International Research Journal of Ayurveda & Yoga Vol. 6(12), pp. 14-20, December, 2023
Available online at http://irjay.com
ISSN: 2581-785X
DOI: 10.47223/IRJAY.2023.61203

ORIGINAL RESEARCH ARTICLE

An Experimental Evaluation of *Karavellaka Phala* (*Momordica Charantia* linn.) for Antimicrobial Effect W.S.R. to *Krimighna Karma*

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ARTICLE INFO

Article history:
Received on: 30-09-2023
Accepted on: 04-12-2023
Published on: 31-12-2023

Key words:
Anti-microbial activity,
Momordica charantia Linn.,
Karavellaka Phala Churna,
Krimighna action

ABSTRACT

Background: *Karavellaka Phala* also advocated for treatment of *Krimi* in various texts which are coated in various *Nighantu* such as *Bhavaprakasha Nighantu*. Hence, *Karavellaka Phala* can be used as antimicrobial agent in the place of antibiotics.

Aims and Objectives: The objective of this study was (1) to evaluate *Krimighna* (Antimicrobial) effect of aqueous and alcoholic extract of *Karavellaka Phala and* (2) to compare antimicrobial action of test drug with standard drug group.

Materials and Methods: The methodology followed in the present study encompasses the following aspects: (1) microscopic and macroscopic study of *Karavellaka Phala,* (2) phytochemical analysis of *Karavellaka Phala,* (3) preparation of test drug extracts with disk diffusion method, and (4) screening of anti-microbial activity of *Karavellaka Phala.* (a) 1 Gram-positive bacteria = *Staphylococcus aureus*, (b) Gram-negative bacteria = *Klebsiella pneumonia*, and (c) Standard Drug Group – Amoxicillin.

Observation and Results: After incubated at 37C for 48 h, the zone of inhibition was measured in mm for each organism with different extracts to evaluate anti-microbial activity of *Karavellaka Phala.*

Conclusion: Alcoholic extract and aqueous extract of *Karavellaka Phala Churna* shows no anti-microbial activity.

1. INTRODUCTION

Nowadays, people are showing much interest in scientifically validating the therapeutic efficacy of herbal drugs. Clinical, pharmacological, and experimental assessing of the drug to test their therapeutic value have been carried out all over the world from time to time.

Trial drug *Karavellaka Phala* (*Momordica charantia* Linn.) is commonly available throughout India. Reference regarding its medicinal uses is available in most of the *Nighantus*[1] and text books of modern period. It is useful in the treatment of various diseases such as *Krimi,*[2] *Swasa, Vrana, Kasa, and Jwara.*

Infectious diseases can be correlated with *Krimi's* as it mentioned in ayurvedic texts. In Ayurveda, *Krimi's* are mentioned from vedic kaal. There are many references found about *Krimi's* in *samhitas*[2] and *nighantus*[3] and these *Krimi's* are responsible for many diseases. Type of *Krimi's* also mentioned in Ayurveda and sign and symptoms also described according to its types. *Krimi's* may be internal or external worms or parasites found in human beings and animals. *Krimi's*[4] create many diseases in the body and so there is a need to search over the control on *Krimi's* infestation.

Nowadays, single drug therapy is becoming popular. Many plants are screened to understand their pharmacological action.[5] The advantage of a single drug over a compound preparation is that it is very easy and convenient from the point of processing, it is economical and will produce specific action of the drug. Thus, it is simple, easy, and convenient for the patient and the physician to fulfill the purpose of treatment. Hence, in this study, single drug *Karavellaka Phala* is selected to evaluate its antibacterial property.

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2. MATERIALS AND METHODS

The study was designed under the following headings:

1. Preparation of test drug.

2. Physicochemical study[6]
   i. Physicochemical analysis of *Karavellaka Phala Churna* (*M. charantia* Linn.).
   ii. Preliminary phytochemical investigation[7] of *Karavellaka Phala Churna* (*M. charantia* Linn.).
   iii. Thin-layer chromatography (TLC) studies of *Karavellaka Phala*.
3. Experimental study, that is, *In vitro* antibacterial activity

2.1. Preparation of Test Drug

a) Source of drug:
Genuine quality *Karavellaka Phala*[8] was collected from their natural habitat near Pune streets of Shanipar Mandai. Botanist and other experts verified the drug sample and its identification was confirmed.

b) Purity, identify, and strength:
For confirmation of purity, identity, strength, and genuinity of the drug *Karavellaka Phala*, the samples were subjected to physicochemical tests[9] according to the standards of database on medicinal plants used in Ayurveda.

c) Preparation of dosage forms:
The work was carried out at Sheetal Analytical Laboratory, Pune.

i. Powdering:
Phala were subjected to powdering using *Khalvayantra* and Pulveriser, respectively, 20 and 120 number sieves were used to get coarse and fine powders, respectively, and were dried and stored in air tight containers.

2.2. Physicochemical Analysis

Physicochemical analysis of *Karavellaka Phala Churna*[10] was done as per the standard methods of API.

2.3. Experimental Study

Methodology:

Disc diffusion Method:

Chemical Requirement:
- Nutrient Broth
- Muller–Hinton

2.4. Mueller–Hinton Agar Contain

- Beef extract: 250 g/L
- Starch: 2.00 g/L
- pH: 25C.

2.5. Aims of Using Mueller–Hinton Agar

- Mueller–Hinton agar is a microbial growth medium that is commonly used for antibiotic susceptibility testing.
  - First, it is a non-selective, non-differential medium, that mean all organism plated on it will grow.
  - In addition, it contains that starch is known to absorb toxins released from bacteria, that they cannot interfere with antibiotics.
  - Second, it is a loose agar. This allows for better diffusion of the antibiotics than me of other plates. A better diffusion leads to a truer zone of inhibition.
  - It contains the above ingredients.
  - Agar is added to microbiological media only as a solidification agent.

2.6. Preparation of Mueller–Hinton Agar

- Suspend 38 g of medium (or the components listed above) in 1 L of purified wat Mix thoroughly.
- Heat with frequent agitation and boil for 1 min to completely dissolve the components. Autoclave at 1210C for 15 min. Cool to 450C.
- Pour cooled Mueller–Hinton agar into sterile Petri dishes on a level, horizontal surface to give uniform death.
- Allow to solidify at room temperature.
- Check prepared Mueller–Hinton agar to ensure the final pH is 7.6+1 at 250C. Prepared media can be stored at 4–80C. Muller–Hinton agar is stable for approximately 70 days from the date of preparation.

2.7. Application of Micro-organism

- With the help of sterile cotton swab all micro-organism spread on nutrient agar culture plate.
- These plate of microorganisms were stained and observed under microscope for morphology and confirmation of the micro-organisms.
- Culture plate then put into incubator for 24 h.

2.8. Boaring of Holes in Agar Plates and Application of Drug

- Boring of holes was done with help of sterile borer
- Take hollow tube of 5 mm diameter heat and press it on nutrient agar plate- and remove it immediately by making the well in the plate. Likewise make five well on each plate.
- Add the test drug Karavellaka Phala Churna (*M. charantia* Linn.).

2.9. SOP/Protocol/Procedure

- Wear PPE's while entering into microbiology lab.
- All procedures should be done under aseptic conditions only.
- Inoculate the required cultures into 10 ml of sterile diluent for enrichment.
- This 24 h old culture of Xanthomonas then centrifuge.
- Centrifuge above culture and prepare the culture and compare it with McFarland 0.5 to get required microbial count 1–5 × 10^5 CFU/mL
- Prepare a Sterile Muller–Hinton agar – 500 mL as per manufacturing instructions of dehydrated culture medium.
- Add 1 ml of above culture into the sterile petri plate then pour the 20–25 mL of Muller–Hinton agar, allowing it to solidify.
  - Make a well with the help of cork borer at center, peel out the Agar medium.
  - Fill this well with the tested sample (app. 0.1–0.2 mL)
  - Allow it stable for 10–15 min. at RT
  - Incubate the plate at 37 deg. for 24 h.
  - After incubation observe for antimicrobial activity of sample by zone of inhibition which was found around the well.
  - Measure the zone using a calibrated Vernier caliper and record the reading in mm diameter.

2.10. Analysis

The data collected were diametrically presented.

3. OBSERVATIONS AND RESULTS

The present chapter contains observations and results found throughout study work, that is, they are divided in following four parts:
- Pharmacognostical study
- Physicochemical study

- Phytochemical study
- Antimicrobial study.

3.1. Pharmacognostical Study

This includes macroscopic, microscopic characters, sensory evaluations, and organoleptic characters.
Botanical name: *M. charantia* Linn.
Family: Cucurbitaceae.
Parts used: fruit and leaves.

3.2. Macroscopic Evaluation of Leaves

This herbaceous, tendril-bearing vine grows up to 5 m (16 ft) in length. It bears simple, alternate leaves 4–12 cm (1.6–4.7 in) across, with three to seven deeply separated lobes. Each plant bears separate yellow male and female flowers. In the Northern Hemisphere, flowering occurs during June to July and fruiting during September to November.

The fruit has a distinct warty exterior and an oblong shape. It is hollow in cross-section, with a relatively thin layer of flesh surrounding a central seed cavity filled with large, flat seeds, and pith. The fruit is most often eaten green, or as it is beginning to turn yellow. At this stage, the fruit's flesh is crunchy and watery in texture, similar to cucumber, chayote or green bell pepper, but bitter. The skin is tender and edible. Seeds and pith appear white in unripe fruits; they are not intensely bitter and can be removed before cooking.

3.3. Microscopic Characters

Transverse section shows alternating bands of larger and smaller polygonal cells consisting of tracheids, fiber tracheids, xylem parenchyma and traversed by xylem rays, numerous xylem vessels distributed throughout in singles or in groups of 2–3, xylem parenchyma rectangular with simple pits, showing tyloses filled with tannin, in isolated preparations, vessels, drum or barrel shaped with well-marked perforation rims and bordered pits, tracheids numerous, long, and thick-walled with tapering ends and simple pits.

3.4. Sensory Evaluation

3.4.1. Karavellaka Phala (M. charantia Linn.) Churna

- Odor – Characteristic
- Taste – Astringent
- Color – brown to chocolate
- Touch/external surface – hard and rough [Table 1].

3.5. Physicochemical Study

Physicochemical Study is shown in Table 2.

3.6. Phytochemical Study

Phytochemical study [Tables 3 and 4].

3.7. Observation of Thin Later Chromatography of *Karavellaka Phala Churna*

- One gram of *Karavellaka Phala Churna* was refluxed with petroleum ether (20 mL) for 24 h and filtered. The extracts were used for the analysis.
- The silica gel slurry was spread on glass plates uniformly with the help of glass slides.
- The sample was spotted with the help of capillary tubes carefully, without allowing spreading.
- The chromatogram developed by the ascending technique. Development was allowed to proceed until the solvent front has traveled the 3/4th distance. Then, dragendroff's solution is sprayed on the plates.
- The distance traveled by the solvent front was noted. Rf value of the spots was then found out using the formula.

$$Rf = \frac{\text{Distance traveled by the solute}}{\text{Distance traveled by the solvent}}$$

3.8. Results of TLC

- Stationary phase: Silica gel
- Salvent system- Butanal: Water: Acetic acid (50:40:10)
- Spraying agents- Dragendorff reagent
- Rf value of *Karavellaka Phala* (alcoholic extract): Short wave: 0.05,0.40, 0.85, and 0.94
- Long wave: 0.05,0.10,0.18, 0.31, 0.66,0.79, 0.88, and 0.94 (water extract): Short wave: 0.51 and 0.95
- Long wave: 0.06, 0.31, and 0.91
- Ratio: 7:3 [Picture 1].

3.9. Antimicrobal Study

Table 5, show distribution of zone of inhibition of *Karavellaka Phala* aqueous and alcoholic extract against *Staphylococcus aureu* Pictures 2 and 3 and Graph 1 show distribution of zone of inhibition of *Karavellaka Phala* aqueous and alcoholic extract and standard drug against *Staphylococcus aureu* Table 6-show distribution of zone of inhibition of *Karavellaka Phala* aqueous and alcoholic extract against *Klebsiella pneumoniae* Pictures 4 and 5 and Graph 2 show distribution of zone of inhibition of *Karavellaka Phala* aqueous and alcoholic extract and standard drug against *Klebsiella pneumoniae*.

4. DISCUSSION

4.1. Discussion on Antibacterial Study

Micro-organisms occur in large number in most natural environments. They are the major causative factors for many infectious diseases such as respiratory tract infection, fever, diarrhea, dysentery, and skin disorders. Antimicrobial activity[11] is a technique in which response of an organism to particular antimicrobial agent can be established. Different methods are employed for evaluation of antimicrobial activity of a drug. In the present study, agar well diffusion method was followed.

Each kind of micro-organism has specific growth requirements. Most of the microbes can be grown in culture medium in the laboratory. In the present study, Mueller–Hinton agar is chosen as a culture media for bacteria. Agar universally used as a solidifying agent is common for bacteria and fungi, and growth of organism was confirmed by turbidity of the media.

For this study, we use antibiotic amoxicillin as a standard.

Amoxicillin is active against a wide range of bacterial infections, mostly Gram-negative bacteria including *Escherichia coli*, Pseudomonas, *Klebsiella pneumonia*, and Proteus, and the Gram-positive *Staphylococcus aureus*.

The results of the present project are found to be very good in case of antibacterial activity against the two organisms selected for study when analyzed by disk diffusion method.

- *S. aureus*
- *K. pneumonia*.

These results suggest that antibacterial activity of *M. charantia* shows no resistance against test bacteria, that is, it shows no zone of inhibition against test bacteria. This result shows it more resistant against bacteria *E. coli* as compared to zone of inhibition. While against test bacteria, *S. aureus* the seeds of *M. charantia* shows no inhibition zone.

The phytochemicals present in *Karvellaka Phala Churna* have shown no anti-bacterial activity near to standard drug.

4.2. Discussion on Physicochemical Study

The drug *Karavellaka phala* (*M. charantia* Linn.) collected for the study showed physicochemical standards according to database on medicinal plants used in Ayurveda and were within normal limits. Macroscopic characters confirmed the genuinity of the drug *Karavellaka Phala* (*M. charantia* Linn.).

Phytochemical analysis carried out for the selected drug showed the presence of alkaloids, glycosides, flavonoids, saponins, steroids, sugars, and proteins. Thus, the genuinity of the drug *Karavellaka Phala* selected for the study was confirmed through physicochemical, phytochemical analysis, and TLC studies.

4.3. Discussion on Study Proper

4.3.1. Experimental study

The methodological selected was agar disc diffusion method, which is one of the sensitive techniques for analytical study of growth response of microorganism. Small size of the sample could be assessed against a single microorganism and it is the commonest, simple, and inexpensive technique which can be followed to test the crude plant extracts.

4.4. Discussion on Results

Antibacterial activity on Gram +ve bacteria - *S. aureus*: Trial drug – *Karavellaka Phala Churna* aqueous and alcoholic extract has no activity (no zone of inhibition) against Gram +ve bacteria *S. aureus*. Standard drug amoxicillin showed greater activity. Trial drug – *Karavellaka Phala Churna* aqueous and alcoholic extract has no activity (no zone of inhibition) against Gram -ve bacteria *K. Pneumonia*. Standard drug amoxicillin showed greater activity.

5. CONCLUSION

Disk diffusion method was followed. It is standard method to screen the herbal extract with antimicrobial property till today. The Mueller-Hinton agar media is ideal for the growth of selected two micro-organisms. The physicochemical results, that is, pH, ash value, acid insoluble ash, alcohol, and water soluble extractive, moisture values are within limits of Ayurvedic pharmacopoeia. The preliminary phytochemical screening of alcoholic extracts of *Karavellaka Phala Churna* shows the presence of flavonoids, alkaloids, and phenols. Against *S. aureus*, *Karavellaka Phala Churn*a extracts show no zone of inhibition against standard amoxicillin, that is, *Karavellaka Phala Churna* extracts which are insensitive against *S. aureus*. Anti-microbial activity against *K. pneumoniae* gave the no zone of inhibition as compare to standard drug Amoxicillin, that is, *Karavellaka Phala Churna* extracts which are insensitive against *K. pneumoniae*. *Karavellaka Phala Churna* shows no antimicrobial activity.

6. ACKNOWLEDGMENT

None.

7. AUTHORS' CONTRIBUTIONS

All the authors contributed equally in design and execution of the article.

8. FUNDING

Nil.

9. ETHICAL APPROVALS

This study does not required ethical clearance as it is experimental study.

10. CONFLICTS OF INTEREST

Nil.

11. DATA AVAIBALITY

This is an original manuscript and all data are available for only research purposes from principal investigators.

12. PUBLISHERS NOTE

This journal remains neutral with regard to jurisdictional claims in published institutional affiliation

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How to cite this article:
Yadav NR. An Experimental Evaluation of *Karavellaka Phala* (*Momordica Charantia* linn.) for Antimicrobial Effect W.S.R. to *Krimighna Karma*. IRJAY. [online] 2023;6(12);14-20
Available from: https://irjay.com
DOI link- https://doi.org/10.47223/IRJAY.2023.61203

Table 1: Organoleptic evaluation of *Karavellaka Phala*

S. No.	Test	Observation
1	Color	Brown
2	Odor	Characteristics
3	Taste	Bitter
4	Touch	Hard, rough

Table 2: Physicochemical study of *Karavellaka Phala Churna*

S. No.	Parameter	Value
2.	pH 5%	6.76
3.	Loss on Drying @ 110^0C	2.82%
4.	Total Ash Content	14.71%
5.	Acid-Insoluble Ash	0.81
6.	Water-Soluble Extract	26.5%
7.	Alcohol-Soluble Extract	

Table 3: Phytochemical study of *Karavellaka Phala* water extract

Sl. No.	Parameter	Results Water
*	Phytochemical Test	Done
1.	Carbohydrates	Present^{++}
2.	Protein	Present^{++}
3.	Glycosides	Present^{+}
4.	Alkoloids	Present^{+++}
5.	Tannins	Present^{+++}

Table 4: *Karavellaka Phala* alcoholic extract

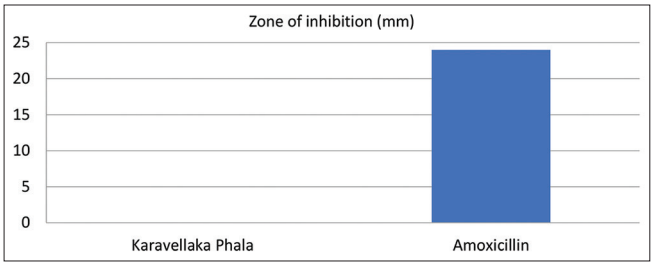
S. No.	Parameter	Results Alcohol
	Phytochemical Test	Done
1.	Carbohydrates	Present^{++}
2.	Protein	Present^{+}
3.	Glycosides	Present^{+}
4.	Alkoloids	Present^{+++}
5.	Tannins	Present^{++}

Table 5: Distribution of zone of inhibition of *Karavellaka Phala* aqueous and alcoholic extract against *Staphylococcus aureus*

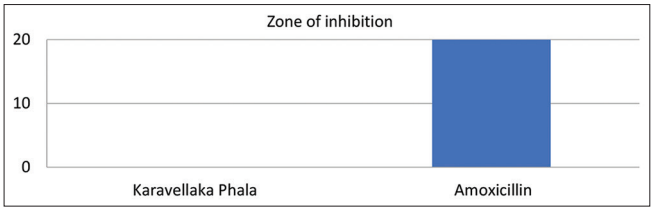
Sample Name	Zone of inhibition (mm)
Karavellaka Phala	0
Amoxicillin	24

Table 6: Distribution of zone of Inhibition of *Karavellaka Phala* Aqueous and alcoholic extract against *Klebsiella pneumoniae*

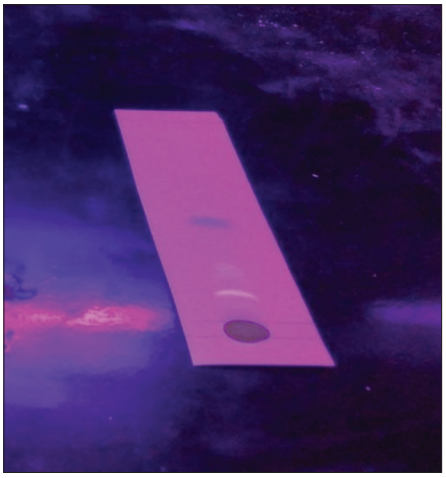
Sample Name	Zone of inhibition
Karavellaka Phala	0
Amoxicillin	20



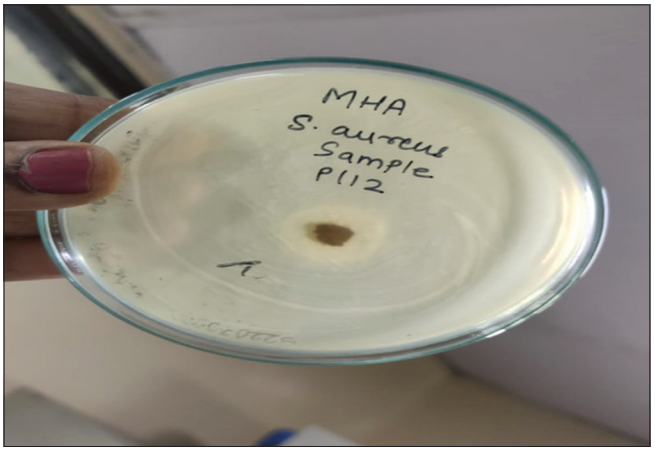
Graph 1: Distribution of zone of inhibition of *Karavellaka Phala* aqueous and alcoholic extract and standard drug against *Staphylococcus aureus*



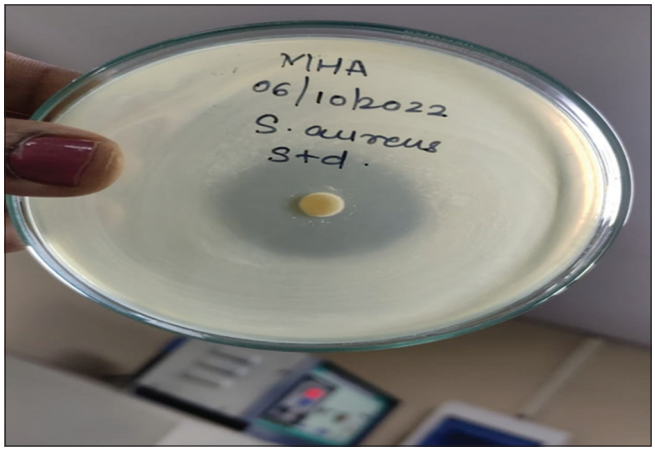
Graph 2: Distribution of zone of inhibition of *Karavellaka Phala* aqueous and alcoholic extract against *Klebsiella pneumoniae*



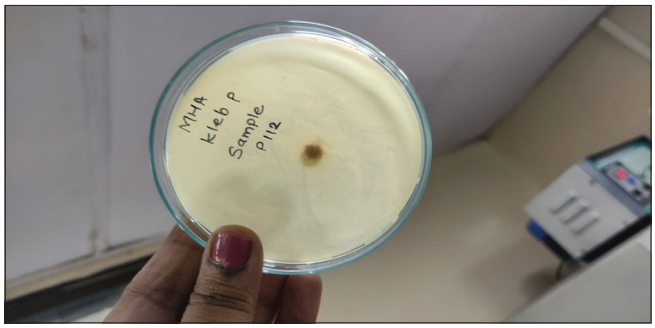
Picture 1: Thin-layer chromatography plate *Karavellaka Phala*



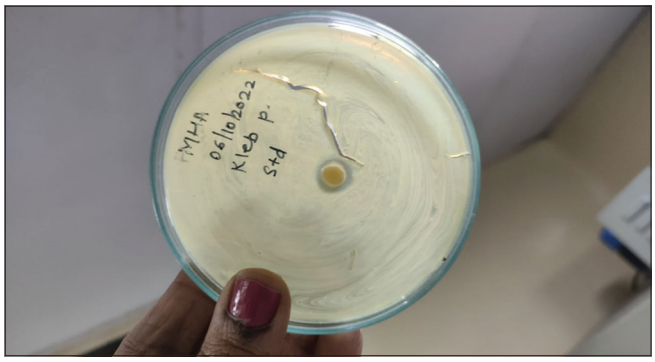
Picture 2: Distribution of zone of inhibition of sample drug against *Staphylococcus aureus*



Picture 3: Distribution of zone of inhibition of standard drug against *Staphylococcus aureus*



Picture 4: Distribution of zone of inhibition of sample drug against *Klebsiella pneumoniae*



Picture 5: Distribution of zone of inhibition of standard drug against *Klebsiella pneumoniae*