples are sensitive and 26 samples are resistant. At 5µg/ml, 16 samples are sensitive and 14 samples are resistant. At 2.5µg/ml, 25 samples are sensitive, 1 sample is moderately sensitive and 4 samples are resistant. At 1.25µg/ml, 28 samples are sensitive and 2 samples are resistant. At 0.625µg/ml, 26 samples are sensitive, 1 sample is moderately sensitive and 3 samples are resistant (Table No.4).

From the above observation and result based on the present study, it was proved that extract of *Gokshura* showed good antimicrobial activity against *Klebsiella pneumoniae*. Maximum zone of inhibition (34mm) was recorded at 2.5µg/ml and 1.25µg/ml and minimum zone of inhibition (10mm) was recorded at 20µg/ml, 10µg/ml, 5µg/ml, 2.5µg/ml and 0.625µg/ml concentrations. For different concentrations of *Gokshura*, it is clear that decreasing the concentrations significantly increase in zone of inhibition. Consecutively, number of sensitive zones increased and number of resistant zones decreased on decreasing the concentrations.

Acharya *Charaka* has mentioned *Gokshura* as krimighna while explaining different dashaimanis. Alcoholic extracts of drugs are enriched with antibacterial constituents viz. Terpenoids, Alkaloids, Saponins, Flavanoids and Phenolic compounds possessing properties like alteration of surface tension of extra cellular medium of bacteria cell wall, ability to complex with extracellular soluble proteins, intruding and destruction of DNA of microbial celletc[5]. In this study, preliminary phyto-chemical screening of *Gokshura* extract has been conducted and confirmed the presence of the Terpenoids, Alkaloids, Tannins, Steroids.

Alcoholic extraction of drug maximizes the bioavailability of the active principles from the plant. It is reported that ethanol contains both, polar and non-polar ends to extract both groups of compounds of a drug. So these phytochemical constituents are completely dissolved in solvent. 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[6]. In other hands, hydrophobic characters of these extracts enable to react with protein of microbial cell membrane and mitochondria to disturb their cell structures and permeability[7]. 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[8]. Meantime for

different concentrations of a same drug, it may exhibit different zone 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[9]. For higher concentrations, even the drug content is more, it may not show significant zone of inhibition.

The variation of susceptibility of the bacteria can be attributed with their intrinsic properties and permeability of cell surface to the extracts. Porosity of cell membrane varies cell to cell and the membrane inhibits cell structure perturbations by phytochemical components because of these unique characteristics.[10]

## CONCLUSION

*Gokshura* showed krimighna (antibacterial) action against *Klebsiella pneumonia* isolated by urine culture and sensitivity. In lower concentrations, the molecular size of the active components will be too small due complete dissolution and thereby it can penetrate easily through cell membrane of bacteria. Therefore it will show maximum zone of inhibition than other higher concentrations.

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