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Antifungal activity of *Vibhitaki* (*Terminalia bellirica* (Gaertn) Roxb.) against dandruff causing organism *Malassezia furfur* – an *in vitro* study

Harsha Uday Kulkarni✉, **Prakash L. Hegde**

Department of Dravyaguna, SDMCAH, Hassan, Karnataka, India

✉ **Corresponding author:** Harsha Uday Kulkarni. **E-mail:** harshauk9@gmail.com

ORCID: Harsha Uday Kulkarni – https://orcid.org/ 0009-0005-0963-9796;
Prakash L. Hegde – https://orcid.org/0009-0002-4034-5831.

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Abstract

Introduction. Dandruff is the commonest scalp disorder. It affects around 50 % of the pre-pubertal age group of any gender. Malazzasia species play a major role in pathogenesis of this condition which is one of the causes for hair fall. Hence It was chosen as *Krimi* (Fungi) for invitro antimicrobial study. The current treatment options for dandruff includes using antifungal shampoos which have certain limitations besides they are known to cause discoloration of hair, Pruritic, dryness of scalp and hair. Hence this study was under taken to find better herbal alternative to manage Dandruff. *Vibhitaki* which is one of the primary constituents of *Triphala* (Classical polyherbal formulation of three fruits) is known to have *keshya* (Beneficial to hair) and *krimighna karma* (Anti-microbial activity). Hence Invitro antifungal activity of Vibhitaki was tested against dandruff causing organism Malassezia furfur.

Aim. To evaluate the Antifungal activity of *Vibhitaki* (*Terminalia bellirica* (Gaertn)Roxb.) against dandruff causing organism Malassezia furfur using well diffusion method.

Materials and methods. Well diffusion method was employed for Invito anti-fungal study. Different forms of the drug were chosen for antimicrobial study viz *Vibhitaki taila* (*Vibhitaki* oil), Aqueous extract, Methanolic extract of *Vibhitaki* fruit and *Narikela taila* (Coconut oil).

Results and discussion. The zone of inhibition was measured to check the Antifungal activity of *Vibhitaki* fruit using suitable internal standard positive control. Methanolic extract of *Vibhitaki* has shown better antimicrobial activity than aqueous extract of *Vibhitaki* against *Malassezia furfur* but it was not that of standard drug's zone of Inhibition. Whereas *Vibhitaki taila* and *Narikela taila* did not show any antimicrobial activity against *Malassezia furfur.*

Conclusion. Methanolic extract and aqueous extract of Vibhitaki (Terminalia bellirica) possess antifungal activity against *Malassezia furfur.*

Keywords: *Vibhitaki*, dandruff, well diffusion method, keshya, krimighna

Conflict of interest. The authors declare that they have no obvious and potential conflicts of interest related to the publication of this article.

Contribution of the authors. Harsha Uday Kulkarni – data collection and study design. Prakash L. Hegde – analysis and interpretation of results.

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Противогрибковая активность вибхитаки (*Terminalia bellirica* (Gaertn) Roxb.) против микроорганизма *Malassezia furfur*, вызывающего перхоть, – исследование *in vitro*

Харша Удай Кулкарни✉, Пракаш Л. Хегде

Индия, Карнатака, Хасан, SDMCAH, Департамент Дравьягуна

✉ **Контактное лицо:** Кулкарни Харша Удай. **E-mail:** harshauk9@gmail.com

ORCID: Харша Удай Кулкарни – https://orcid.org/0009-0005-0963-9796;
Пракаш Л. Хегде – https://orcid.org/0009-0002-4034-5831.

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Резюме

Введение. Перхоть является самым распространенным заболеванием кожи головы. Она поражает около 50 % людей препубертатного возраста любого пола. Виды *Malazzasia* играют важную роль в патогенезе этого состояния, которое является одной из причин выпадения волос. Поэтому он был выбран в качестве *Krimi* (грибков) для *in vitro* антимикробного исследования. Текущие варианты лечения перхоти включают использование противогрибковых шампуней, которые имеют определенные ограничения, кроме того, как известно, они вызывают обесцвечивание волос, зуд, сухость кожи головы и волос. Поэтому было проведено это исследование, чтобы найти лучшую растительную альтернативу для борьбы с перхотью. Вибхитаки, который является одним из основных компонентов Трифалы (классическая политравяная формула из трех плодов), как известно, обладает кешья (полезным для волос) и кримигхна карма (антимикробной активностью). Поэтому противогрибковая активность вибхитаки *in vitro* была протестирована против вызывающего перхоть микроорганизма *Malassezia furfur.*

Цель. Оценить противогрибковую активность вибхитаки (*Terminalia bellirica* (Gaertn) Roxb.) против вызывающего перхоть микроорганизма *Malassezia furfur* с использованием метода диффузии в плотную питательную среду.

Материалы и методы. Для исследования противогрибковой активности *in vito* использовался метод диффузии. Для исследования антимикробной активности были выбраны различные формы препарата: вибхитаки тайла (масло вибхитаки), водный экстракт, метанольный экстракт плодов вибхитаки и нарикела тайла (кокосовое масло).

Результаты и обсуждение. Зона ингибирования была измерена для проверки противогрибковой активности плодов вибхитаки с использованием подходящего внутреннего стандарта положительного контроля. Метанольный экстракт вибхитаки показал лучшую антимикробную активность, чем водный экстракт вибхитаки, в отношении *Malassezia furfur*, но она не соответствовала зоне ингибирования стандартного препарата. В то же время, вибхитаки тайла и нарикела тайла не проявили антимикробной активности в отношении *Malassezia furfur.*

Заключение. Метаноловый и водный экстракты вибхитаки (*Terminalia bellirica*) обладают противогрибковой активностью против *Malassezia furfur.*

Ключевые слова: вибхитаки, перхоть, метод диффузии, кешья, крымигна

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Introduction

Vibhitaki (*Terminalia bellirica* Roxb.) is commonly found tree in deciduous forests throughout the greater part of India [1]. It is abundantly available in all seasons and cost effective. It is widely used drug in many formulations. It is one of the essential ingredients of *Triphala* which is commonly used in *Urdhwajatrugata vikaras* (Diseases of the head, neck, and upper chest region). *Vibhitaki* possesses *karmas* like *Keshya* (Nourishing hair), *Krimighna* (Anti-microbial activity), *Swarya* (Nourishing voice), *Chardighna(Anti emetic)* [2]. It is used in *Kasa* (Cough), *Netra roga* (Eye diseases), *Kesha vikara* (Disorders affecting hair), *Krimiroga* (Infections caused by microbes), *Swara vikara* (Hoarseness of voice), *Trishna* (Polydipsia), *Chardhi* (Vomiting), *Mukharoga* (Diesease of oral cavity) and *Akshiroga* (Eye diseases). *Vibhitaki* fruit has exhibited Antifungal activity against *Cryptococ-*

cus fungi and also has stopped the growth of drug-resistant fungi [3]. The fruit contains 20–30 % of tannins, gallic acid, ellagic acid, termilignan, thaninilignan, anolignan B. The lignans isolated from *Vibhitaki* possess Anti fungal, Antimalarial activities [4]. Gallic acid which is major phytoconstituent of this drug also possesses Antifungal and Antibacterial activity [5].

Darunaka (Dandruff) is a *Kapalagata roga* (Scalp disorder) mentioned under *Kshudra roga*(minor diseases). It's symptoms can be correlated to Dandruff, which is the commonest scalp disorder. It affects around 50 % of the pre-pubertal age group of any gender [6]. The prevalence of dandruff in population varies between 30–95 percent [7]. Malazzasia species play a major role in pathogenesis of this condition along with stress, use of shampoos, oily nature of scalp [8]. *Malassezia furfur* is a species of yeast (Type of fungus) that is found on the skin surface of human and some other mammals. It is associated with a variety of dermatological conditions caused by fungal infections, notably Seborrheic dermatitis, Tinea versicolor, Dandruff, Malassezia folliculitis, Pityriasis versicolor. Majority of the population experiencing dandruff also suffer from severe hairfall which casts negative impact on their self esteem and confidence. Currently available treatment options for the management of dandruff include therapeutic use of Antidandruff shampoos containing keratolytics, Antimicrobials like Zinc pyrithione, Selenium Sulphide, Salicylic acid [9]. However these agents have certain limitations either due to poor clinical efficiency or due to compliance issues. Furthermore, these drugs are unable to prevent reoccurrence of dandruff which is the commonest problem. Prolonged usage of antifungal shampoos (e.g Ketoconazole shampoo) induce discoloration of hair, Pruritic, dryness of scalp and hair [10]. So it is imperative to search for the herbal alternatives that are safe and ecofriendly.

Though many drugs are mentioned as *Krimighna* (Anti microbial), only few works are done on establishing *Krimighna* action (Anti microbial) of specific drug on specific organism. Since *Vibhitaki* is known to possess both *Karma*'s (Actions) like *krimighna* (Antifungal activity) and *Keshya* (Nourishing hair), It's *krimighna karma* (Antifungal activity) on dandruff causing organism was taken for study. *Vibhitaki taila*, aqueous and alcoholic extracts of *Vibhitaki and Narikela taila* (Coconut oil) were tested against *Malassazia furfur*. Since *Narikela taila* (Coconut oil) was used as the base oil in the preparation of *Vibhitaki taila* it was be tested against *Malassezia furfur*.

Aim and Objective of the study

To evaluate the *Krimighna karma* (Antifungal activity) of *Vibhitaki taila*, Methanolic extract of *Vibhitaki*, Aqueous extract of *Vibhitaki*,and *Narikela taila* (Coconut oil) on dandruff causing organism Malassezia furfur.

Methodology

A. Collection of drug and authentification of the drug.
B. Preparation of extracts.
C. Preparation of *Vibhitaki taila* (*Vibhitaki* oil).
D. Antifungal activity – well diffusion method.

A. Collection of drug and authentification of the drug (Figure 1).

Vibhitaki fruits (*Terminalia bellirica* (Gaertn) Roxb.) were purchased from "Gadgil Vanoushadhi Sangraha" pharmacy Belagavi in the month of August, 2024. *Vibhitaki* fruits were authenticated at Department of Dravyaguna Vijnana, Sri Dharmasthala Manjunatheshwara College of Ayurveda and Hospital, Hassan (Figure 2).

Figure 1. *Vibhitaki* tree bearing fruits (*Terminalia bellirica* (Gaertn) Roxb.)

Figure 2. *Vibhitaki* fruits

B. Preparation of extracts.

8 gm of *Vibhitaki* fruit (*Terminalia bellirica* (Gaertn) Roxb.) coarse powder was used to prepare each Aqueous and methanolic extracts. 100 ml of Distilled water and 100ml Methanol was taken in conical flasks to prepare extracts. 8 gms of *Vibhitaki* coarse powder was added to each conical flasks containing 100 ml of distilled water and 100 ml of methanol. Both the conical flasks after securing with rubber cork were placed on spinix orbital shaker with 16cpm for 24 hours for uniform mixing. After mixing they were kept undisturbed for overnight. Next morning both the solutions were filtered using filter paper into separate beakers and about 40 ml of filtrate was obtained. These filtrates were transferred to pre-weighed, labelled and dried separate China dishes. They were placed on water bath for 6–8 hours. Once the liquid portion got evaporated dishes containing extracts were allowed to cool in desiccator and weighed later. The extracts were scraped using spatula and stored in separate prelabelled sterile airtight containers.

C. Preparation of *Vibhitaki taila* (*Vibhitaki* oil).

Vibhitaki taila (*Vibhitaki* oil) was prepared as per classical method prescribed for *Taila kalpana* (Medicated oil), in the Department of Dravyaguna Vijnana, Sri Dharmasthala Manjunatheshwara College of Ayurveda and Hospital, Hassan.

Procedure

Table 1 gives the ingredients of *Vibhitaki* taila with the quantity indicated.

Table 1. Ingredients of *Vibhitaki taila* with quantity

Ingredients	Means	Number of batches	Quantity
***Kalka* (Paste)**	*Vibhitaki kalka* (Soft paste/Bolus of *Vibhitaki* fruit)	1 part	12.5 gm
***Taila* (Oil)**	*Narikela taila* (Coconut oil)	4 parts	50 gm
***Kwatha* (Decoction)**	*Vibhitaki kwatha* (Decoction of *Vibhitaki* fruit)	16 parts	200 ml

Preliminary procedure

1. ***Vibhitaki kwatha* preparation (Decoction of *Vibhitaki* fruit).**
2. ***Vibhitaki kalka* preparation (Preparation of Soft paste / Bolus of *Vibhitaki* fruit powder).**

1. *Vibhitaki kwatha* preparation (Preparation of Decoction of *Vibhitaki* fruit).

Dried *Vibhitaki* fruits were taken and pounded in *Khalwa yantra* (Mortar and Pestle) to obtain coarse powder. 100 gm of coarse powder was taken and mixed with 16 parts of water (1.6 l) and boiled over low flame. The decoction is reduced to 1/8th part (200 ml). It is allowed to cool and filtered.

2. *Vibhitaki Kalka* preparation (Preparation of Soft paste/Bolus of *Vibhitaki* fruit).

Dried *Vibhitaki* fruits were taken and pounded in *Khalwa yantra* (Mortar and Pestle). Obtained *Choorna* (*Powder*) was filtered through a clean cotton cloth to get fine powder. Fine powder is mixed with few drops of *Vibhitaki Kashaya* (Decoction of *Vibhitaki* fruit*)* to prepare *kalka* (Bolus/paste) (12.5 gm).

Narikela taila (cold pressed coconut oil) was obtained from local mill at Hassan.

Taila Kalpana (Preparation of Vibhitaki taila)

Vibhitaki taila was prepared as per classical *Sneha kalpana vidhi* [11] (Classical method of preparation of medicated oil).

- All the above-mentioned ingredients (Table 1) were weighed and taken.
- Clean stainless-steel vessel was taken in which 50 gm of *Narikela taila* (Coconut oil) was added.
- It was placed over stove under low flame.
- Later *Vibhitaki kalka* (Paste/bolus of *Vibhitaki* fruit*)* was added to the oil and stirred it thoroughly using spatula for uniform mixing.
- *Vibhitaki kwatha* (*Vibhitaki* decoction) was added slowly to the mixture containing oil and *kalka* (Paste/bolus).
- It is boiled over low flame, stirring was done if required until *kwatha* (Decoction) was completely evaporated.
- Clear layer of *Vibhitaki taila* (*Vibhitaki* oil) was seen on the top layer under which *kalka* (Paste) had precipitated.
- It was heated until *Sneha Siddhi lakshanas* [12] (Appearance of foam) were obtained.
- It was allowed to cool and filtered through clean cotton cloth and about 30 ml of filtrate (*Vibhitaki taila*) was obtained.
- Stored in Air tight container.

D. Anti-fungal study.

Aim and objective.

To evaluate the antifungal activity of *Vibhitaki* on dandruff causing organism Malassezia furfur.

Antifungal activity of *Vibhitaki* was evaluated using Dixon's agar well diffusion method on *Malassezia furfur* at Sri Dharmasthala Manjunatheshwara Centre for research in Ayurveda & Allied science, Udupi (Table 2).

Table 2. Requirements for Antimicrobial study

Ingredients	Devices
Test strains – Malassezia furfur	Micro pipettes
Test samples	Spatula
Distilled water	Well boring pipettes
Methanol	Cotton swabs
Normal saline	Petri plates
Test tubes	Incubator
Laminar air flow	

Test samples used for antimicrobial study:

- ✓ Aqueous extract of *Vibhitaki* fruits.
- ✓ Methanolic extracts of *Vibhitaki* fruits.
- ✓ *Vibhitaki taila* (*Vibhitaki oil*).
- ✓ *Narikela taila* (Coconut oil).

Method – Agar well diffusion method

Steps involved:

1. Preparation of Agar media.
2. Preparation of Inoculum.
3. Preparation of Agar plates.
4. Dilution of test drug and standard drug.
5. Dispensing test samples, control and standard drugs.

1. Preparation of dixon's agar media.

- 32 gm of Part A (Standard mixture) was taken and added to 100 ml distilled water.
- It was stirred continuously for uniform mixing.
- 4.5 ml of Part B (Standard mixture) was added to above mentioned mixture and 100 ml distilled water was added.
- The mixture was stirred to obtain homogenous solution and it was made up to 300 ml by adding additional 100 ml distilled water.
- The mixture was heated in oven for 2–3 min to obtain homogenous solution. After which it was autoclaved for 1 hour.
- Stored in cold storage.

2. Preparation of inoculum

- *Malassezia furfur* (MTCC 1765) was procured from Microbial Type Culture Collection and Gene Bank (MTCC), IMTECH, Chandigarh India.
- Slant preparation was done on which streaks of *Malassezia* were made and incubated for 7 days.
- Under sterile condition one loopful of 7 days old culture from the slants was transferred to 10 ml buffer sodium chloride peptone solution and mixed well to prepare a homogeneous inoculum in laminar airflow chamber.

3. Preparation of agar plates.

- Autoclaved sterile agar plates were taken, were labelled with name of sample, Organism, Sample number, date of preparation.
- Numbering was done on backside of agar plates from 1 to 6 at equal intervals to dispense samples of different concentrations.
- Agar media was heated to adequate temperature and 20 ml of media was poured in each agar plate and 1 ml of inoculum was added to each plate swirled for uniform distribution.
- Plates were left undisturbed and allowed to cool in sterile condition.

4. Dilution of test samples and standard drug.

- Extracts were weighed and 2 mg of extract was collected in 2 separate sterile containers.
- 2 ml of distilled water and 2 ml of methanol was added to separate containers having respective extracts.
- 150 mg tablet of fluconazole was powdered and dissolved in 1ml of distilled water.
- All three containers were subjected to mixing on cyclomixer (Table 3).

Table 3. Dilution ratio of samples in solvent media

S.no	Test sample	Dilution	Media
1	Fluconazole – 150 mg	1000 µl	Distilled water
2	Aqueous extract of *Vibhitaki* – 2 mg	2000 µl	Distilled water
3	Methanolic extract of *Vibhitaki* – 2 mg	2000µl	Methanol

5. Dispensing test samples, control and standard drugs (Table 4).

- Under sterile condition in laminar air flow chamber wells were bored in agar plates using sterile well cutting pipettes.
- Six wells were bored on each agar plates corresponding to its numbers marked on its back.

Table 4. Different Volumes Of Test Samples, Cotrol And Standard Drug Dispensed

S.no/Samples	Aqueous extract of *Vibhitaki*	Methanolic extract of *Vibhitaki*	*Vibhitaki taila* (*Vibhitaki* oil)	*Narikela taila* (Coconut oil)
1	25 µl	25 µl	25 µl	25 µl
2	50 µl	50 µl	50 µl	50 µl
3	75 µl	75 µl	75 µl	75 µl
4	100 µl	100 µl	100 µl	100 µl
5	Distilled water (Vehicle Control) – 100 µl	Methanol (Vehicle Control) – 100 µl	Coconut oil (Vehicle Control) – 100 µl	125 µl
6. Standard drug	Fluconazole – 20 µl	Fluconazole – 20 µl	Fluconazole – 20 µl	Fluconazole – 20µl

- Samples of Different volumes, Control drug and standard drugs were dispensed into wells using separate sterile micro pipettes.
- They were carefully placed in incubator at 25 °C for observation for 7 days.

Observations and results

Observations on *Krimighna Karma* (*Anti-fungal activity*) shown for *Vibhitaki taila*, methanol and aqueous extracts of ***Vibhitaki* (*Terminalia bellirica* Roxb).**

Zone of inhibition of *Vibhitaki taila*, methanol and aqueous extracts of ***Vibhitaki* (*Terminalia bellirica* Roxb.)** with different concentrations were determined using the Agar well Diffusion Method. The zone of inhibition observed during the study was recorded in Table 5.

Table 5. Zone of inhibition of methanol extract of *Vibhitaki* against *M. furfur* at different volumes

Sample	Volumes, ml	Zone of inhibition – (Radius in mm)	
Methanol extract of *Vibhitaki* (10 mg/ml)	25	6	6
	50	8	9
	75	9	10
	100	10	10
Internal Control (Methanol)	100	0	0
Standard (Fluconazole) 150 mg / 2 ml	10	18	18

Average zone of inhibition of methanolic extract of *Vibhitaki* is 8.8 mm and of standard drug (Fluconazole) is 18mm. No zone of inhibition was seen in control group (methanol) (Figure 3).

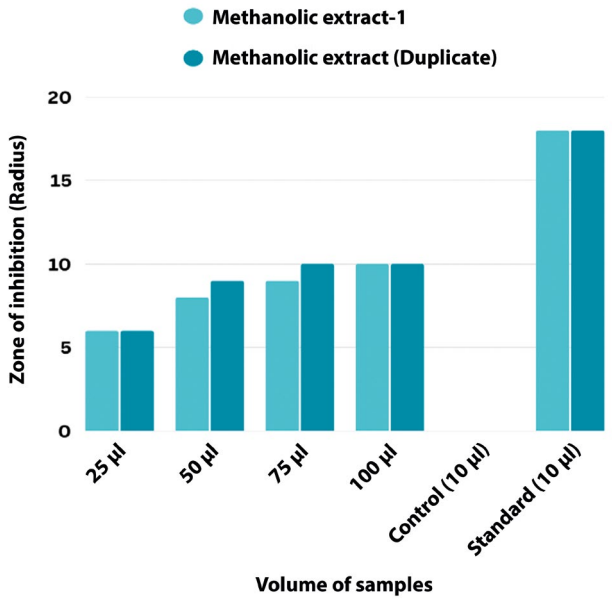

Figure 3. Graph representing zone of inhibition of methanolic extract of Vibhitaki, control and standard drug.

Zone of Inhibition (Radius in mm); Control (Methanol); Standard (Fluconazole)

Result: Zone of inhibition of methanolic extract was more than control group but not as that of standard group (Table 6).

Table 6. Zone of inhibition of aqueous extract of *Vibhitaki* against *M. furfur* at different volumes

Sample	Volumes, ml	Zone of inhibition – (Radius in mm)	
Aqueous extract of *Vibhitaki* (10 mg/ml)	25	0	0
	50	6	5
	75	6	6
	100	7	7
Internal Control (DD water)	100	0	0
Standard (Fluconazole) 150 mg / 2 ml	10	14	15

Average zone of inhibition of aqueous extract of *Vibhitaki* is 6.1 mm and of standard drug (Fluconazole) is 14.5 mm. No zone of inhibition was seen in control group group (distilled water) (Figure 4).

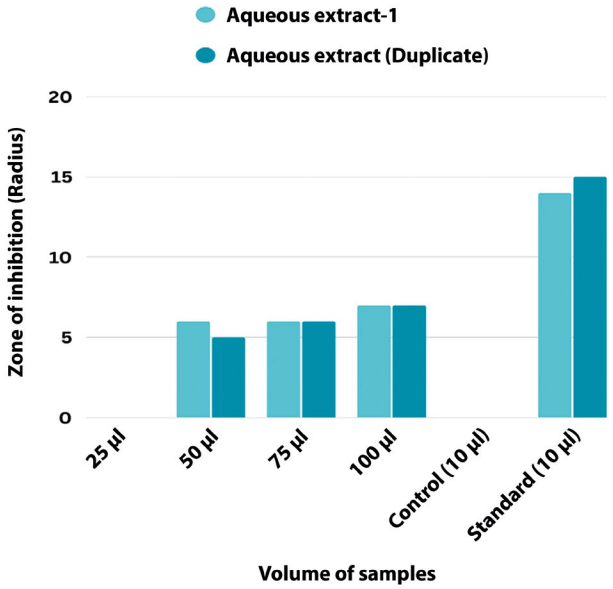

Figure 4. Graph representing zone of inhibition of Aqueous extract of Vibhitaki, control and standard drug.

Zone of Inhibition (Radius in mm); Control (Methanol); Standard (Fluconazole)

Result: Zone of inhibition of aqueous extract was more than control group but not as that of standard group (Table 7).

Result: No zone of inhibition was observed in Vibhitaki taila group of different concentrations (Table 8).

Table 7. Zone of inhibition of *Vibhitaki* taila against *M. furfur* at different volumes

Sample	Volumes, ml	Zone of inhibition – (Radius in mm)	
Vibhitaki taila	25	0	0
	50	0	0
	75	0	0
	100	0	0
Control (Coconut oil)	100	0	0
Standard (Fluconazole) 150 mg / 1 ml	10	17	18

Table 8. Zone of inhibition of Narikrla taila against *M. furfur* at different volumes

Sample	Volumes, ml	Zone of inhibition – (Radius in mm)	
Narikela taila	25	0	0
	50	0	0
	75	0	0
	100	0	0
	125	0	0
Standard (Fluconazole) 150 mg / 2 ml	10	17	18

Result: No zone of inhibition was observed in Vibhitaki taila group of different concentrations.

Statistics

Arithmetic mean is applied for statistical analysis of this study**.**

Average of ZOI of each test samples, control and the standard drugs (Table 9).

Table 9. Average Zone of inhibition of Methanolic extract,Control and standard drug

Test sample	Arithmetic mean
Methanolic extract of *Vibhitaki*	8.5
Control (Methanol)	0
Standard (F)	18

Result: The average of ZOI of Aqueous extract group was more than the control group but not as that of standard group (Table 10).

Table 10. Average Zone of inhibition of Aqueous extract,Control and standard drug

Test sample	Arithmetic mean
Aqueous extract of *Vibhitaki*	4.625
Control (Distilled water)	0
Standard (Fluconazole)	14.5

Result: The average of ZOI of Methanolic extract group was more than the control group but not as that of standard group (Table 11).

Result: The average of ZOI of Narikela taila group was more than the control group but not as that of standard group (Table 12).

Table 11. Average Zone of inhibition of Vibhitaki taila,Control and standard drug

Test sample	Arithmetic mean
Vibhitaki taila	0
Control (Narikela taila)	0
Standard (Fluconazole)	17.5

Table 12. Average Zone of inhibition of Narikela taila, Control and standard drug

Test sample	Arithmetic mean
Narikela taila	0
Control (Coconut oil)	0
Standard (Fluconazole)	17.5

Results

1. The methanolic extract of *Vibhitaki* has shown positive antimicrobial effect *Malassezia furfur* fungi in comparison with the control group but not as much as the standard group.
2. The aqueous extract of *Vibhitaki* has shown positive antimicrobial activity in comparison with the control group but not as that of standard drug.
3. The *Vibhitaki taila* has not shown positive antimicrobial activity in comparison with the control group.
4. The *Narikela taila* has not shown positive antimicrobial activity in comparison with the control group.

By all the above observations we found that Methanolic extract of *Vibhitaki* has shown better antimicrobial activity than aqueous extract of *Vibhitaki* against *Malassezia furfur*. Where as *Vibhitaki taila* and *Narikela taila* did not show any antimicrobial activity against *Malassezia furfur.*

Discussion

Discussion of the study can be classified under following headings:

- ✓ Discussion on samples chosen
- ✓ Based on in vitro antimicrobial study
- ✓ Based on the mode of action

Discussion on samples chosen

Aqueous extract of *Vibhitaki.*

It dissolves all the water-soluble constituents in the fruits. To some extent it can be considered as *Kashaya Kalpana* hence it was chosen as sample for study.

Methanolic extract of *Vibhitaki.*

The lignans and gallic acid, phytoconstituents responsible for antimicrobial activity known to have more solubility in methanol than ethanol. Hence Methanolic extract was chosen for this study [13].

***Vibhitaki Taila* (*Vibhitaki oil*).**

Taila Kalpana is one such dosage form in which fat-soluble constituents of drug are extracted. *Vibhitaki taila* was prepared as per the *Sneha Kalpana* using *Narikela taila* (Coconut oil). Since *Vibhitaki* is both *Keshya*

(Nourishing hair) and *Krimihara* (Antifungal). It can be used on daily bases as regular hair oil. Hence *Vibhitaki taila* was chosen as a sample to test for its invitro antifungal activity.

***Narikela taila* (Coconut oil).**

Since *Narikela taila* was used to prepare *Vibhitaki taila* it was also chosen as sample to test against Malassezia furfur.

Discussion on Antimicrobial study

- Antifungal activity of *Vibhitaki* was tested against Malassezia furfur fungi in different concentrations.
- Samples taken for antifungal activity were *Vibhitaki taila, Narikela taila*, Alcoholic extract and Aqueous extract of *Vibhitaki.*
- Antifungal activity was seen only in Aqueous and Methanolic extracts of *Vibhitaki*.
- There was absolutely no antifungal activity seen in *Vibhitaki* and *Narikela taila.*
- At the lowest concentration of Aqueous extract sample there was no inhibitory activity seen since the concentration of drug is minimum in lowest concentration. At the higher concentration there was antifungal activity observed. Average of 7 mm radius of inhibition was seen in aqueous extract at higher concentration which is comparatively lesser than standard (14.5 mm).
- Methanolic extracts showed comparatively better antifungal activity than Aqueous extract. There was inhibitory activity seen even in lowest concentration of drug. Highest activity was observed at higher concentration (10 mm) but lesser than the standard which was 18mm.
- Lignans and gallic acid which are major phytoconstituents in *Vibhitaki* are more soluble in Methanol than water. Hence Antifungal activity is better observed in Methanolic extract.
- In taila samples no zone of inhibition was seen. It puts light on a possibility that *Taila* (Oil media) was not suitable solvent media to test invitro antifungal activity.

Statistical analysis:

- Arithmetic mean of Zone of Inhibition of Methanolic extract and Aqueous extract of *Vibhitaki* was higher than the control but lesser than the standard group whereas Zone of Inhibition of *Vibhitaki taila* and *Narikela taila* was nil.

Based on mode of action.

The Phytochemical investigation of aqueous extract of *Vibhitaki* revealed the presence of Tannins, Carbohydrates. The Methanolic extract revealed the presence of Tannins, Glycosides, Flavonoids, Tannins, Carbohydrates, Phenolic compounds and Alkaloids.

Tannins – The mode of antimicrobial action of tannins is potentially due to inactivation of microbial adhesins and cell envelope transport proteins. Besides their efficacy against bacteria, tannins have been reported to be inhibitory on fungi and yeasts [14].

Flavonoids – Flavonoids often inhibit fungal growth with various underlying mechanisms, including plasma membrane disruption, the induction of mitochondrial dysfunction, and inhibiting the following: cell wall formation, cell division, RNA and protein synthesis, and the efflux mediated pumping system [15].

Alkaloids – inhibition of nucleic acid and protein synthesis. A key component of alkaloids' antibacterial activity is their substantial capacity to interfere with the creation of proteins and nucleic acids inside bacterial cells. Another important antibacterial action of alkaloids is their capacity to change the permeability of bacterial cell membranes. A number of alkaloids are potent efflux pump inhibitors (EPIs), which are essential in the battle against bacterial resistance [16].

Saponins – the antifungal activity of the saponin is associated with their aglycone moieties and the number and structure of monosaccharide units in their sugar chains [17].

Hence presence of phytoconstituents like Flavonoids, Tannins, Phenolic compounds and Alkaloids will contribute to the antifungal activity of *Vibhitaki.*

Conclusion

- *Vibhitaki* was evaluated for its antifungal activity using well diffusion method, against Dandruff causing organism Malassezia furfur. The samples chosen were *Vibhitaki taila* (Vibhitaki oil), Aqueous extract, Methanolic extract and *Narikela taila* (Coconut oil) .
- Among all the samples Methanolic extract showed maximum zone of inhibition of 10 mm at 100 ml volume however it was not as that of standard drug which was 18 mm.
- Aqueous extract showed moderate zone of inhibition of 7 mm at 100 ml volume however it was not as that of standard drug which was 14.5 mm.
- While *Vibhitaki taila* (*Vibhitaki oil)* and *Narikela taila* (Coconut oil) did not exhibit any antifungal activity against Malassezia furfur.
- Thus, it can be concluded that Methanolic and aqueous extracts of *Vibhitaki* possess antimicrobial activity *(Krimighna karma*) against the Malassezia furfur fungi.

Scope for further study

1. Clinical study can be undertaken using *Vibhitaki taila (Vibhitaki oil)* as test medicine in dandruff cases associated with hair loss.
2. This study can be conducted using samples with higher concentration and with other extracts of *Vibhitaki* fruit.

Limitations

Lipid media was not suitable solvent to test *in vitro* antimicrobial activity of the drug.

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