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

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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

Greentree Group

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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[1]. 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[2] contains alkaloids flavonoids, phenol, tannins, saponins, sugar, glycosides, steroids, Carbohydrates, glycosides, carboxylic acid, Resin and anthraquinone[3] which are responsible for its various pharmacological properties and has been widely used in Ayurveda.

Dermatophytoses are superficial fungal infections of the skin[4]. 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*[5]. The infection is commonly designated as ringworm or *tenia*. In *Bavaprakasha, madanpala* and *kaiyadeva nigantus* has mentioned, *Chakramarda (Cassia tora. Linn) beeja* to possess *krimighna* properties. *Chakramarda seeds (Cassia tora Linn)* mainly contain anthraquinane, glycosides, cassiaside, rubrofusarin & toralactone[6]. *Tila* (*Sesamum indicum)* seeds contain flavonoids, phenolic acids, alkaloids, tannins[7]. All these properties impart antimicrobial, antipruritic, antioxidant and antiulcer activities[8].

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.

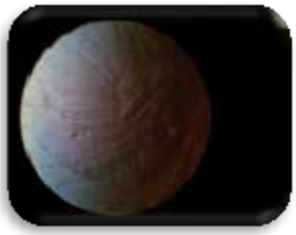
IDENTIFICATION OF FUNGAL ORGANISMS

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

Collection of Skin Scrapping Sample

Added 2-3 drops of 10% KOH



Observed under Microscope

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 37^0C temperature.

Figure 2 Fungal cultures by streak culture technique

Inoculated by Streak culture method

Incubated in culture Conditions

Observed for Fungal growth

IDENTIFICATION OF FUNGAL COLONY MORPHOLOGY:

Rate of growth, pigmentations, 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
- Microscope

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

Take sample of fungal isolates grown in culture & placed on glass slide

Add one drop of LPCB & teased well and placed the cover slips

Observed under 40X microscope

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 SDA[10] (Sabouraud's dextrose agar) media by Cork bourer well diffusion method[11], 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 minutes[12]. 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 bourer (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

Took Isolated sample by using cotton swab & Inoculated it on sensitivity plate.

Well was made with the help of Cork bourer

Wells were marked

Different concentrations of extract of Chakramarda were filled into the wells with the help of micropipette

Incubated the petri plate in upright

ASSESSMENT CRITERIA:

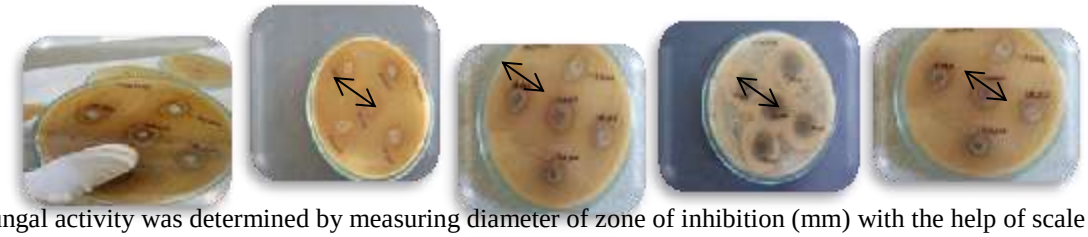
Assessment was recorded as below;

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



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

RESULTS

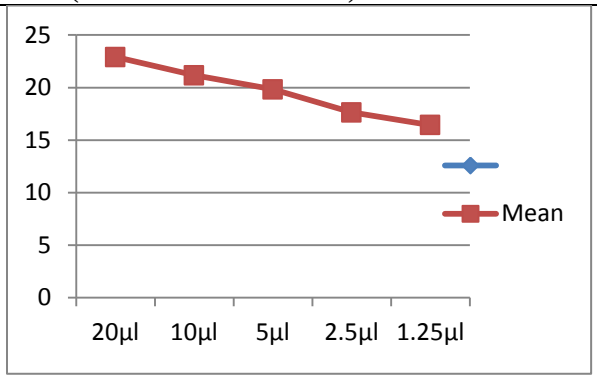
In vitro anti-fungal study of Dermatophytoses against five different concentration of alcoholic extract of *Chakramarda* seeds was evaluated by agar well diffusion method.

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

Different Concentrations of alcoholic extract	20µl	10µl	5µl	2.5µl	1.25µl
N	30	30	30	30	30
Mean (Zone of inhibition in mm)	22.900	21.167	19.833	17.633	16.433



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 Dermatophytoses (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

Concentrations	20µl			10µl			5µl			2.5µl			1.25µl		
Sensitivity	S	M	R	S	M	R	S	M	R	S	M	R	S	M	R
No of samples	24	5	1	21	7	2	20	7	3	17	7	6	15	8	7

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

Zone of inhibition	N	Mean	F value	P value	Statistical significance
20µl	30	22.900	139.11	0.000	HS
10µl	30	21.167	127.52	0.000	HS
5µl	30	19.833	118.49	0.001	HS
2.5µl	30	17.633	115.32	0.001	HS
1.25µl	30	16.433	93.78	0.008	S

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 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 37^0c 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 Kushta*. 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). Henc,e *Chakramarda* is effective against *Dadru kushta* (dermatophytoses).

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