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

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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 37°C 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 and text books of modern period. It is useful in the treatment of various diseases such as Krimi, 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 and nighantus 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 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. 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.

2. MATERIALS AND METHODS

The study was designed under the following headings:
1. Preparation of test drug.

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2. Physicochemical study
   i. Physicochemical analysis of Karavellaka Phala Churna (M. charantia Linn.).
   ii. Preliminary phytochemical investigation 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 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 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.

2.2. Physicochemical Analysis
   Physicochemical analysis of Karavellaka Phala Churna 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: 25°C.

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 1210°C for 15 min. Cool to 45°C.
   • Pour cooled Mueller–Hinton agar into sterile diluent for 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 250°C. Prepared media can be stored at 4–80°C. 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 microorganisms.
   • 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
3.1. Pharmacognostical Study
This includes macroscopic, microscopic characteristics, 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.

3.8. Results of TLC
• Stationary phase: Silica gel
• Solvent 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. Antimicrobial Study
Table 5, show distribution of zone of inhibition of *Karavellaka Phala* aqueous and alcoholic extract against *Staphylococcus aureus*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 aureus* 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 pneumoniae*, 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. pneumoniae.*
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 *Karvellaka 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 *Karvellaka 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 *Karvellaka 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**


**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 *Karvellaka Phala Churna* shows the presence of flavonoids, alkaloids, and phenols. Against *S. aureus*, *Karvellaka Phala Churna* extracts show no zone of inhibition against standard amoxicillin, that is, *Karvellaka 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, *Karvellaka Phala Churna* extracts which are insensitive against *K. pneumoniae*. *Karvellaka 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 AVAILABILITY**

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 jurisdical claims in published institutional affiliation

**REFERENCES**


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Table 1: Organoleptic evaluation of Karavellaka Phala

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Test</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Color</td>
<td>Brown</td>
</tr>
<tr>
<td>2</td>
<td>Odor</td>
<td>Characteristics</td>
</tr>
<tr>
<td>3</td>
<td>Taste</td>
<td>Bitter</td>
</tr>
<tr>
<td>4</td>
<td>Touch</td>
<td>Hard, rough</td>
</tr>
</tbody>
</table>

Table 2: Physicochemical study of Karavellaka Phala Churna

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameter</th>
<th>Value</th>
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<tbody>
<tr>
<td>2.</td>
<td>pH 5%</td>
<td>6.76</td>
</tr>
<tr>
<td>3.</td>
<td>Loss on Drying @ 110°C</td>
<td>2.82%</td>
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<tr>
<td>4.</td>
<td>Total Ash Content</td>
<td>14.71%</td>
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<td>5.</td>
<td>Acid-Insoluble Ash</td>
<td>0.81</td>
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<tr>
<td>6.</td>
<td>Water-Soluble Extract</td>
<td>26.5%</td>
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<td>7.</td>
<td>Alcohol-Soluble Extract</td>
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Table 3: Phytochemical study of Karavellaka Phala water extract

<table>
<thead>
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<th>Sl. No.</th>
<th>Parameter</th>
<th>Results Water</th>
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<tbody>
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<td>*</td>
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</tr>
<tr>
<td>1.</td>
<td>Carbohydrates</td>
<td>Present**</td>
</tr>
<tr>
<td>2.</td>
<td>Protein</td>
<td>Present**</td>
</tr>
<tr>
<td>3.</td>
<td>Glycosides</td>
<td>Present'</td>
</tr>
<tr>
<td>4.</td>
<td>Alkoloids</td>
<td>Present***</td>
</tr>
<tr>
<td>5.</td>
<td>Tannins</td>
<td>Present+++</td>
</tr>
</tbody>
</table>

Table 4: Karavellaka Phala alcoholic extract

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameter</th>
<th>Results Alcohol</th>
</tr>
</thead>
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<td>Phytochemical Test</td>
<td>Done</td>
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<tr>
<td>1.</td>
<td>Carbohydrates</td>
<td>Present+++</td>
</tr>
<tr>
<td>2.</td>
<td>Protein</td>
<td>Present'</td>
</tr>
<tr>
<td>3.</td>
<td>Glycosides</td>
<td>Present'</td>
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<tr>
<td>4.</td>
<td>Alkoloids</td>
<td>Present+++</td>
</tr>
<tr>
<td>5.</td>
<td>Tannins</td>
<td>Present+++</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Karavellaka Phala</td>
<td>0</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>24</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Zone of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Karavellaka Phala</td>
<td>0</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>20</td>
</tr>
</tbody>
</table>
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*