AYUSHDHARA
ISSN: 2393-9583 (P)/ 2393-9591 (O)
An International Journal of Research in AYUSH and Allied Systems

Research Article

ANTIFUNGAL ACTIVITY OF *BHURJAH* (BETULA UTILIS D. DON) BARK IN HUMAN PATHOGENIC FUNGI

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Article info

Article History:
Received: 16-05-2024
Accepted: 17-06-2024
Published: 10-07-2024

KEYWORDS:
Antifungal activity, *Bhurjah*, *Betula utilis D.Don,* Birch oil, Inhibition zone, MIC.

ABSTRACT

Aim: To evaluate the antifungal activity of *Bhurjah* (*Betula utilis* D.Don) bark against six fungal strains. Water extract, alcohol extract of bark and essential oil (Birch oil) were screened against six fungal strain *Trichophyton rubrum* (7859), *Epidermophyton floccosum* (7880), *Microsporum fulvum* (7685), *Aspergillus flavus* (2590), *Actinomucour elengi* (963) and *Candida albicans* at four different concentrations (25µl, 50µl, 75µl, 100µl) using "Agar well diffusion method". The MIC was tested using micro-PDA dilution method at concentrations ranging from (25µl, 50µl, 75µl, 100µl). *Bhurjah* essential oil showed significantly more inhibitory activity than fluconazole in case of 7880 (p value <0.05) and almost similar inhibitory activity as fluconazole in case of 7685 (p value >0.05). *Bhurjah* essential oil is shown to be effective against the growth of both *M. fulvum* with MIC value of 2.5% (v/v) and *E. floccosum* with MIC value of 2.5% (v/v) as determined by microbroth dilution. *Bhurjah* essential oil showed antifungal activity against two fungal strains *E. floccosum* (7880), *M. fulvum* (7685). Therefore *Bhurjah* essential oil can be used as antifungal agents for dermatophytosis causing strains i.e. *E. floccosum* (7880), *M. fulvum* (7685).

INTRODUCTION

Ayurveda is one of the most recognized traditional medical systems that has endured and grown over the centuries. The resources of plant, animal, metal, and mineral origin found in the Materia Medica of Ayurveda have been prescribed for therapeutic purposes. According to reports, people around the world, including those in nations in the Asia Region, have been using traditional medical systems for healthcare for centuries. Traditional remedies have gained more attention from the public recently for many different kinds of purposes.[1]

Pathogenic microorganisms such as bacteria, fungi, algae and viruses are the reason behind various dread illnesses in humans and other animals. Fungal infections are the most prominent one ranging from mild infection on skin to severe life-threatening infections in bloodstreams and organs. Dermatophytes (three genera: *Trichophyton, Epidermophyton and Microsporum*) are fungi that grow on keratin causing various infections. Fungus can cause various infections such as tinea pedis (athlete's foot), rashes, itching or scaling on skin, nails, hair and white coating or patches on mucous membrane known as thrush in mouth and throat. Some other pathogenic fungi responsible for skin infections are *Candida* causing cutaneous candidiasis and mucormycosis caused by *Actinomucor*. These fungi are majorly opportunistic that invade when the body's natural defense system is weak. Thus, immunocompromised patients are at greater risk of getting infected by opportunistic pathogens.[2]

Antimicrobial resistance is an important facet that WHO is concerned about.[3] Antimicrobial resistance (AMR) is the ability of a microorganism (like bacteria, viruses, and some parasites) to stop antimicrobials from working against them. As a result, standard treatments become ineffective, new resistant microorganisms are spreading globally, threatening

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https://doi.org/10.47070/ayushdhara.v11i3.1576

our ability to treat common infectious diseases.[4] As a result, it is necessary to assess the antifungal activity of herbal medicine due to the growing antifungal drug resistance that has been observed against the current therapeutic regimen. At present time, as per need of hour, herbal based antifungal drugs may be beneficial for mankind.

Commonly referred to as Himalayan birch, *Bhurjah* is also known as *Bhojpatra*. *B. utilis D. Don*, a member of the Betulaceae family. The forest vegetation, which extends to an altitude of 4200 meters, is capped by the *Bhurjah* trees, which are present throughout the main Himalayan range.[5] It is a medium-sized tree that can reach a height of 20 meters. *Bhurjah* (*Bhojpatra*), also known as the stem bark of *B. utilis*, was used as paper in ancient times for writing literature. It has been reported to contain betulin, lupeol, oleanolic acid, acetyloleanolic acid, betulic acid, lupenone, sitosterol, methyl betulonate and other essential oils which possess medicinal properties.[6]

Herbal compounds are the source of numerous therapeutic agents. *Bhurjah* (*B. utilis*) is described as *Bhootrakshakara, Bhootghana, Rakshashghanash, Kushtha* in Ayurvedic literature hence it may be evaluated as antifungal drug against different strain of fungus (full detail regarding this plant was explained in my previous published article). [7]

Pentacyclic triterpenoid such as Betulin, Lupeol, Oleonolic acid and β-sitosterol+ are natural bioactive compounds present in the bark of birch trees that exhibit antimicrobial activities.[8] Betulin majorly found in the betulaceae family show effective activity against bacteria *Escherichia coli*, *Staphylococcus aureus* and *Enterococcus faecalis* and *Candida*.[9] Lupeol shows antibacterial activity against gram-positive bacteria and antifungal activity against *Aspergillus*.[10] Oleonolic acid present in free acid form or triterpenoid saponins shows antimicrobial properties against human bacterial pathogens *Staphylococcus*, *Bacillus*, *Enterococcus* and *Pseudomonas*.[11,12] Oxygenated terpenoids such as caryophyllene oxide found in *B. utilis D. Don* essential oil is reported to have antimicrobial effect and are more active due to their hydrogen bonding capability with water and thus show more microbial inhibition.[13] Triterpenoids are active compounds known to act against microbes by causing squandering of bacterial membrane structuring with damaging effects like DNA binding, mitochondrial disruption and CDP-choline pathway. These are the secondary metabolites of plants with low toxic effects to eukaryotic cells, which favors the use of these metabolites or plants producing them as antimicrobial medications.[14,15]

MATERIALS AND METHODS

Collection and Identification of Plant and Essential Oil

The bark of the *B. utilis* D. Don was collected from Chamoli district, Uttarakhand state, India. The plant was identified and authenticated by RHMD, CSIR-NISCAIR with Ref. No. NISCAIR/RHMD/Consult/ 2021/4137-38 at CSIR- National Institute of Science Communication and Policy Research, Raw Material Herbarium and Museum, Delhi. The birch essential oil, batch no HI/essential oil/23031501 used in this study was acquired from Hiya India Biotech Pvt. Ltd, New Delhi, India. The purity of *Bhurjah* essential oil is 90%, refractive index at 20°C is 1.4997 and specific gravity at 25°C is 0.926.

Preparation of Ethanol and water Extract

To prepare ethanol and water extract, about 500mg, coarse powder of *Bhurjah (B. utilis*) bark was macerated with 6ml of ethanol and distilled water in two different closed glass tubes for 72 hours (3 days), shaking frequently. It was filtered for screening of antifungal activity.

Test Organisms

Fungal strains *T. rubrum* (MTCC 7859), *E. floccosum* (MTCC 7880), *M. fulvum* (MTCC 7685), *A. flavus* (MTCC 2590) and *A. elengi* (MTTC 963) were purchased from Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India and *Candida albicans* from ACBR University of Delhi, for the testing of the antifungal effect of *Bhurjah (B. utilis* D. Don*)* bark and essential oil.

The fungal strains used in this study are human pathogenic fungi and were stored at 4°C and subcultured once in a month.

Assessment of Antifungal activity

The fungal growth inhibiting activity of water extract, alcohol extract and essential oil of *Bhurjah* was determined by well-diffusion assay. Fungal suspensions were grown overnight in PDA broth at 37°C. 100µl of cultures were spread evenly over the entire agar surface using a sterile glass spreader. After drying the plates for 15-20 min, wells were made into the agar plates. Then different concentrations ranging from 25µl, 50µl, 75µl, 100µl of water extract, alcohol extract and essential oil of *B. utilis* were added to respective wells. The plates were left for some time to let the test samples diffuse well into the agar. Then, the plates were incubated at 37°C. Antifungal activity was detected by measuring the zone of inhibition that appeared after the incubation period. The assessment has been taken after experimenting 3 times.

Minimum Inhibitory Concentrations

From the antifungal activity of birch oil, *M. fulvum* (MTCC 7685) and *E. floccosum* (MTCC 7880) were selected for evaluation. The minimum inhibitory concentration was determined using the broth dilution method in 96-well. Fungal suspensions were grown overnight in Potato Dextrose Agar (PDA) broth. Five different dilutions of concentrations of microbial culture with birch essential oil ranging from 0 to 100µl inoculated in 900µl PDA broth were kept in incubation for 24 hrs at 37°C. Anti-fungal agent fluconazole stock solution 0.005mg/ml in DMSO was taken as positive controls. Diluted series i.e., well in which broth without microorganism were poured served as negative control and microbial control i.e. well having broth with microorganism only was taken as positive control. The MIC values were evaluated spectro-photometrically at 600nm wavelength.

Statistical Analysis

The experiments were repeated three times and the statistical analysis (p-value) calculated using the T-test using Microsoft Excel 2016 Software.

Results

Determination of Antifungal Activity

Water extract and alcohol extract of *Bhurjah* (*B. utilis*) bark showed no significant antifungal activity against these fungal strains. The *Bhurjah* essential oil showed antifungal activity against *M. fulvum* (MTCC 7685) and *E. floccosum* (MTCC 7880) fungal strains in well diffusion assay determined by well-diffusion method and the zone of inhibition was enumerated using Image J software (as shown in Fig. 2).

Table 1: Area of inhibition (cm²) as indicator of antifungal activity of *B. utilis* (100µl) essential oil against six fungal strains

Strain MTCC no.	Area of Inhibition (mm²) 100µl of essential oil
T. rubrum	-
M. fulvum *E. floccosum*	304.123 ± 2.078 127.86 ± 0.171
A. flavus *A. elengi* *C. albicans*	- - -

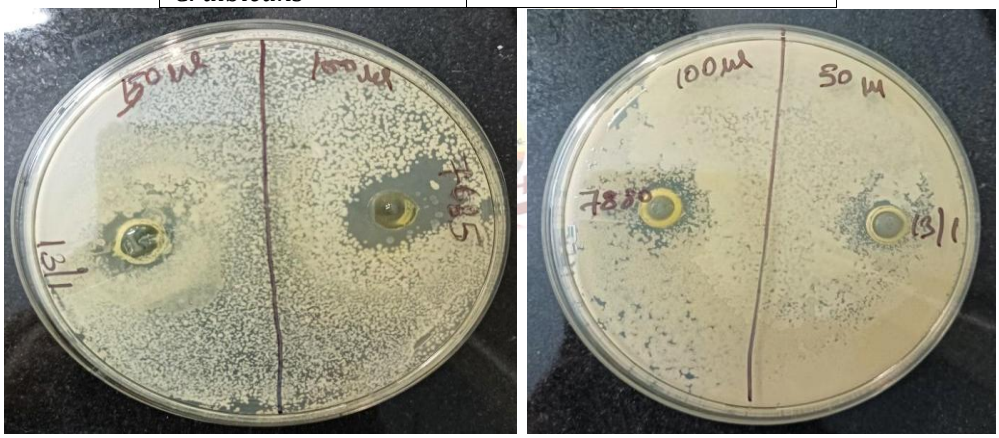

Fig. 2: Effect of *B. utilis* essential oil determined by zone of inhibition in well diffusion assay for (A) MTCC 7685 and (B) MTCC 7880 fungal strains

MIC value determination

The Minimum Inhibitory Concentration (MIC) of *B. utilis* essential oil was determined against fungus *M. fulvum* (MTCC 7685) and *E. floccosum* (MTCC 7880) along with comparative evaluation against standard antifungal medication fluconazole.

Table 2: IC50 value (µl/ml) of *B. utilis* essential oil and fluconazole against fungus MTCC 7685 and MTCC 7880

Microorganism	IC50 value (v/v)	
	Essential oil	Fluconazole
M. fulvum	<0.025	<0.025
E. floccosum	<0.025	>0.1

In this study, *Bhurjah* essential oil is shown to be effective against the growth of both *M. fulvum* with MIC value of 2.5% (v/v) and *E. floccosum* with MIC value (v/v) for both as determined by microbroth dilution. The

comparative analysis of fluconazole and *Bhurjah* essential oil fungal growth inhibition activity was determined for both *M. fulvum* and *E. floccosum.* As for the essential oil activity in comparison to fluconazole, essential oil shows significantly more inhibitory activity than fluconazole in case of 7880 (p value <0.05) and almost similar inhibitory activity as of fluconazole in case of 7685 (p value >0.05) is shown in fig. 3 for MTCC 7880 and in fig. 4 for MTCC 7685.

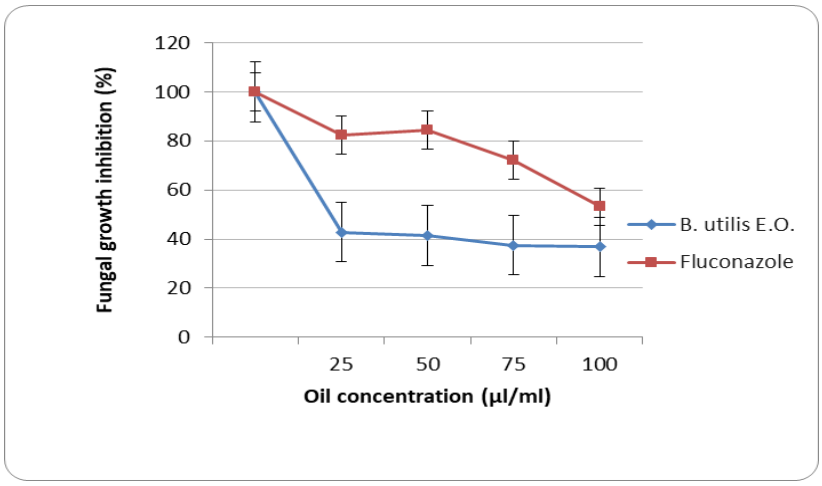

Fig. 3: Effect of *B. utilis* essential oil at four concentrations against *E. floccosum* (MTCC 7880)

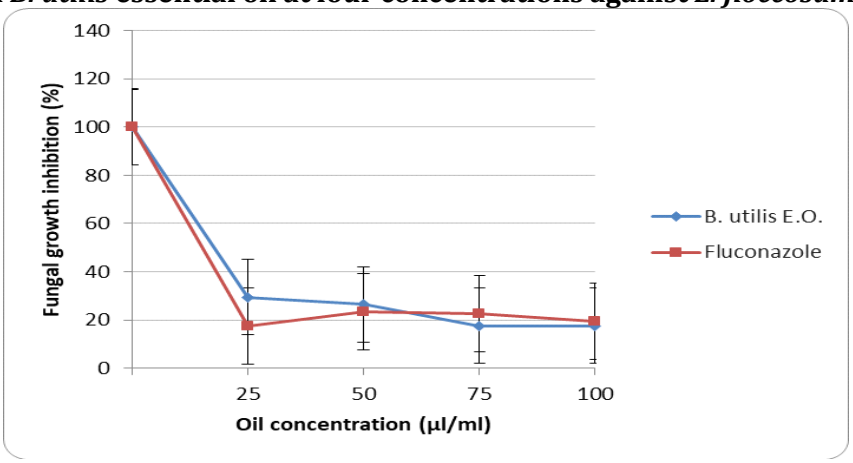

Fig. 4: Effect of *B. utilis* essential oil at four concentrations against *M. fulvum* (MTCC 7685)

DISCUSSION

Essential oils are a type of volatile oil made up of a number of volatile and non-volatile elements that are extracted from plants and can be used as powerful weapons against pathogenic microbes (bacteria, fungi, and viruses) as well as provide defense against insects and herbivores. Essential oil components like terpenes/terpenoids disrupt cell membrane, lead to cell death and inhibit sporulation and germination in fungi. The inhibiting effect of different essential oils on the genes and proteins responsible for motility, structural integrity and virulence in various treated microorganisms have also been reported. Essential oil also decreases synthesis of enzymes involved in the process of invading the host tissue and pathogenicity by affecting the gene expression or degrading the proteins. By down-regulating the genes and hyphal cell wall proteins responsible for biofilm, essential oils are able to inhibit quorum sensing and biofilm formation.[16,17]

In this study, water extract, alcohol extract and essential oil of *Bhurjah (B. utilis)* were screened against six pathogenic fungal strains *T. rubrum*, *E. floccosum*, *M. fulvum*, *A. flavus*, *A. elengi* and *C. albicans.* The water and alcohol extracts obtained from *Bhurjah (B. utilis)* bark show no significant activity against pathogenic fungal strains and *Bhurjah* essential oil exhibited activity against two dermatophytes *E. floccosum* and *M. fulvum*. The components of essential oil were determined by GC-MS, found to be a complex mixture of various terpenes and other compounds. The majorly present active secondary metabolites present were terpenes which are reported to be present in other Betula species also, among terpenes caryophyllene, a dominantly present compound is more active and have been shown to have antimicrobial properties. Other components such as δ-cadinene, pinene, humulene and murrolene are reported to have antimicrobial effects. The overall activity of essential oil is combinatorial and cannot be attributed to a single metabolite.

CONCLUSION

In conclusion, result suggest that essential oil from *B. utilis* bark possesses antifungal activity and can be used as an effective antifungal agent against two dermatophytes *i.e. E. floccosum*, *M. fulvum*.

Acknowledgements

Authors are thankful to Dr. Rajesh Sharma, HOD of Department of Dravyaguna, A & U Tibbia college, Karol Bagh, New Delhi, for encouragement and support. Author Sandhya Pal gratefully acknowledges the fellowship received from the Department of Biotechnology (DBT/2022-23/CBR/2146). Dr. Meenakshi Sharma would like to extend gratitude to the financial support of a grant received from ICMR, Government of India, Delhi, [No./ICMR/52/06/2022-BIO/BMS].

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Cite this article as:
Monika Kunwar, Shalini Varshney, Sandhya Pal, Meenakshi Sharma, Yogita Munjal. Antifungal Activity of Bhurjah (Betula Utilis d. Don) Bark in Human Pathogenic Fungi. AYUSHDHARA, 2024;11(3):143-147.
https://doi.org/10.47070/ayushdhara.v11i3.1576
Conflict of interest: None Declared

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