

Evaluation of Antimicrobial Activity of *Bruhat Marichyadi Taila* and its Ointment Formulation *in vitro*

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Abstract

Introduction: *Bruhat marichyadi taila* is a traditional Ayurvedic preparation indicated for various *twacha vikara* (skin diseases). Although traditionally used in oil form, its solid conversion into an ointment may increase sanative efficacy and tolerant compliance. The present study was undertaken to evaluate and compare the antimicrobial activity of *Bruhat marichyadi taila* and its ointment formulation *in vitro*. **Materials and Methods:** *Bruhat marichyadi taila* (Sample A) was prepared according to definitive Ayurvedic guidelines and then formulated into an ointment (Sample B) using low paraffin, stale paraffin, and beeswax. Both formulations were evaluated for antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, and *Aspergillus niger* using the agar considerably diffusion method at concentrations of 100 µL and 200 µL. **Results:** It was observed that the ointment formulation (Sample B) showed higher zones of inhibition against entirely tested microorganisms than the oil (Sample A). Sample A showed bottom antibacterial activity (1–2 mm) and moderate antimycotic activity (6–11 mm), whereas Sample B demonstrated enhanced antibacterial (4–8 mm) and antimycotic activity (10–17 mm). **Discussion:** The improved antimicrobial activity of the ointment formulation may be attributed to better drug retention, enhanced diffusion, and uninterrupted release of existing phytoconstituents from the ointment base. The solid form also improves stability and ensures chronic contact with microbial cells. **Conclusion:** *Bruhat marichyadi taila* ointment exhibits superior antimicrobial activity compared to its oil form and offers advantages with respect to stability, ease of application, and tolerant compliance. The formulation may serve as a further operative local dosage form for the treatment of contagious skin disorders.

Key words: Agar well diffusion, antimicrobial study, *Aspergillus niger*, *Bruhat marichyadi taila*, *Candida albicans*, *Escherichia coli*, ointment formulation, *Staphylococcus aureus*

INTRODUCTION

Skin serves as the covering for domestic organs and protects the body from various foreign interferences. It is the largest organ of the human body. Among the five “*Gyanindriyas*” mentioned in Ayurvedic *samhitas*, it is related to “*Sparsha Gyan*,” or the sense of touch, and therefore plays a pivotal role in maintaining somatic and mental health. The skin forms a normal tutelary barrier, and any disruption of this barrier may lead to several health problems. Due to their obvious appearance, skin disorders importantly contribute to pain, suffering, disability, financial hardship, and interpersonal stigma.

In Ayurveda, various skin conditions are collectively referred to as *kushtha*

(skin diseases), and several traditional remedies are described for *twak vikara* (skin afflictions). The term *kushtha* includes a full range of dermatologic manifestations that may progress speedily if untreated. *Escherichia coli*, *Staphylococcus aureus*, *Aspergillus niger*, and *Candida albicans* are general organisms causative for simple skin infections, each characterised by different morphology and disease course. Secondary infections occur over pre-existing skin lesions.

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With the increasing prevalence of dermatologic disorders, the need for improved and further operative treatment modalities continues to rise.

Bruhat marichyadi taila is a traditional Ayurvedic oil indicated for foreign application. Mentioned in *Bhaishajya Ratnavali*, *Yogaratanakar Samhita*, and different definitive compendia, it is wise advantageous in *kustha*, *dadru*, *kandu*, *dustavrana*, *pama*, *visfot*, *vyanga*, *mandalkustha*, and *shwitra*.^[1,2] It is also used in dermatologic conditions such as eczema and psoriasis. The formulation contains ingredients including *Sarshap taila*, *Marich*, *Snuhi Ksheera*, *Haridra*, *Nimba*, *Daruharidra*, *Vidanga*, and *Pippalimoola*. *Brassica juncea* (*Sarshap taila*)^[3] possesses phenolic compounds and glucosinolates that contribute to antimicrobial activity by altering bacterial membrane permeability.

The essential oil of *Piper nigrum* (*Maricha*)^[4] contains α -pinene, β -pinene, limonene, caryophyllene, phenolics, and flavonoids, which disrupt microbial membranes and inhibit biofilm formation. *Euphorbia neriifolia* (*Snuhi*)^[5] exhibits broad-spectrum antimicrobial activity due to triterpenoids such as euphol, taraxerol, and β -amyrin. *Curcuma longa* (*Haridra*)^[6] demonstrates antibacterial and antifungal effects principally because of curcuminoids and changeable oils such as ar-turmerone. *Azadirachta indica* (*Nimba*)^[7] also possesses potent antimicrobial properties attributed to limonoids such as nimbin, nimbidin, and nimbolide.

Tinospora cordifolia (*Guduchi*)^[8] contains alkaloids, terpenoids, tannins, and flavonoids, with compounds such as moupinamide showing antibacterial and biofilm inhibitory activity. *Berberis aristata* (*Daruharidra*)^[9] derives its antimicrobial effect principally from berberine, while *Holarrhena antidysenterica* (*Kutaja*)^[10] and *Embelia ribes* (*Vidanga*)^[11] contain phytochemicals that inhibit microbial growth. *Piper longum* (*Pippali*)^[12] also exhibits antimicrobial potential due to essential oils and piperine derivatives. Collectively, these findings support the role of plant derived phytochemicals in the development of novel antimicrobial agents.

Up to date pharmaceutic advancements, such as ointment formulations, can improve ease of application and sanative delivery compared to tralatitious oils.^[13] Therefore, in the present study, *Bruhat marichyadi taila* was prepared according to definitive methods and further converted into a solid ointment using low paraffin, stale paraffin, and beeswax. The objective of the study was to compare the antimicrobial efficacy of the tralatitious oil formulation (Sample A) with its ointment form (Sample B) *in vitro*. Antimicrobial activity was evaluated against representative skin pathogens *S. aureus*, *E. coli*, *C. albicans*, and *A. niger* using the agar well diffusion method.

MATERIALS AND METHODS

Materials

Bruhat marichyadi taila was formulated from *Murchita Katu taila* (mustard oil) and a wide spectrum of herbal and mineral ingredients. Table 1 lists the composition of the oil. *Gomutra* (cow's urine) was also used as per classical procedure. For the ointment, bases included soft paraffin, beeswax, and hard paraffin blended with *Bruhat marichyadi taila* [Table 2]. All herbs and reagents were procured and authenticated from standard Ayurvedic suppliers. The composition and quantities of the ingredients used for the preparation of Bruhatmarichyadi Taila are presented in Table 1, while the individual ingredients used in the formulation are depicted in Figure 1.

Formulation of *Bruhat Marichyadi Taila* (Oil)^[1]

Taila siddha procedure

1. An iron vessel was taken for the preparation process
2. 540 mL of *Murchita Katu taila* was taken as the base oil [Table 1]
3. The necessary volume of *Gomutra* was added to the base oil
4. *Kalka* was added to the mixture, prepared from entirely powdered ingredients (total 264 g, [Table 1])
5. The mixture was heated on a low flame and until the smooth *Gomutra* content had entirely evaporated
6. Upon reaching the end point, the prepared oil was filtered through a cloth
7. All *Taila Siddhi Lakshanas* were achieved
8. The prepared oil sample was sent to the laboratory for an antimicrobial study.

The sequential steps involved in preparing Bruhatmarichyadi Taila, including the addition of ingredients, heating, and completion of the preparation, are shown in Figure 2.

Observation of *Sneha Pāka Lakṣaṇas*^[15]

Varti pariksha

The *Kalka* transformed into a soft, pliable mass that could be easily rolled between the fingers to form a *varti* (wick-like mass).

Shabda pariksha

When the *varti* was placed over fire, it did not produce any cracking sound, confirming proper *Sneha Pāka*.

The observed *Sneha Pāka Lakṣaṇas*, including *Varti Pariksha* and *Shabda Pariksha*, confirming the proper completion of *Sneha Pāka*, are depicted in Figure 3.

Table 1: Proportion of contents for preparation of *Bruhat marichyadi Taila*^[1,14]

S. No.	Dravya	Botanical name	Part used	quantity
1.	<i>Murchit katu taila</i>	<i>Brassica juncea</i> (L) Czern. and Coss.	Seeds	540 mL
2.	<i>Maricha</i>	<i>Piper nigrum</i> Linn.	Fruit	8 g
3.	<i>Snuhi ksheer</i>	<i>Euphorbia neriifolia</i>	Latex	8 g
4.	<i>Gomutra</i>	<i>Cow urine</i>	-	2480 mL
5.	<i>Jyotishmati</i>	<i>Celastrus paniculatus</i>	Seeds	8 g
6.	<i>Dantimool</i>	<i>Baliospermum montanum muell.</i>	Roots	8 g
7.	<i>Arka ksheer</i>	<i>Calotropis procera</i>	Latex	8 g
8.	<i>Shakrudrasa (Gobar rasa)</i>	<i>Cow dung juice</i>	-	8 g
9.	<i>Devdaru</i>	<i>Cedrus deodara</i>	Heartwood and Bark	8 g
10.	<i>Haridra</i>	<i>Carcuma longa</i> Linn.	Rhizome	8 g
11.	<i>Daruharidra</i>	<i>Berberis aristata</i>	Stem, Root	8 g
12.	<i>Jatamansi</i>	<i>Nardostachys jatamansi</i>	Root	8 g
13.	<i>Kushtha</i>	<i>Saussurea costus</i> (Fale) Lipsch.	Root	8 g
14.	<i>Chandan</i>	<i>Santalum album</i> Linn.	Wood	8 g
15.	<i>Indrayana</i>	<i>Citrullus colocynthis</i>	Root	8 g
16.	<i>Karveer</i>	<i>Nerium indicum</i> Mill.	Root	8 g
17.	<i>Shudha hartal</i>	<i>Arsenic trisulphide</i>	-	8 g
18.	<i>Shudha Mansheela</i>	<i>Arsenic disulphide</i>	-	8 g
19.	<i>Chitrak</i>	<i>Plumbago zeylanica</i>	Root	8 g
20.	<i>Shudha langali</i>	<i>Gloriosa superba</i> Linn.	Tuberous Root	8 g
21.	<i>Khadira</i>	<i>Acacia catechu</i> Willd.	Bark and Heartwood	8 g
22.	<i>Vidang</i>	<i>Embelia ribes</i>	Fruits	8 g
23.	<i>Chakramard</i>	<i>Cassia tora</i>	Seeds	8 g
24.	<i>Shirish</i>	<i>Albizia lebbbeck</i>	Bark	8 g
25.	<i>Kutaja</i>	<i>Holarrhena antidysenterica</i> (Linn.)	Bark	8 g
26.	<i>Nimba</i>	<i>Azadirachta indica</i> A. Juss.	Leaves	8 g
27.	<i>Saptaparna</i>	<i>Alstonia scholaris</i>	Bark	8 g
28.	<i>Trivrutt</i>	<i>Operculina turpethum</i>	Root	8 g
29.	<i>Guduchi</i>	<i>Tinospora cordifolia</i>	Stem	8 g
30.	<i>Amlavetas</i>	<i>Garcinia pedunculata</i>	Fruit	8 g
31.	<i>Karanja</i>	<i>Pongamia pinnata</i> (L.) Pierre	Seeds, leaves, bark, roots	8 g
32.	<i>Pippali</i>	<i>Piper longum</i>	Root	8 g
33.	<i>Vacha</i>	<i>Acorus calamus</i> Linn.	Rhizome	8 g
34.	<i>Shudha vatsanabh</i>	<i>Aconitum ferox</i>	Root	16 g

Table 2: Proportion of contents for preparation of *Bruhat marichyadi ointment*^[16]

S. No.	Ingredients	Quantity
1.	<i>Bruhat marichyadi taila</i>	50 mL
2.	Soft paraffin	31.25 g
3.	bees wax	2 g
4.	Cetosteryl alcohol	2 g
5.	Hard paraffin	3 g
6.	Volatile oil	QS

The composition and quantities of the ingredients used for the preparation of Bruhatmarichyadi Ointment are presented in Table 2, and the individual components of the ointment formulation are shown in Figure 4.

Formulation of *Bruhat Marichyadi Ointment*^[17]

1. The prepared *Bruhat marichyadi Taila* was gently heated using a water bath
2. The required base ingredients, beeswax, soft paraffin,



Figure 1: Contents for preparation of *Bruhat marichyadi taila*



Figure 1: (Continued)

cetosteryl alcohol, and hard paraffin, were added in the specified proportions [Table 2]

3. The mixture was continuously stirred until all the base components were completely melted and uniformly blended with the oil
4. The finished ointment was then poured into containers, followed by proper labelling
5. The prepared ointment sample was sent to the laboratory for antimicrobial study.

The sequential formulation process of Bruhatmarichyadi Ointment, including melting and blending of the ointment

base with Bruhatmarichyadi Taila followed by filling into containers, is shown in Figure 5.

Experimental Study

Antimicrobial study (in vitro)

The *in vitro* antimicrobial activity of *Bruhat marichyadi taila* (Sample A) and its ointment formulation, *Bruhat marichyadi ointment* (Sample B), was evaluated using the agar well diffusion method against microorganisms commonly associated with skin infections. The test organisms included



Figure 2: Preparation of *Bruhat marichyadi taila*



Figure 3: *Sneha paka lakṣaṇas*

Gram-positive bacteria (*S. aureus*, ATCC 6538), Gram-negative bacteria (*E. coli*, ATCC 8739), and fungal strains (*C. albicans*, ATCC 14053 and *A. niger*, ATCC 11414).

Sterile agar plates were inoculated with standardized microbial suspensions. Wells measuring 6–8 mm in diameter were aseptically bored into the agar medium. The test samples were prepared at a concentration of 10 mg/mL using an appropriate solvent, and volumes of 100 μ L and 200 μ L were introduced into the respective wells. The plates were incubated under suitable conditions for bacterial and fungal growth for 48–72 h.

Following incubation, antimicrobial activity was assessed by measuring the zones of inhibition in millimetres (mm). All experiments were performed once under identical conditions, along with appropriate positive and negative controls. Standard antibacterial and antifungal drugs,

namely streptomycin and fluconazole, were procured from authenticated pharmaceutical suppliers and used as positive controls in the study.

Ethical Consideration

This study did not involve any human participants or experimental animals; therefore, ethical approval was not required.

RESULTS

Tables 3 and 4 summarize the zones of inhibition (in mm) produced by Sample A (*Bruhat marichyadi taila*) and Sample B (*Bruhat marichyadi ointment*) against the four test microorganisms. Sample B consistently exhibited larger inhibition zones than Sample A at both concentrations tested. For *S. aureus*, Sample A produced zones of 1 mm and 2 mm (100 μ L and 200 μ L, respectively); Sample B demonstrated increased inhibition zones of 4 mm and 6 mm at 100 μ L and 200 μ L concentrations, respectively [Figure 6]. Similarly, against *E. coli*, Sample A showed only 1–2 mm, whereas Sample B showed 4–8 mm. For the fungi, *C. albicans* was inhibited 6–10 mm by Sample A and 10–15 mm by Sample B. *A. niger* showed 8–11 mm zones with Sample A versus 15–17 mm with Sample B. No inhibition was seen in negative controls. The standard antibiotics (streptomycin for bacteria, fluconazole for fungi) produced much larger zones. These results demonstrate that the ointment formulation had greater antimicrobial activity than the oil alone against all strains tested.^[18]



Figure 4: Contents for preparation of *Bruhat marichyadi* ointment

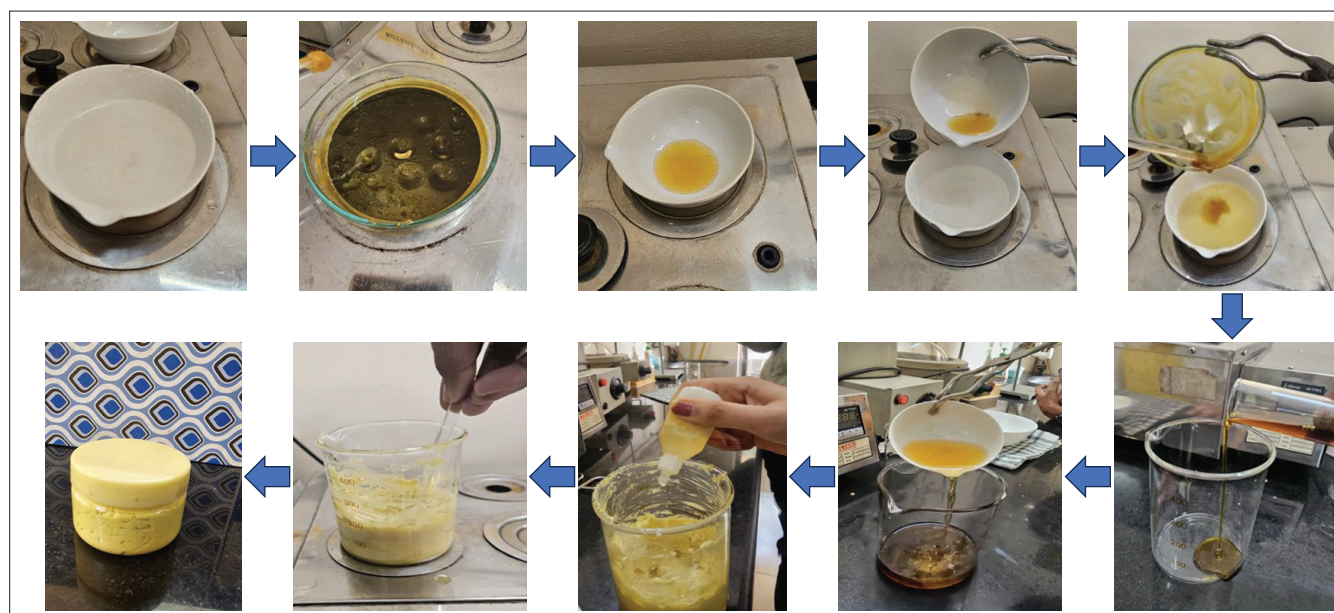


Figure 5: Formulation of *Bruhat marichyadi* ointment

Table 3: Zone of inhibition (mm) of *Bruhat marichyadi taila* (Sample A) and *Bruhat marichyadi* ointment (Sample B) against test organisms. Data from agar well diffusion

Sr. No	Parameter	Control (DMSO)	Standard streptomycin	Sample A (<i>Bruhat marichyadi taila</i>)		Sample B (<i>Bruhat marichyadi</i> ointment)	
1.	Concentration	-	1 mg/mL	100 μ L	200 μ L	100 μ L	200 μ L
2.	Zone of inhibition (mm) <i>Staphylococcus aureus</i>	-	25	01	02	04	06
3.	Zone of inhibition (mm) <i>Escherichia coli</i>	-	35	01	02	06	08

Table 4: Zone of inhibition (mm) of *Bruhat marichyadi taila* (Sample A) and *Bruhat marichyadi* ointment (Sample B) against test organisms. Data from agar well diffusion

Sr. No.	Parameter	Control (DMSO)	Standard fluconazole	Sample A (<i>Bruhat marichyadi taila</i>)		Sample B (<i>Bruhat marichyadi</i> ointment)	
1.	Concentration	-	1 mg/mL	100 μ L	200 μ L	100 μ L	200 μ L
2.	Zone of inhibition (mm) <i>Candida albicans</i>	-	29	06	10	10	15
3.	Zone of inhibition (mm) <i>Aspergillus niger</i>	-	32	08	11	15	17

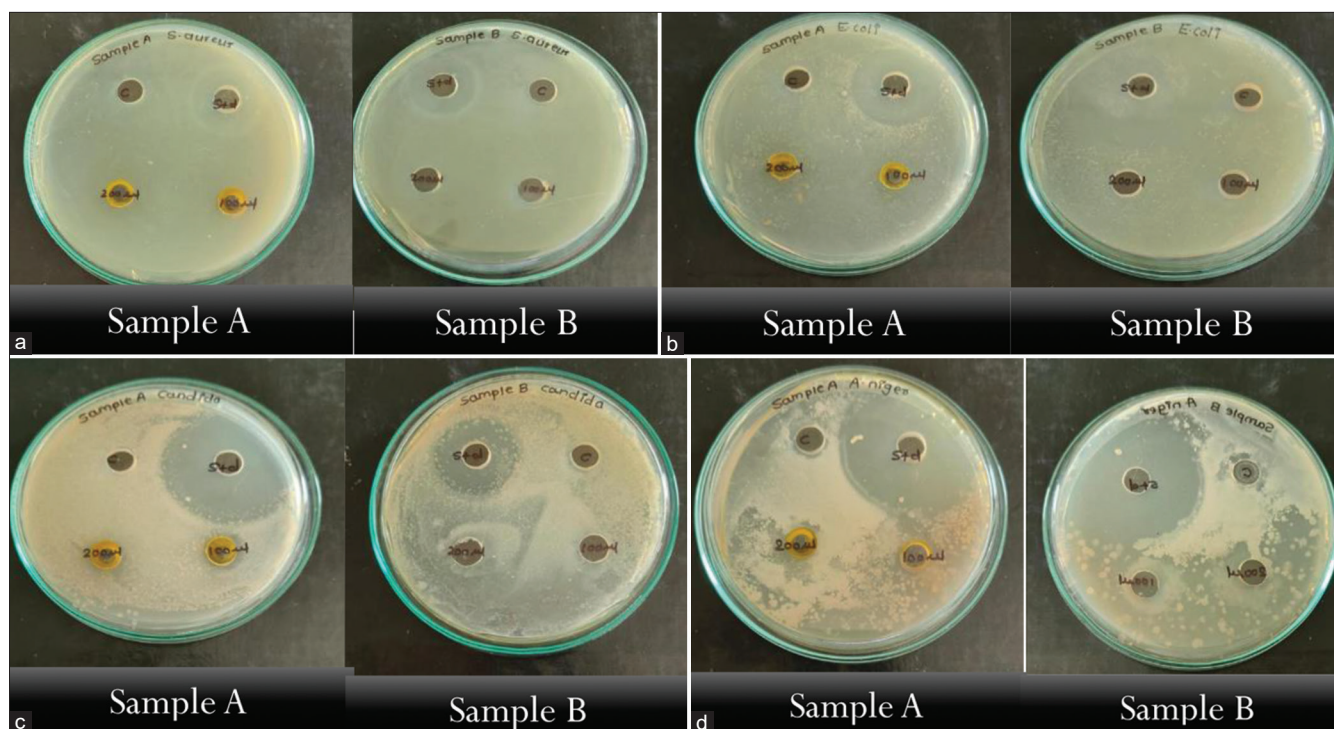


Figure 6: (a) *Staphylococcus aureus* ATCC no. 6538 (b) *Escherichia coli* ATCC no- 8739 (c) *Candida albicans* ATCC No- 14053 (d) *Aspergillus niger* ATCC No- 11414. The antimicrobial activity of *Bruhat marichyadi taila* (Sample A) and *Bruhat marichyadi ointment* (Sample B) against test organisms

DISCUSSION

The findings of the present study demonstrate that the ointment formulation of *Bruhat marichyadi taila* (Sample B) exhibited superior *in vitro* antimicrobial activity compared to the oil formulation (Sample A) against Gram-positive (*S. aureus*), Gram-negative (*E. coli*), and fungal pathogens (*C. albicans* and *A. niger*). Sample A showed bottom antibacterial activity and relatively mild antifungal activity, whereas Sample B produced big zones of inhibition against entirely tested microorganisms, indicating improved antimicrobial potential following conversion into an ointment dosage form.

The increased activity of the ointment formulation may be attributed to several pharmaceutical and physicochemical factors. The ointment base containing low paraffin, stale paraffin, and beeswax may facilitate improved dispersion and uninterrupted release of existent phytoconstituents into the agar medium, thereby allowing chronic contact with the test microorganisms. In contrast, the pasty nature of the oil formulation may have limited its diffusion within the agar matrix, resulting in relatively infernal antimicrobial activity.

Mustard oil, a pivotal component present in both formulations, contains allyl isothiocyanate, which is known for its antibacterial and antifungal properties. Although this constituent contributes to the antimicrobial activity of both

formulations, the ointment matrix may provide improved retention and delivery of existent constituents. Furthermore, beeswax and paraffin act as stabilizing agents that may help reduce degradation of phytoconstituents and improve formulation stability.

A pivotal observation of the study was the relatively greater antifungal activity exhibited by both formulations. This observation correlates with the traditional Ayurvedic indication of *Bruhat marichyadi taila* in fungal skin disorders such as *Dadru Kushtha* (ringworm). In addition, the mild antibacterial activity observed against *S. aureus* and *E. coli* suggests mathematical supportive use in localized bacterial skin infections and dirty wounds.

From a pharmaceutical perspective, formulation stability is a pivotal consideration in local preparations. Although medicated oils (*Taila*) possess intrinsic stability, prolonged exposure to light, heat, and moisture may contribute to aerobic degradation over time. Conversion into an ointment formulation may improve physicochemical stability by reducing exposure to environmental factors.

The solid nature of the ointment formulation also offers hard nosed advantages such as ease of application, improved retention at the site of administration, reduced chances of leakage, and better tolerant acceptability. These characteristics may enhance its suitability as a local dosage form.

The present study was limited to a preliminary *in vitro* evaluation performed under exclusive experimental conditions. Further studies involving continual trials, advanced microbiological investigations, stability studies, and *in vivo* evaluation are required to validate the sanative potential of the formulation.

Overall, the findings suggest that conversion of *Bruhat marichyadi taila* into an ointment formulation may improve antimicrobial activity, formulation stability, and local applicability. The study supports the relevance of definitive Ayurvedic formulations while highlighting the importance of pharmaceutical modification for improved sanative utility.

CONCLUSION

The present study concludes that the ointment formulation of *Bruhat marichyadi taila* demonstrated relatively better *in vitro* antimicrobial activity than the oil formulation against Gram-positive, Gram-negative, and fungal pathogens. The improved activity may be attributed to increased dispersion, sustained release of existent phytoconstituents, and improved physicochemical stability, making the formulation further right for local application compared to the traditional oil preparation.

The relatively higher antifungal activity observed in the study supports the definitive Ayurvedic indication of *Bruhat marichyadi Taila* in *Kushtha* (skin disorders), peculiarly fungal infections such as *Dadru Kushtha* (ringworm). The mild antibacterial activity also suggests mathematical supportive use in localized bacterial skin infections and *Dushta Vrana* (infected wounds).

Furthermore, the ointment formulation offers hardnosed advantages, including ease of application, improved retention at the site of administration, and better tolerant acceptability. However, further studies involving continual experimental trials, advanced microbiological investigations, stability studies, and nonsubjective evaluation are required to establish the sanative efficacy and safety of the formulation.

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