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In Vitro Anti-Dandruff Activity of Karnasphotha (*Cardiospermum helicacabum* Linn.) against *Malassezia furfur*

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ABSTRACT

Background: Dandruff is a common skin condition that mainly affects the scalp. Almost half of the population at the pre-pubertal age and of any gender and ethnicity will be affected. It was discovered that the responsible agent is a scalp specific fungus, *Malassezia globosa*. *Karnasphotha* (*Cardiospermum helicacabum* Linn.) commonly known as Balloon vine from Sapindaceae family is traditionally used for dandruff. So here an attempt is made to study the in vitro efficacy of *Karnasphota* (*Cardiospermum helicacabum* Linn.) decoction against dandruff. **Aims and Objectives:** In vitro Antidandruff activity of *Karnasphota* Mula and Beeja by Agar cup method and Biocidal activity. **Methodology:** In vitro Antidandruff activity of *Karnasphota* (*Cardiospermum helicacabum* Linn.) Bija and Mula Kwatha against dandruff by – Agar cup method and Biocidal activity. **Result:** The *Karnasphotha Moola* showed good antidandruff activity against *Malassezia furfur* in different concentrations. Whereas *Karnasphotha Beeja* showed no antidandruff activity against *Malassezia furfur* in any concentrations. **Conclusion:** The *Karnasphotha Moola* is having significant Antidandruff properties.

Key words: Dandruff, *Malassezia globosa*, *Karnasphota*, Agar cup method, Biocidal activity.

INTRODUCTION

Dandruff is a common skin condition that mainly affects the scalp.^[1] Almost half of the population at the pre-pubertal age and of any gender and ethnicity will be affected.^[2] No population in any geographical region would have passed through freely without being affected by dandruff at some stage in their life.^[3] The

word dandruff (dandruff, dandriffe) is of Anglo-Saxon origin, a combination of 'tan' meaning 'tetter' and 'drof' meaning 'dirty'. Dandruff affects aesthetic value and often causes itching.^[4] The severity of dandruff may fluctuate with season as it often worsens in winter.^[5] The extrapolated prevalence of Dandruff in India is 195,785,036.^[6]

Symptoms include flaking and sometimes mild itchiness.^[7] Older literature cites the fungus *Malassezia furfur* (previously known as *Pityrosporum ovale*) as the cause of dandruff. It was discovered that the responsible agent is a scalp specific fungus, *Malassezia globosa*,^[8] that metabolizes triglycerides present in sebum by the expression of lipase, resulting in a lipid byproduct: oleic acid. During dandruff, the levels of *Malassezia* increase by 1.5 to 2 times its normal level.^[9] Oleic acid penetrates the top layer of the epidermis, the stratum corneum, and evokes an inflammatory response in susceptible people which disturbs homeostasis and results in erratic cleavage of stratum

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corneum cells. The main symptoms of dandruff are an itchy scalp and flakiness.^[10] Red and greasy patches of skin and a tingy feeling on the skin are also symptoms.

Treatment available is shampoos with combination of special ingredients to control dandruff.^[11] Antifungal treatments including ketoconazole, zinc pyrithione, antimicrobials-selenium disulfide, steroids and regulators of keratinization have been found to be effective. In spite of this the reoccurrence of dandruff is reported.

Ayurveda explains many remedies to combat hair problems under *Keshya Karma*. One among is *Karnasphotha*. *Karnasphotha* (*Cardiospermum helicacabum* Linn.) commonly known as Balloon vine from Sapindaceae family, A climbing tendril bearing herb with wiry stem throughout the plains of India. Branches slender and leaves deltoid, 2-ternate, petioles 2-3.8 cm long and flowers are white. Seeds are globose.^[12] Chemically plant has: saponin, quebrachitol, apigenin, proanthocyanidin and stigmasterol.^[13] *Rajanighantukara* explains the properties of *Karnasphotha* as *Katu Tikta Rasayukta*, *Shita Virya*, and *Karma* as *Sarvavishapaha* and indicated in *Graha* and *Bhuta Roga*.^[14] Ayurvedic pharmacopoeia of India has explained the *Karma* of *Karnasphotha* (seed and root) as *Keshya*.^[15] Traditionally, the herb is used in hair oils for treating dandruff, alopecia and for darkening hair. The leaf paste is applied on domestic animals to kill lice and other insects.^[16] *Sahasrayoga* under *Taila Yoga Prakarana*, explains the formulation named *Nili Bhringadi Taila* containing Swarasa of *Karnasphotha* (*Shatakaratulata*).^[17]

So here an attempt is made to study the in vitro efficacy of *Karnasphotha* (*Cardiospermum helicacabum* Linn.) decoction against dandruff.

OBJECTIVES

1. Collection and preparation of *Kwatha* of *Karnasphotha* (*Cardiospermum helicacabum* Linn.) by decoction method of Root and Seed as per API.
2. Collection of Dandruff flakes from subjects and isolation of Fungi.

3. In vitro Antidandruff activity – Agar cup method and Biocidal activity.

Hypothesis

H₀ : *Karnasphotha* (*Cardiospermum helicacabum* Linn.) *Kwatha* is not having antidandruff effect.

H₁: *Karnasphotha*(*Cardiospermum helicacabum* Linn) *Kwatha* is having antidandruff effect.

METHODOLOGY

Type of study: Experimental study

Collection of study drug

Root and Seeds of *Karnasphotha* (*Cardiospermum helicacabum* Linn.) were collected from fields of Bailhongal and authenticated by Dr. S.V. Bagade, Professor and HOD Department of Dravyaguna Vignana, SNVVS's SGV Ayurvedic Medical College Hospital, Research Center, Bailhongal.

Image 1: *Karnasphotha*



Image 2: *Karnasphotha Moola*



Image 3: Karnasphotha Beeja



Preparation of Kwatha Churna:

The root and seeds were shade dried weighed and powdered coarsely following the SOP of API.

Image 4: Weighing of Karnasphotha Beeja



Image 5: Kwatha Churna of Karnasphotha Beeja



Image 6: Weighing of Karnasphotha Moola



Image 7: Kwatha Churna of Karnasphotha Beeja

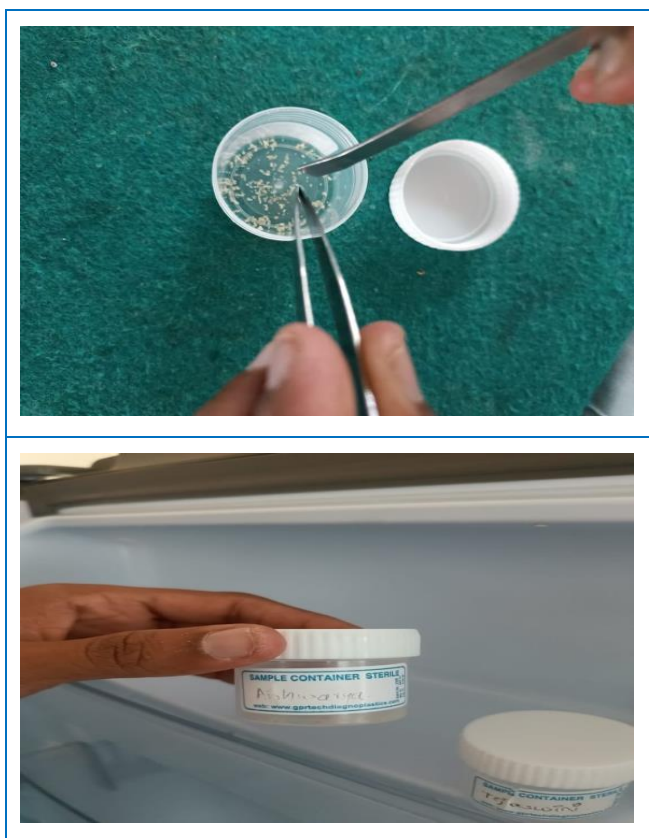


Collection of Dandruff

Samples of dandruff were collected from volunteers by scraping the scalp with sterile scalpel and stored in sterile container.

Image 8,9,10: Showing the collection of dandruff





Isolation of Fungi in Pure culture

The dandruff collected was inoculated on sterile Sabouraud dextrose agar plate. It was incubated at 32°C to 37°C for 3-5 days. Characteristic white growths around the flakes were indicative of an organism causing dandruff.

Growth and Identification

The organism was identified based on cultural, microscopic and biochemical methods. The colonies were identified as *Malassezia furfur*, identified fungal species of *Malassezia furfur* was isolated by pure culture in Sabouraud dextrose agar medium with added chloramphenicol. The agar provides a selective media for the growth of medically significant fungi while the antibiotic chloramphenicol serves to inhibit the growth of unwanted bacterial floras (Sabouraud, 1892).

Table 4: Chemicals and Media

| Chemical | Lot/Cat No. | Manufacturer |
|-------------------------------|-------------|----------------|
| Sabouraud dextrose agar (SDA) | MM1067 | Himedia, India |

| | | |
|-----------------|-------|----------------|
| Sodium chloride | MB023 | Himedia, India |
|-----------------|-------|----------------|

Table 5: Equipments

| SN | Name of the Instrument | Make | Instrument ID |
|----|--|----------------------------|----------------------------------|
| 1. | Weighing Balance | Orion Automation Systems | RRS/INS/MCR/05 |
| 2. | Autoclave (Sterilization) | Ascension Innovation India | RRS/INS/MCR/10 |
| 3. | Autoclave (decontamination) | Ascension Innovation India | RRS/INS/MCR/20 |
| 4. | Bacteriological Incubator -II | Biovision India /BVL 01 | RRS/INS/MCR/21 |
| 5. | Biological Safety Cabinet-II | Thermocon | RRS/INS/MCR/27 |
| 6. | Fungal incubator | Ascension Innovation India | RRS/INS/MCR/08 |
| 7. | Micropipettes 2-200 µl 100-1000 µl | Gilson Gilon | RRS/INS/MCR/11 RRS/INS/MCR/25 |
| 8. | Refrigerator | LG India / GL 191PMEH | RRS/INS/MCR/02 |

Preparation of test sample

Test sample was prepared at 100%, 75%, 50%, and 25% in distilled water for conducting zone of inhibition.

Method^[40-42]

Evaluation of Antidandruff Activity against *M. furfur* By Zone of Inhibition By Agar Cup Plate Method.

On sterile SDA plates were prepared and wells are made using sterile borer. Plates were spread with inoculum of *M. furfur*. In each plate 100%, 75%, 50% and 25% of test sample (*Karnasphotha Moola*) was loaded using micropipette. All plates were incubated at 30 ±2°C for 72hours. After incubation, the zone of

inhibition around the well was measured and antimicrobial activity was determined.

The same procedure repeated for *Karnasphotha Beeja*.

RESULT

Image 11 A & B: Antidandruff activity of test sample by zone of inhibition using agar cup plate method.

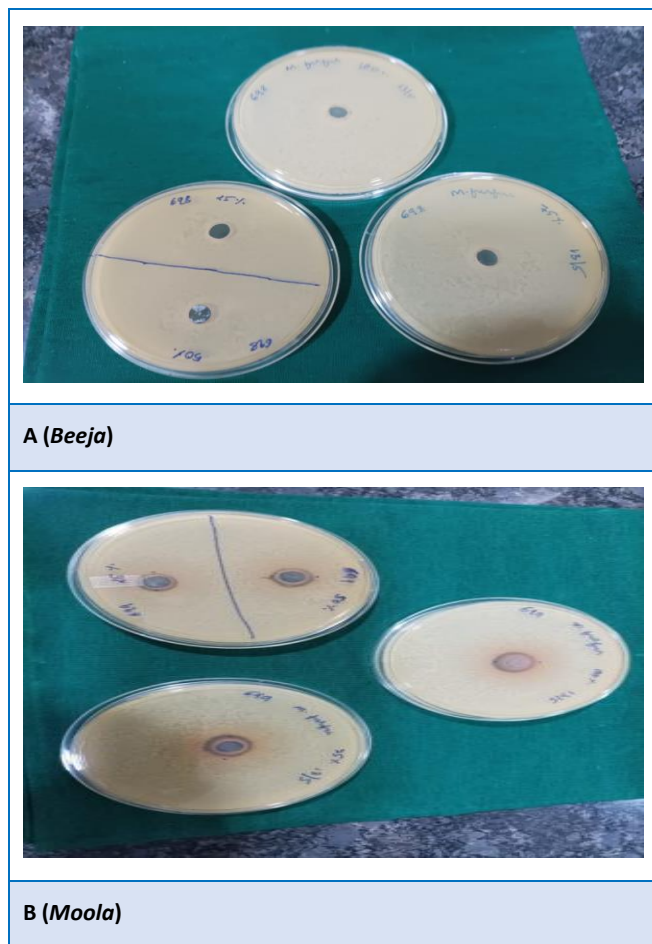


Table 6: Antidandruff activity of test sample by zone of inhibition using agar cup plate method

| Samples | Sample Code | conc. of test sample in (%) | zone of inhibition in (mm) |
|---------------------------|-------------|-----------------------------|----------------------------|
| <i>Karnasphotha Beeja</i> | RR230698 | 100 | No inhibition |
| | | 75 | No inhibition |
| | | 50 | No inhibition |
| | | 25 | No inhibition |
| | RR230699 | 100 | 17mm |

| | | |
|---------------------------|----|------|
| <i>Karnasphotha Moola</i> | 75 | 16mm |
| | 50 | 14mm |
| | 25 | 13mm |

The *Karnasphotha Moola* showed good antidandruff activity against *Malassezia furfur*. The antidandruff activity was found to be concentration dependent. In 100% concentration, the highest inhibition zone of 17 mm diameter clear zone was noticed against *Malassezia furfur*. In 75% concentration, *Karnasphotha Moola* showed activity of 16 mm inhibition zone, in 50% concentration, *Karnasphotha Moola* showed activity of 14 mm inhibition zone and in 25% concentration, *Karnasphotha Moola* showed activity of 13 mm inhibition zone against *Malassezia furfur*.

Whereas *Karnasphotha Beeja* showed no antidandruff activity against *Malassezia furfur* in any concentrations.

DISCUSSION

The overall goal of the present study was to know which part of the plant *Karnasphotha* is having antidandruff property. API explains *Karma* of *Moola* and *Beeja* as *Keshya* but present study confirms that *Moola* of *Karnasphotha* is having antidandruff property against *Malassezia furfur*.

Karnasphotha is having *Katu, Tikta Rasa Katu Vipaka* acts as *Prakruti Vighatana Chikitsa* for *Krimi* and hence same can be proposed for its antidandruff activity. Its *Prakruti Vighata* action prevents new formation of dandruff and also removes *Nidana* for it. Its *Vishahara* property helps in healing dandruff.

CONCLUSION

The antidandruff activity of *Karnaspota Moola* was tested by MIC against *M. furfur* indicated as recorded in Table No.6. Hence, it shall be concluded that the test sample is having significant Antidandruff properties. The antidandruff activity of *Karnaspota Beeja* was tested by MIC tested against *M. furfur* indicated as recorded in Table No.6. Hence, it shall be concluded that the test sample is not having significant Antidandruff properties.

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