Journal of Pharmaceutical and Scientific Innovation

www.jpsionline.com (ISSN : 2277 -4572)

Research Article

# AN OBSERVATIONAL EXPERIMENTAL ASSESSMENT OF ANTIBACTERIAL ACTIVITY OF GUDUCHI AGAINST *KLEBSIELLA PNEUMONIAE* BY URINE CULTURE AND SENSITIVITY IN PITTAJAMUTRAKRICHRA (URINARY TRACT INFECTION)

Anand S [1*], Ajantha [2], Shashirekha K S [3], Geetha Nayak S [1]

[1] P.G Scholar, Department of Roga Nidana Avum Vikriti Vigyana, Sri Dharmasthala Manjunatheshwara College of Ayurveda and Hospital, Hassan, India

[2] Professor, Department of Roga Nidana Avum Vikriti Vigyana, Sri Dharmasthala Manjunatheshwara College of Ayurveda and Hospital, Hassan, India

[3] Micro biologist, Department of Roga Nidana Avum Vikriti Vigyana, Sri Dharmasthala Manjunatheshwara College of Ayurveda and Hospital, Hassan, India

*Corresponding Author Email :dranandpala@gmail.com

**DOI :10.7897/2277-4572.091165**

Received on :14/11/19 Revised on :10/12/19 Accepted on :22/12/19

**ABSTRACT**

Urinary tract infections in human beings are associated with high morbidity and long term complications. Lakshanas of Pittaja mutrakrichra and symptoms of urinary tract infections are analogues. In majority, causative organisms of urinary tract infections are gram-negative bacteria viz. *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis* etc. *Klebsiella pneumoniae* is the foremost micro-organism causing nosocomial infections including Urinary tract infection. Krimighna action of Ayurveda drug has to be re-investigated in line with modern scientific parameters to generate evidence and thereby to facilitate administration in patient against specific causative bacteria as Upashaya. This is achieved through tools like culture and sensitivity *in vitro*. Guduchi (*Tinospora cordifolia* Miers.) is indicated in mutrakrichra and said to possess krimighna property. Present work is planned to envisage various attributes of the microorganism *Klebsiella pneumoniae* by culture and sensitivity with Guduchi in patients suffering from *Pittaja Mutrakrichra* with special reference to Urinary tract infection. Patients of Pittaja mutrakrichra were subjected for urine culture and those with positive results for *Klebsiella pneumoniae* were further used. Alcoholic extract of Guduchi was prepared by Soxhlet method. Further sensitivity test was performed by Agar well diffusion method and zone of inhibition was measured. Alcoholic extract of Guduchi has showed better antibacterial effect against *Klebsiella* pneumoniae. Alcoholic extract contains active phytochemical components and these components aid in antibacterial action against cell membrane of micro-organism.

**Keywords:** Urine culture and Sensitivity, *Klebsiella pneumoniae*, Antibacterial action of alcoholic extract of Guduchi

## INTRODUCTION

Mutrakrichra is defined as krichrata of mutrapravruthi or difficulty during micturition affecting the Basthi, one among the trimarma. Pittaja mutrakrichra is characterized by krichrata of mutrapravriti, muhurmuhurpravriti, sarakta, sadaha, saruja, peetamutra pravruthi and same presentation is encountered in Urinary tract infection[1,2]. It is caused by infection from bacteria *Klebsiella pneumoniae,* one among the pathogenic organism, others include *Escherichia Coli, Proteus mirabilis* etc. The pathological process of urinary tract infection induced by *Klebsiella pneumonia* bacteria may affect any part of urinary system[3]. Ayurveda explains drugs possessing krimighna action, but indication of specific drug on specific causative micro-organism and stage of disease is missing and very few studies have been accomplished in this regard. Therefore it is the need of the hour to use diagnostic tools like 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 checked on causative microorganisms *in vitro* and confirmed whether the drug shows sensitivity. Thereby preliminary evidence *in-vitro* study can be generated scientifically, so that drug can be later used in patients as Upashaya. Sensitivity is done in order to find out the anti-microbial activity of a drug that possesses krimighna property against a particular microorganism and to define the anti-microbial property of that particular drug for known concentrations. Guduchi is a common drug used in urinary disorders and said to possess krimighna property[4]. Hence, adoption of new approaches like culture and sensitivity methods would strengthen existing Ayurveda knowledge and help in achieving improved diagnostic and curative abilities. Therefore, present study is undertaken to study various attributes of the bacteria *Klebsiella pneumoniae*, its laboratory diagnosis, its culture and sensitivity with Guduchi by culture and sensitivity method with urine of patients suffering from Pittaja mutrakrichra (Urinary Tract Infection).

### Aims and objectives

To study the sensitivity of Guduchi (*Tinospora cordifolia* Miers.) against *Klebsiella pneumoniae* by urine culture and sensitivity method in Pittaja Mutrakrichra (Urinary Tract Infection)

## MATERIALS AND METHODS

Plant collection and Authentication was initially conducted. Stems of Guduchi (*Tinospora cordifolia* Miers.) were collected from a shop. The authentication of the raw drug was done at Sri Dharmasthala Manjunatheshwara College of Ayurveda and

Hospital, Hassan (No. SDMCAH-DG/2018/22). It was cleaned, dried and a coarse powder was prepared.

Alcoholic extract of Guduchi by Hot Extraction was prepared using Soxhlet method[5]. Coarse powder of Guduchi (50 gm) was placed inside a thimble in a filter paper that was loaded into the main chamber of the Soxhlet extractor. The Soxhlet extractor was placed onto a flask containing the extraction solvent (Ethanol = 500 ml). The Soxhlet was then equipped with a condenser. The solvent was heated and the solvent vapour travelled up a distillation arm, and flooded into the chamber housing the thimble of solid. The condenser ensures that any solvent vapour cools and drips back down into the chamber housing the solid material. The chamber containing the solid material slowly filled with warm solvent. When the Soxhlet chamber was almost full, the chamber was automatically emptied by a siphon side arm, and the solvent was run back down to the distillation flask. This cycle was repeated for 14 siphons in 1 day (Figure 1-7).

Preliminary Phytochemical Screening and HPTLC of alcoholic extract of Guduchi was conducted at Sri Dharmasthala Manjunatheshwara Centre for Research in Ayurveda and Allied Sciences, Kuthpady, Udupi (Table 2 and 3/Figure 13 and 14).

30 patients fulfilling diagnostic and inclusion criteria were included for study from OPD and IPD of Sri Dharmasthala Manjunatheshwara College of Ayurveda and Hospital, Hassan and other referrals. Study was approved by Institutional ethical committee and study was carried out as per the ethical standards approved in study.

Patients complaining of Krichramutrata (dysuria) associated with one or more following symptoms of Pittaja mutrakrichra (Urinary Tract Infection)

**Table 1: Diagnostic criteria**

| Pittaja mutrakrichra | Urinary tract infection |
|---|---|
| Muhurmuhu mutra pravruthi | Frequency/ urgency |
| Basthisoola | Supra pubic pain |
| Mutra daha | Burning sensation |
| Sarujamutrata | Painful micturition |
| Peeta mutrata | Yellowish urine |
| Saraktamutram | Haematuria |

**Inclusion criteria**

Patients between the ages of 18 – 70 years of either gender fulfilling the diagnostic criteria were included for the study

**Exclusion criteria**

Patient with chronic kidney failure or any other disease, that may interfere in course of study

Culturing, isolation and identification of bacteria *Klebsiella pneumonia* was conducted. The mid-stream sample of urine from the patients of urinary tract infection was collected and microscopical examination was done for microscopic characterization of the bacteria. The inoculum was transferred to MacConkey agar plate and culturing was done by Streak culture method under incubation for 24 hours with culture condition. The cultured organism was subjected to microscopical examination, Sting test and Gram's staining for the identification of *Klebsiella pneumoniae* bacteria followed with sub-culturing and bio-chemical-serological tests for confirmation (Figure 8-9).

Evaluation of Sensitivity was conducted. Sensitivity test was performed by Agar well diffusion method. Workplace was cleaned in laminar air flow using 70% of ethyl alcohol and UV was switched on for 20 minutes. One loop full of *Klebsiella pneumoniae* from 24 hours culture was transformed into the Mueller-hinton agar media with a sterile non-toxic cotton swab and swabbing done over the media (lawn culture). 6 equidistant wells were made on the plate with sterile cork borer. These wells in the Petri dish were filled with different concentrations (20, 10, 5, 2.5, 1.25 and 0.625 µg/ml) of alcoholic extract of Guduchi. Plates were kept for incubation at 37◦C for 24 hours. After the incubation period, the zone of inhibition was measured and tabulated (Figure 10-12).

Next Assessment was done. After incubation; zone of inhibition was identified with presence of "*Halo"*around the wells. These "*Halo"* was measured with ruler and the results were tabulated as sensitive zone, moderately sensitive / intermediate sensitive zone and resistant zone.

**OBSERVATIONS AND RESULT**

**Figure 1: Raw drug Guduchi stems**


**Figure 2: Powdered Guduchi stems**

**Figure 3: Soxhlet extraction of Guduchi**

**Figure 4: Soxhlet extraction of Guduchi**


**Figure 5: Extract collected after hot extraction**

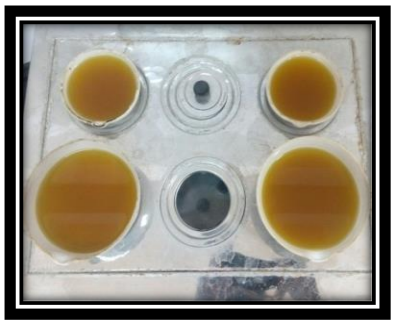


**Figure 6: Extract over water bath**

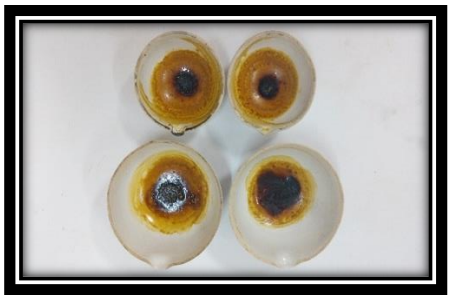


**Figure 7: Alcoholic extract of Guduchi**

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

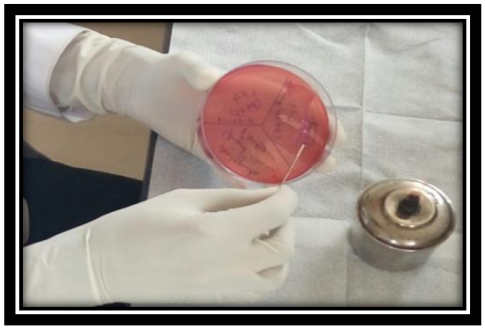


**Figure 9: Culturing of urine sample over MacConkey agar media**

**Figure 10: Dilutions of different concentrations of Guduchi alcoholic extract**

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

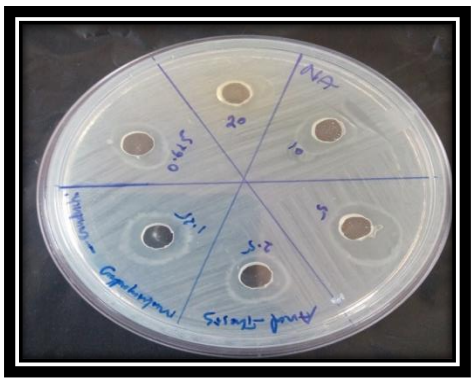


**Figure 12: Sensitivity shown by alcoholic extract of Guduchi at different concentrations**

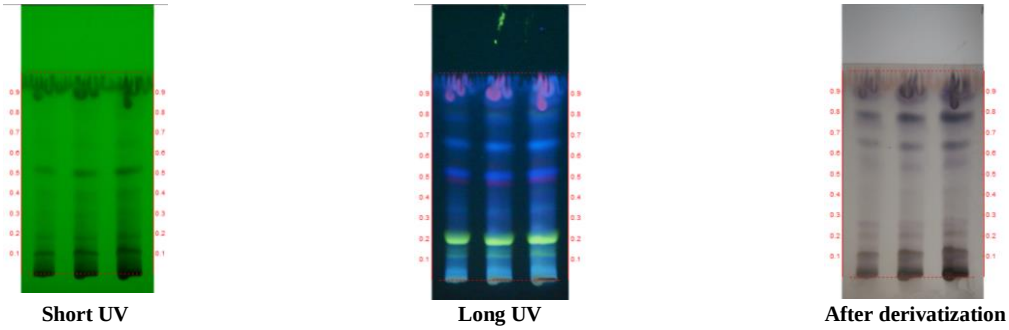
**Table 2: Observation on Preliminary phytochemical tests and HPTLC of Alcoholic extract of Guduchi**

| Tests | Color if positive | Result for Alcoholic extract of Guduchi |
|---|---|---|
| **Alkaloids** | | |
| Dragendorff'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- burchard test | Bluish green color | Bluish green color |
| 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** | | |
| Molisch 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 |
| **Flavonoids** | | |
| Shinoda's test | Red or pink | Red color |
| **Saponins** | | |
| With $NaHCO_3$ | Stable froth | Stable froth |
| **Triterpenoids** | | |
| Tin and thionyl chloride test | Pink color | 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 ninhydrin reagent | Purple colour | Brown color |
| **Resin** | | |
| With aqueous acetone | Turbidity | Turbidity |
| **Quinone** | | |
| Conc. sulphuric acid | Pink/purple/red | Yellow color |

**Table 3: Results of preliminary phytochemical screening of Alcoholic extract Guduchi**

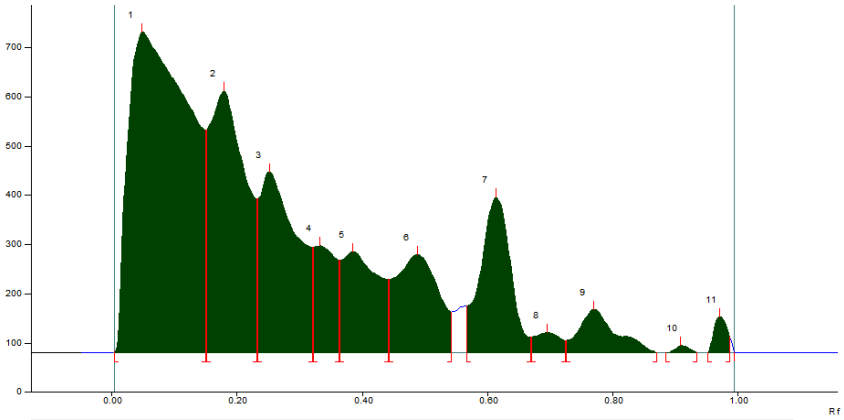
| Test | Inference |
|---|---|
| | **Guduchi** |
| Alkaloid | + |
| Steroid | + |
| Carbohydrate | + |
| Tannin | + |
| Flavonoids | + |
| Saponins | + |
| Terpenoid | + |
| Coumarins | + |
| Phenols | - |
| Carboxylic acid | - |
| Amino acids | - |
| Resin | + |
| Quinone | - |

(+)– present; (-) – negative



**Short UV** **Long UV** **After derivatization**

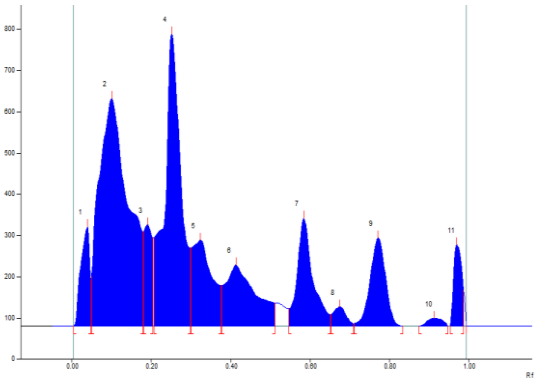
**Figure 13: HPTLC Photo-documentation of sample of Alcoholic extract of Guduchi**



Track 3, ID: Guduchi extract

| Peak | Start Position | Start Height | Max Position | Max Height | Max % | End Position | End Height | Area | Area % |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.00 Rf | 2.5 AU | 0.05 Rf | 651.2 AU | 24.10 % | 0.15 Rf | 51.6 AU | 45677.5 AU | 36.63 % |
| 2 | 0.15 Rf | 452.4 AU | 0.18 Rf | 531.0 AU | 19.65 % | 0.23 Rf | 12.5 AU | 22222.3 AU | 17.82 % |
| 3 | 0.23 Rf | 312.6 AU | 0.25 Rf | 367.0 AU | 13.58 % | 0.32 Rf | 13.1 AU | 15737.1 AU | 12.62 % |
| 4 | 0.32 Rf | 213.5 AU | 0.33 Rf | 216.8 AU | 8.03 % | 0.36 Rf | 87.2 AU | 5369.9 AU | 4.31 % |
| 5 | 0.36 Rf | 187.2 AU | 0.39 Rf | 204.9 AU | 7.59 % | 0.44 Rf | 48.2 AU | 8731.9 AU | 7.00 % |
| 6 | 0.44 Rf | 148.4 AU | 0.49 Rf | 199.2 AU | 7.37 % | 0.54 Rf | 82.7 AU | 9812.8 AU | 7.87 % |
| 7 | 0.57 Rf | 95.1 AU | 0.61 Rf | 315.2 AU | 11.67 % | 0.67 Rf | 31.8 AU | 10893.7 AU | 8.74 % |
| 8 | 0.67 Rf | 32.0 AU | 0.70 Rf | 41.4 AU | 1.53 % | 0.73 Rf | 24.8 AU | 1212.6 AU | 0.97 % |
| 9 | 0.73 Rf | 24.8 AU | 0.77 Rf | 87.9 AU | 3.25 % | 0.87 Rf | 0.2 AU | 3710.2 AU | 2.98 % |
| 10 | 0.89 Rf | 0.1 AU | 0.91 Rf | 14.1 AU | 0.52 % | 0.94 Rf | 0.3 AU | 222.2 AU | 0.18 % |
| 11 | 0.95 Rf | 0.1 AU | 0.97 Rf | 72.9 AU | 2.70 % | 0.99 Rf | 31.5 AU | 1102.5 AU | 0.88 % |

**At 254 nm**



Track 3, ID: Guduchi extract

| Peak | Start Position | Start Height | Max Position | Max Height | Max % | End Position | End Height | Area | Area % |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.00 Rf | 0.7 AU | 0.04 Rf | 241.0 AU | 8.48 % | 0.05 Rf | 10.9 AU | 3843.2 AU | 4.18 % |
| 2 | 0.05 Rf | 121.7 AU | 0.10 Rf | 552.4 AU | 19.44 % | 0.18 Rf | 28.7 AU | 29412.6 AU | 32.00 % |
| 3 | 0.18 Rf | 229.5 AU | 0.19 Rf | 245.1 AU | 8.63 % | 0.20 Rf | 14.5 AU | 3722.0 AU | 4.05 % |
| 4 | 0.21 Rf | 214.7 AU | 0.25 Rf | 708.7 AU | 24.93 % | 0.30 Rf | 89.3 AU | 21185.3 AU | 23.05 % |
| 5 | 0.30 Rf | 189.5 AU | 0.32 Rf | 208.9 AU | 7.35 % | 0.38 Rf | 98.5 AU | 7586.4 AU | 8.25 % |
| 6 | 0.38 Rf | 98.6 AU | 0.41 Rf | 147.9 AU | 5.21 % | 0.51 Rf | 56.2 AU | 8170.5 AU | 8.89 % |
| 7 | 0.55 Rf | 42.7 AU | 0.59 Rf | 261.5 AU | 9.20 % | 0.65 Rf | 28.3 AU | 7423.7 AU | 8.08 % |
| 8 | 0.65 Rf | 28.7 AU | 0.68 Rf | 46.9 AU | 1.65 % | 0.71 Rf | 6.4 AU | 1106.4 AU | 1.20 % |
| 9 | 0.71 Rf | 6.5 AU | 0.77 Rf | 213.2 AU | 7.50 % | 0.84 Rf | 0.0 AU | 5967.0 AU | 6.49 % |
| 10 | 0.88 Rf | 0.0 AU | 0.91 Rf | 19.3 AU | 0.68 % | 0.95 Rf | 0.4 AU | 529.9 AU | 0.58 % |
| 11 | 0.95 Rf | 0.2 AU | 0.97 Rf | 197.0 AU | 6.93 % | 0.99 Rf | 87.5 AU | 2955.6 AU | 3.22 % |

**At 366 nm**

**Figure 14: Densitometric scan of sample of Alcoholic extract of Guduchi**

**Table 4: Observation on Antibacterial activity shown at different concentrations of alcoholic extracts of Guduchi against *Klebsiella pneumonia***

| 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 Guduchi | **0** | 13 | 43.3 | 12 | 40 | 8 | 26.7 | 2 | 6.7 | 2 | 6.7 | 1 | 3.3 |
| | **10** | 5 | 16.7 | 2 | 6.7 | 2 | 6.7 | 1 | 3.3 | - | - | - | - |
| | **12** | - | - | 1 | 3.3 | - | - | - | - | - | - | - | - |
| | **14** | - | - | 1 | 3.3 | 1 | 3.3 | - | - | - | - | - | - |
| | **16** | - | - | 1 | 3.3 | - | - | 1 | 3.3 | - | - | - | - |
| | **18** | 1 | 3.3 | 1 | 3.3 | 1 | 3.3 | - | - | - | - | - | - |
| | **20** | 8 | 26.7 | 6 | 20 | 6 | 20 | 6 | 20 | 3 | 10 | - | - |
| | **22** | 1 | 3.3 | 4 | 13.3 | 4 | 13.3 | 4 | 13.3 | 6 | 20 | 5 | 16.7 |
| | **24** | 1 | 3.3 | 1 | 3.3 | 5 | 16.7 | 11 | 36.7 | 9 | 30 | 7 | 23.3 |
| | **26** | - | - | - | - | 2 | 6.7 | 2 | 6.7 | 7 | 23.3 | 5 | 16.7 |
| | **28** | 1 | 3.3 | 1 | 3.3 | 1 | 3.3 | 2 | 6.7 | 1 | 3.3 | 5 | 16.7 |
| | **30** | - | - | - | - | - | - | - | - | 1 | 3.3 | 2 | 6.7 |
| | **32** | - | - | - | - | - | - | 1 | 3.3 | - | - | 3 | 10 |
| | **34** | - | - | - | - | - | - | - | - | - | - | 1 | 3.3 |
| | **36** | - | - | - | - | - | - | - | - | 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 sample Sensitivity shown against alcoholic extract of Guduchi
* N = Total no. of samples

**Table 5: Sensitivity at different concentrations of alcoholic extract of Guduchi against *Klebsiella pneumoniae***

| Concentrations | 20 µg/ml | | | 10 µg/ml | | | 5 µg/ml | | | 2.5 µg/ml | | | 1.25 µg/ml | | | 0.625 µg/ml | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **S** | **M** | **R** | **S** | **M** | **R** | **S** | **M** | **R** | **S** | **M** | **R** | **S** | **M** | **R** | **S** | **M** | **R** |
| No. of samples | 3 | 9 | 18 | 5 | 8 | 17 | 13 | 6 | 11 | 21 | 6 | 3 | 25 | 3 | 2 | 29 | 0 | 1 |

S (Sensitive) = 22-20 mm, M (Moderately sensitive) = 18-16 mm, R (Resistant) = 14-12 mm

**Table 6: Mean values of zone of inhibition in millimetre at Different concentrations of alcoholic extract of Guduchi against *Klebsiella pneumonia***

| Different concentrations of alcoholic extract of Guduchi | 20 µg/ml | 10 µg/ml | 5 µg/ml | 2.5 µg/ml | 1.25 µg/ml | 0.625 µg/ml |
|---|---|---|---|---|---|---|
| **N** | **30** | **30** | **30** | **30** | **30** | **30** |
| **Mean of zone of inhibition in mm** | **10.07** | **11.33** | **15.33** | **21.27** | **22.80** | **25.80** |

## DISCUSSION

Sensitivity of alcoholic extract of different concentrations of Guduchi (20, 10, 5, 2.5, 1.25 and 0.625 µg/ml) against *Klebsiella pneumoniae* in 30 samples isolated by urine culture and sensitivity has drawn the following data (Table 4).

At 20 µg/ml, Guduchi showed that maximum zone of inhibition was 28 mm in 1 (3.3%) sample, 24 mm in 1 (3.3%) sample, 22 mm in 1 (3.3%) sample, 20 mm in 8 (26.7%) samples, 18 mm in 1 (3.3%) sample and minimum zone of inhibition 10 mm was observed in 5 (16.7%) samples and there was no zone of inhibition observed in 13 (43.3%) samples.

At 10 µg/ml, it showed that maximum zone of inhibition was 28 mm in 1 (3.3%) sample, 24 mm in 1 (3.3%) sample, 22 mm in 4 (13.3%) samples, 20 mm in 6 (20%) samples, 18 mm in 1 (3.3%) sample, 16 mm in 1 (3.3%) sample, 14 mm in 1 sample (3.3%), 12 mm in 1 sample (3.3%) and minimum zone of inhibition 10 mm was observed in 2 (6.7%) samples and there was no zone of inhibition observed in 12 (40%) samples.

At 5 µg/ml, it showed that maximum zone of inhibition was 28 mm in 1 (3.3%) sample, 26 mm in 2 (6.7%) samples, 24 mm in 5 (16.7%) samples, 22 mm in 4 (13.3%) samples, 20 mm in 6 (20%) samples, 18 mm in 1 (3.3%) sample, 14 mm in 1 (3.3%) sample and minimum zone of inhibition 10 mm was observed in 2 (6.7%) samples and there was no zone of inhibition observed in 8 (26.7%) samples.

At 2.5 µg/ml, it showed that maximum zone of inhibition was 32 mm in 1 (3.3%) sample, 28 mm in 1 (3.3%) sample, 26 mm in 2 (6.7%) samples, 24 mm in 11 (36.7%) samples, 22 mm in 4 (13.3%) samples, 20 mm in 6 (20%) samples, 16 mm in 1 (3.3%) sample and minimum zone of inhibition 10 mm was observed in 1 (3.3%) sample and there was no zone of inhibition observed in 2 (6.7%) samples.

At 1.25 µg/ml, it showed that maximum zone of inhibition was 36 mm in 1 (3.3%) sample, 30 mm in 1 (3.3%) sample, 28 mm in 1 (3.3%) sample, 26 mm in 7 (23.3%) samples, 24 mm in 9 (30%) samples, 22 mm in 6 (20%) samples and minimum zone of inhibition 20 mm was observed in 3 (10%) samples and there was no zone of inhibition observed in 2 (6.7%) samples.

At 0.625 µg/ml, it showed that maximum zone of inhibition was 36 mm in 1 (3.3%) sample, 34 mm in 1 (3.3%) sample, 32 mm in 3 (10%) samples, 30 mm in 2 (6.7%) samples, 28 mm in 5 (16.7%) samples, 26 mm in 5 (16.7%) samples, 24 mm in 7 (23.3%) samples and minimum zone of inhibition 22 mm was observed in 5 (16.7%) samples and there was no zone of inhibition observed in 1 (3.3%) sample.

Out of 30 samples of *Klebsiella pneumoniae*, at 20 µl concentration, 3 samples are sensitive, 9 samples are moderately sensitive and 18 samples are resistant. At 10 µl, 5 samples are sensitive, 8 samples are moderately sensitive and 17 samples are resistant. At 5 µl, 13 samples are sensitive, 6

samples are moderately sensitive and 11 samples are resistant. At 2.5 µl, 21 samples are sensitive, 6 samples are moderately sensitive and 3 samples are resistant. At 1.25 µl, 25 samples are sensitive, 3 samples are moderately sensitive and 2 samples are resistant. At 0.625 µl, 29 samples are sensitive and 1 sample is resistant (Table 5).

From the observation, it is evident that the alcoholic extract of Guduchi showed good antimicrobial activity against *Klebsiella pneumoniae*. Alcoholic extract of Guduchi had shown various zones of inhibition against *Klebsiella pneumoniae* ranging from 36 mm to 10 mm against various concentrations (20 µl to 0.625 µl). Maximum zone of inhibition (36 mm) was recorded for alcoholic extract of Guduchi at 1.25 µl and 0.625 µl and minimum zone of inhibition (10 mm) was recorded at 20 µl, 10 µl, 5 µl and 2.5 µl concentrations. For different concentrations of Guduchi, it is evident that decreasing the concentrations significant increase in zone of inhibition. Consecutively number of sensitive zones is increasing and number of resistant zones decreasing when decreasing the concentrations.

Guduchi was selected for this study because Acharya Bhavaprakasha has mentioned Guduchi as krimighna and indicated in the treatment of mutrakrichra. Cytological components of *Klebsiella pneumoniae* comprises of group of virulent factors such as polysaccharide, lipopolysaccharide, fimbriae and outer membrane proteins mainly[6]. These factors play pivotal role for pathogenesis in an infected person. Depends on type of strains and species, virulence level may vary. Cell constituents aid in attachment of bacteria to host cell, invasion into host cell and prevention of phagocytosis from other immunomodulator helper cells[7].

Alcoholic extracts of drugs are enriched with antibacterial constituents viz. Terpenoids, Alkaloids, Saponins, Flavonoids 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 cell etc[8]. In the present study, preliminary phyto-chemical screening of alcoholic extract of Guduchi has been conducted and revealed the presence of the same (Table 2, Table 3).

Alcoholic extract shows more significant zone of inhibition than aqueous and chloroform extracts because it maximizes the bioavailability of the active principles from the plant. And ethanol contains of both the polar and non-polar ends to extract both groups of compounds of a drug. So these phytochemical compounds are completely dissolved in solvent. While doing sensitivity, phytochemical constituents interact with enzymes and proteins of cell membrane of bacteria, causing its disruption to disperse a flux of protons towards cell exterior which will cause cell death or inhibit amino acid biosynthesis of microbial cell[9]. And in other hands, hydrophobic characters of these extracts enable to react with protein of microbial cell membrane and mitochondria to disturbing their cell structures and permeability. Likewise for different strains of gram negative bacteria it has been proposed that the mechanism of the antimicrobial effects involves the inhibition of various cellular processes followed by an increase in plasma membrane permeability and finally ion leakage from the cells[10]. 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 it can penetrate easily through cell membrane of bacteria[11]. So it will show maximum zone of inhibition than other higher concentrations. For higher concentrations, even the drug content is more, it may not show significant zone of inhibition. While dilution, the active components completely will be dissolved with losing effect of active principles. So the drug will be incapable to forward with antibacterial action even it would reach at surface of cell membrane. Active phytochemical contents fail to thrive with antimicrobial action depends on cytological characteristics of organism. 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.

## CONCLUSION

Alcoholic extract of Guduchi showed krimighna (antibacterial) action against *Klebsiella pneumoniae* isolated from urine culture and sensitivity. In lower concentrations, the molecular size of the active components will be too small via complete dissolution and thereby it can penetrate easily through cell membrane of bacteria. So it will show maximum zone of inhibition than other higher concentrations. In the present urine culture and sensitivity study on *Klebsiella pneumoniae*, as the concentration of the alcoholic extract of Guduchi decreased, the antibacterial activity increased.

## REFERENCES


1. Ramkaran Sharma, Agnivesa's Caraka Samhitha chikitsasthana, 26th chapter. Chaukhambha Sanskrit series Varanas 26th chapter; 2012. p. 477-478.
2. Leo AS, Braun E, Neil KJ, Wilson JD, Martin JB, Kasper DL, *et al*. Harrison's principles of internal medicine. 17th ed. New York: Health Professions Division; 2008. p. 1620.
3. Maria Damian, Codruta-Romanita Use in, Andi-Marian Palade, Stefania Ceciu, Maria Cosman. Molecular Epidemiology and Virulence Characteristics of *Klebsiella pneumoniae* Strains Isolated from Hospital-Associated Infections. The Open Epidemiology Journal; 2009. p. 69-78. DOI: 10.2174/1874297100902010069.
4. KR Srikantha Murthy, Bhavaprakasha of Bhavamisra, Purvakhanda, Guduchyadivarga, Chowkhamba Krishnadas Academy; 2004. p. 228.
5. KR Khandelwal and Vrunda K Sethi, Practical Pharmacognosy- Techniques and Experiments, Nirali Prakashan, Pune; 2016.
6. Podschun R and U Ullmann. *Klebsiella* spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods and pathogenicity factors. Clin. Microbiol. Rev 1998; 11: 589–603.
7. Podschun R and U Ullmann. *Klebsiella* spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. Clin. Microbiol. Rev 1998; 11: 589–603.
8. Hazera Khatun, Suman Kundu, Kazi MM. Mohiuddin Ahmed. Guduchi (*Tinospora cordifolia* (Wild)), A Traditional Indian Herbs and Its Medicinal Importance-An Ayurvedic Approach with Contemporary View. International Journal of Ayurvedic and Herbal Medicine; 2016. p. 2260-2267.
9. Ashraf A Mostafa, Abdulaziz A. Al-Askar, Khalid S. Almaary Turki M. Dawoud, Essam N. Sholkamy, Marwah M. Bakri. Antimicrobial activity of some plant extracts

against bacterial strains causing food poisoning diseases. Saudi Journal of Biological Sciences; 2018. p. 361-366. https://doi.org/10.1016/j.sjbs.2017.02.004.

10. LM Kaur, NK Aggarwal and R Dhiman. Antimicrobial Activity of Medicinal Plant: *Parthenium hysterophorus* L. Research Journal of Medicinal Plants 2016; 10: 106-112. DOI: 10.3923/rjmp.2016.106.112.
11. Rosina Khan, Barira Islam, Mohd Akram, Shazi Shakil, Anis Ahmad, S. Manazir Ali, Mashiatullah Siddiqui, Asad U. Khan. Antimicrobial Activity of Five Herbal Extracts Against Multi Drug Resistant (MDR) Strains of Bacteria and Fungus of Clinical Origin. Journal of Molecules; 2009. p. 586-597. DOI:10.3390/molecules14020586.

**How to cite this article :**

Anand S. *et al*. An observational experimental assessment of Antibacterial activity of Guduchi against *Klebsiella pneumoniae* by Urine culture and sensitivity in Pittaja mutrakrichra (Urinary tract infection). J Pharm Sci Innov . 2020;9(1):31-38.
http://dx.doi.org/10.7897/2277-4572.091165

Source of support :Nil, Conflict of interest :None Declared