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Evaluation of anti-fungal activity of Chakramarda Seeds (Alcoholic extract) on Clinical Pathogens (Dermatophytosis)

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ABSTRACT

Dermatophytoses have become a significant health problem affecting children, adolescents and adults world-wide. In India 5 out of 1000 people suffer from Tinea infections. In the present study evaluation of antifungal activity of Chakramarda on Tinea (Dermatophytoses) was performed and for the same culture and sensitivity skin scrapping samples were collected from the edge (active border area) of lesions. Culture was done by collected skin scrapping of patient suffering from Dadru Kushta. Sabouraud’s dextrose agar (SDA) slant media was inoculated via sterile technique and culturing was done by streak culture method and it was subjected to macroscopic and microscopic examination for the identification of fungi. Sensitivity test was done using SDA media by Cork Borer well diffusion method, with five different concentrations of alcoholic extracts of Chakramarda seeds. After the incubation period, the zone of inhibition was measured in mm. Experimental study with five different concentrations (1.25µl, 2.5µl, 5µl, 10µl, 20µl) of Alcoholic extract of Chakramarda showed progressive increase in zone of inhibition with increase in concentration. Therefore, it was concluded that as concentration of Chakramarda drug increases the antifungal activity also increases. Alcoholic extract of Chakramarda seeds has anti-fungal (Krimighna) action against Dermatophytoses (Tinea).

KEYWORDS

Dermatophytoses, Dadru Kushta, Chakramarda seeds, Skin scrapping, Antifungal activity, Krimighna action
INTRODUCTION

In Ayurveda references are available regarding testing of drug and food on animal for evaluating their safety before administration to the human beings. Sushruta Samhita Sutrasthana has dealt with this by devoting a separate chapter Yogya vidhi. It is recommended that any procedure to be performed on human being should primarily undergo trial on animals or other models, having similar characteristics. Hence before using Chakramardha, in the form of taila, on humans in dermatophytoses, an experiment to evaluate the efficacy of Chakramardha on Dermatophytoses in vitro is essential.

Chakramarda (Cassia tora Linn) belonging to family Fabaceae contains alkaloids flavonoids, phenol, tannins, saponins, sugar, glycosides, steroids, Carbohydrates, glycosides, carboxylic acid, Resin and anthraquinone which are responsible for its various pharmacological properties and has been widely used in Ayurveda. Dermatophytoses are superficial fungal infections of the skin. Though it is not very harmful & has no fatal ill effects, it is considerably troublesome to the patients. Dermatophytoses is a common cutaneous fungal disease caused by species belonging to the genera Trichophyton, Microsporum and Epidermophyton. The infection is commonly designated as ringworm or tenia.

In Bavaprakasha, madanpala and kaiyadeva nigantus has mentioned, Chakramarda (Cassia tora. Linn) beija to possess krimighna properties. Chakramarda seeds (Cassia tora Linn) mainly contain anthraquinane, glycosides, cassiaside, rubrofusarin & toralactone. Tila (Sesamum indicum) seeds contain flavonoids, phenolic acids, alkaloids, tannins. All these properties impart antimicrobial, antipruritic, antioxidant and antiulcer activities.

OBJECTIVE OF THE STUDY

To evaluate antifungal activity of Chakramarda on Tinea (Dermatophytoses) by culture and sensitivity.

COLLECTION OF SPECIMEN:

A total number of 30 patients fulfilling diagnostic and inclusion criteria were selected from OPD of Sri Dharmasthala Manjunatheshwara College of Ayurveda & Hospital, Hassan. Skin scrapping samples were collected from the edge of lesions with sterile scalpel blade. The scrapping was collected in a clean sterile fold of black paper. The collected specimens were bought
to the ACR laboratory, Hassan for Identification of fungal organisms.

IDENTIFICATION OF FUNGAL ORGANISMS

Fungal elements were detected in the clinical specimens by direct microscopic examination of skin scrapping from the lesion by wet mounting method. The scrapping sample was treated with 10% KOH which digests the keratin material so that the fungal hyphae were clearly seen under the microscope.

**Figure 1** Identification of fungi by using 10% of KOH

FUNGAL CULTURE

Skin scrapping samples were collected from the edge of lesions and culture was done by streak culture method. Sabouraud’s dextrose agar (SDA) slant culture media was inoculated via sterile technique. Incubated for 3-4 weeks at 37°C temperature.

**Figure 2** Fungal cultures by streak culture technique

IDENTIFICATION OF FUNGAL COLONY MORPHOLOGY:

Rate of growth, pigmentation, texture and colony surface was assessed for macroscopic appearance of the Colony.

**Figure 3** Fungal colony morphology

| Various pigments, Waxy/ velvety and cottony texture, Radial grooves surface | Rose brown Waxy Radial groove | Dark Brown Velvety Cottony | Brown Waxy Cerebri form |
MICROSCOPIC EXAMINATION OF FUNGI:
To study the microscopic appearance of the fungal isolates, it was grown in culture by Lactophenol cotton blue.

Requirements-
- Lactophenol Cotton Blue
- Glass slides
- Teasing needle

Few filaments of fungal colony was teased out from the culture tube and Lactophenol Cotton Blue mount was made on a slide and viewed under microscope. Septate or aseptate, hyaline, narrow or wide and conidia were observed.

Figure 4 Microscopic examinations of fungi

ASSAY FOR THE ANTIFUNGAL ACTIVITY USING AGAR WELL DIFFUSION METHOD
The screening of antifungal activity of Alcoholic extract of Chakramarda seeds were carried out in the study. Sensitivity test was done using SDA¹⁰ (Sabouraud’s dextrose agar) media by Cork boucher well diffusion method¹¹, with five different concentrations (20µl, 10µl, 5µl, 2.5µl, 1.25µl) of alcoholic extracts of Chakramarda seeds.

Requirements-
- SDA Media plates
- Micro pipette

Five different concentration of alcoholic extracts of Chakramarda seeds
- Incubator

Procedure for Anti-fungal Sensitivity Test-
The work place was cleaned in laminar air flow using 70% ethyl alcohol and UV for 20 minutes¹². One loop of fungal organism was inoculated to SDA Plate from fungal isolates grown in culture.

Five equidistant wells were made on the plates with the help of sterile Cork boucher (6 mm diameter). Initially 5 wells in each SDA plate was charged with five different concentrations (20µl, 10µl, 5µl, 2.5µl, 1.25µl) of alcoholic extracts of Chakramarda seeds in respective wells, with the help of
micropipette and then incubated in upright position at 37°C for 4-6 days. After the incubation period, antifungal activity was determined by measurement of diameter of zones of inhibition (mm).

**Figure 5** Procedure for Anti-fungal Sensitivity Test

![Procedure for Anti-fungal Sensitivity Test](image)

Different concentrations of extract of Chakramarda seeds were filled into the wells with the help of micropipette. Incubated the petri plate in upright position at 37°C for 4-6 days. After the incubation period, antifungal activity was determined by measurement of diameter of zones of inhibition (mm).

**ASSESSMENT CRITERIA:**

Assessment was recorded as below:

1. Sensitive (S) zone - Between 20 – 24 mm zone of inhibition
2. Moderately sensitive (M S) zone - Between 15 – 19 mm zone of inhibition
3. Resistant (R) zone - Below 15 mm zone of inhibition

**Figure 6** Assessment of fungal activity

![Assessment of fungal activity](image)

Fungal activity was determined by measuring diameter of zone of inhibition (mm) with the help of scale.

**RESULTS**

In vitro anti-fungal study of Dermatophytoses against five different concentration of alcoholic extract of Chakramarda seeds was evaluated by agar well diffusion method. Based on zone of inhibition of fungal growth of Dermatophytoses against five different concentration of alcoholic extract of Chakramarda seeds, optimal zone of inhibition was evident between 24 mm to 15 mm zone of inhibition.
Here S- Sensitivity (Between 20mm to 24 mm), MS- Moderate sensitivity (Between 15mm to 19 mm), R-Resistant (Below 15 mm).

**Table 1** Mean values of Zone inhibition at Different Concentrations of alcoholic extract of *Chakramarda* Seeds

<table>
<thead>
<tr>
<th>Different Concentrations of alcoholic extract</th>
<th>20µl</th>
<th>10µl</th>
<th>5µl</th>
<th>2.5µl</th>
<th>1.25µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Mean (Zone of inhibition in mm)</td>
<td></td>
<td>22.900</td>
<td>21.167</td>
<td>19.833</td>
<td>17.633</td>
</tr>
</tbody>
</table>

Mean values of zone of inhibition by alcoholic extract of *Chakramarda* in five different concentration i.e. 20µl, 10µl, 5µl, 2.5µl, 1.25µl against Dermatophyses (Tinea) are 22.900mm, 21.167mm, 19.833mm, 17.633mm and 16.433mm respectively.

**Table 2** Sensitivity test for Alcoholic extract of five Different Concentrations of alcoholic extract of *Chakramarda* Seeds

<table>
<thead>
<tr>
<th>Concentrations</th>
<th>20µl</th>
<th>10µl</th>
<th>5µl</th>
<th>2.5µl</th>
<th>1.25µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>S</td>
<td>M</td>
<td>R</td>
<td>S</td>
<td>M</td>
</tr>
<tr>
<td>No of samples</td>
<td>24</td>
<td>5</td>
<td>1</td>
<td>21</td>
<td>7</td>
</tr>
</tbody>
</table>

Out of 30 samples of fungal organisms 24 samples are sensitive, 05 samples are moderately sensitive and one sample is resistant at 20µl of alcoholic extract of *Chakramarda*. At 10µl of concentration of alcoholic extract of *Chakramarda* 21 samples are sensitive, 07 samples are moderately sensitive and 02 are in resistant. Twenty samples are sensitive, 07 samples are moderately sensitive and 03 samples are resistant at 5µl of alcoholic extract of *Chakramarda*. At 2.5µl of concentration of alcoholic extract of *Chakramarda* 17 samples are sensitive, 07 samples are moderately sensitive and 06 are resistant. 15 samples are sensitive, 08 samples are moderately sensitive and 07 samples are resistant at 1.25µl concentration of alcoholic extract of *Chakramarda*.

**Table 3** Statistical significance of zone of inhibition of different concentration of alcoholic extract of *Chakramarda*

<table>
<thead>
<tr>
<th>Zone of inhibition</th>
<th>N</th>
<th>Mean (Zone of inhibition in mm)</th>
<th>F value</th>
<th>P value</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>20µl</td>
<td>30</td>
<td>22.900</td>
<td>139.11</td>
<td>0.000</td>
<td>HS</td>
</tr>
<tr>
<td>10µl</td>
<td>30</td>
<td>21.167</td>
<td>127.52</td>
<td>0.000</td>
<td>HS</td>
</tr>
<tr>
<td>5µl</td>
<td>30</td>
<td>19.833</td>
<td>118.49</td>
<td>0.001</td>
<td>HS</td>
</tr>
<tr>
<td>2.5µl</td>
<td>30</td>
<td>17.633</td>
<td>115.32</td>
<td>0.001</td>
<td>HS</td>
</tr>
<tr>
<td>1.25µl</td>
<td>30</td>
<td>16.433</td>
<td>93.78</td>
<td>0.008</td>
<td>S</td>
</tr>
</tbody>
</table>

The zone of inhibition of different concentration of alcoholic extract of *Chakramarda*. It was observed that 20µl and 10 µl concentration were found to sensitive with P value 0.000, which was statistically highly significant. 5 µl concentration and 2.5 µl concentration of alcoholic extract of *Chakramarda* were found to moderately sensitive.
Chakramarda were found to be sensitive with P value 0.001, which was statistically highly significant. 1.25 µl concentration of Chakramarda was found to be sensitive with P value 0.008 which was statistically significant. Hence it can be concluded that five different concentration of alcoholic extract of Chakramarda is having anti-fungal action against Dermatophytoses. Further it is evident that as the concentration of alcoholic extract of Chakramarda increased the zone of inhibition also increased.

DISCUSSION

Chakramarda is used in management of Dadru kushta and said to possess krimighna property. Hence, adoption of new approaches like culture and sensitivity methods would help in achieving improved diagnostic and curative abilities. Therefore in the present study, culture and sensitivity test was planned to evaluate anti-fungal effect of Chakramarda by sensitivity test. Present study was done on skin scrapping of patients who were suffering from Dadru Kushta (Dermatophytoses or Tinea). Collected skin scrapping was examined under low and high power microscope for fungal structure by using 10% KOH (potassium hydroxide) (Figure 1). Isolation of fungi was done by streak culture technique using SDA slant media and after inoculation slant was incubated in an incubator at 37°C for 3 to 4 weeks Figure 2). The growth of fungal organisms along with change in the colour, texture, surface within 21 days was assessed to confirm the presence of Dermatophytoses(Figure 3). Further identification of fungi was done by macroscopic & microscopic examination) (Figure 4). In the present study, anti-fungal activity of alcoholic extract of Chakramarda was analyzed against Dermatophytoses. Evaluation was done at five different concentrations of alcoholic extract of Chakramarda. Here anti-fungal study was done using SDA (Sabouraud’s dextrose agar) media by Cork bourer well diffusion method, with five different concentrations (20µl, 10µl, 5µl, 2.5µl, 1.25µl) of alcoholic extracts of Chakramarda seeds) (Figures 5 & 6). At 20µl concentration of alcoholic extract of Chakramarda, Out of 30 samples, 24 (79.99%) samples had shown sensitive zone of inhibition with mean value of zone of inhibition 22.90mm (Table 1 & 2). which is statistically highly significant. Thus at 20µl concentration of alcoholic extraction of Chakramarda is sensitive against (Dermatophytoses) Dadru kushta. At 10µl
concentration of alcoholic extract of *Chakramarda*, Out of 30 samples, 21 (70%) samples had shown sensitive zone of inhibition with mean value of zone of inhibition 21.16mm. which is statistically highly significant. Thus at 10µl concentration of alcoholic extraction of *Chakramarda* is sensitive against (Dermatophytoses) *Dadru kushta*. At 5µl concentration of alcoholic extract of *Chakramarda*, Out of 30 samples, 20 (66.67%) samples had shown sensitive zone of inhibition with mean value of zone of inhibition 19.83mm. which is statistically highly significant. Thus at 5µl concentration of alcoholic extraction of *Chakramarda* is sensitive against (Dermatophytoses) *Dadru kushta*. At 2.5µl concentration of alcoholic extract of *Chakramarda*, Out of 30 samples, 17 (56.66%) samples had shown sensitive zone of inhibition with mean value of zone of inhibition 17.633mm. which is statistically highly significant. Thus at 2.5µl concentration of alcoholic extraction of *Chakramarda* is sensitive against (Dermatophytoses) *Dadru kushta*. At 1.25µl concentration of alcoholic extract of *Chakramarda*, Out of 30 samples, 15 (50%) samples had shown sensitive zone of inhibition with mean value of zone of inhibition 16.433mm. which is statistically significant. Thus at 1.25µl concentration of alcoholic extraction of *Chakramarda* is sensitive against (Dermatophytoses) *Dadru kushta*. On consideration of mean values of zone of inhibitions at 20µl, 10µl, 5µl and 2.5µl concentrations were found to be statistically highly significant and 1.25µl concentration was found to be statistically significant (Table 3). This study clearly shows that alcoholic extract of *Chakramarda* has anti-fungal action against Dermatophytoses (Tinea) isolated from the skin scrapping samples of patients suffering from *Dadru Kusha*. Hence it can be concluded that *Chakramarda* is having anti-fungal action against Dermatophytoses (Tinea).
CONCLUSION

Microbiological experimental study with five different concentrations (1.25µl, 2.5µl, 5µl, 10µl, 20µl) of Alcoholic extract of Chakramarda showed progressive increase in zone of inhibition with increase in concentration. Therefore it is concluded that as concentration of Chakramarda drug increases the antifungal activity also increases. Alcoholic extract of Chakramarda seeds possess anti-fungal (Krimighna) action against Dermatophytoses (Tinea). Hence Chakramarda is effective against Dadru kushta (dermatophytoses).
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