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In Vitro Evaluation of Antifungal Potential of Balliospermum montanum (Wild.) Muell. Arg. Against Dermatophytes

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Abstract:

Fungal infections are one of the most difficult diseases to manage in humans. Eukaryotic fungal pathogens have many similarities with their host cells, which hinders the development of antifungal compounds. Therefore, it is desirable to exploit the pharmaceutical potential of medicinal plants to discover antifungal drugs. In this study, the antidermatophytic activity of water, ethanol, chloroform and hexane extracts of Baliospermum montanum leaves and stem was investigated against selected dermatophytic fungi i.e. Trichophyton verrucosum, Trichophyton rubrum, Epidermophyton floccosum and Microsporum canis by poisoned food technique. Four solvents, two polar (water and ethanol) and two non-polar (chloroform and hexane) were used to perform the extraction. Out of four solvent extracts ethanol extract of leaf and stem showed 23±0.57mm zone of inhibition and 04.16±0.44% growth inhibition, while stem showed 18±0.88mm zone of inhibition and 33.33±0.60% growth inhibition against Trichophyton rubrum. Most of the samples exhibited good antidermatophytic activity.

Keywords: Antidermatophytic activity, Dermatophytic fungi, Plant extracts, Dermatophytosis, Baliospermum montanum.

Introduction:

The incidence of dermatophytes has increased dramatically in the last 10 years. Hot and humid climate, working conditions and other predisposing factors play an important role in increasing the infection in humans, but the development of strains resistant to various antifungal drugs is also an important factor in increasing the spread of the infection (Ayatollahi and Kazemi, 2015). Over time, griseofulvin, oral imidazoles, ketoconazole, oral azoles fluconazole, itraconazole and topical allylamines such as terbinafine, butenafine and naftifine have been widely used to treat dermatophytosis (Matsuda *et al.*, 2016). Fluconazole, itraconazole and terbinafine are effective in treating dermatophytosis when used for systemic treatment (Vandeputte *et al.*, 2012). Despite the availability of a wide range of antifungal agents, treatment failures have been widely reported. This may be multifaceted and the reasons are the severity of dermatophytosis, causative agents, immunocompromised patients and some antifungal drugs can alter blood levels, inadequate drug administration and discontinuation of therapy (Bhatia and Sharma, 2016).

These antifungal agents are effective for the treatment of dermatophytosis but these antifungal agents also have many side effects on the patients as these drugs require long treatment to cure and some dermatophyte species acquire resistance to antifungal drugs after prolonged use. Therefore, there is a need to find new cost-effective alternatives with good

antidermatophytic activity and are not harmful to human health. Traditional medicine has been known to treat various human diseases for centuries in many parts of the world without harmful effects. Several studies have found that some medicinal plants are very useful in treating various skin diseases including dermatophytes, because these medicinal plants are natural, therefore low cost, high availability, low side effects and valuable resources (Sharma and Malik, 2015). Medicinal plants are a good source of low cost phytochemicals, which have high availability and low side effects (Ayatollahi and Kazemi, 2015).

Balliospermum montanum (Willd.) Muell.- Arg. is a sensitive medicinal plant of the family Euphorbiaceae. The roots, leaves and seeds of the plant are used medicinally. The root contains phorbol esters related to dipteran hydrocarbons such as montanin, balliospermine, 12-deoxyphorbol 13-palmitate, 12-deoxy-5-hydroxyphorbol 13-myristate and 12-deoxy-16-hydroxyphorbol 13-palmitate. The leaves contain 8-sitosterol, 8-D-glucoside and hexacosamol. The presence of steroids, terpenoids and flavonoids has also been reported in the plant (Pasqua *et al.,* 2009). The root is astringent, thermogenic, purgative, helminthic, carminative and anti-inflammatory. It is useful in stomachache, constipation, calculus, piles, helminthic manifestations, scabies, skin disorders, wounds and jaundice (Shama *et al.,* 2000). The paste of the roots is applied to painful swellings and piles. The leaves cure asthma and bronchitis. They are also used for dropsy. The seeds are astringent, rubifacient, hydragogue and stimulant which are useful in inflammation and flatulence. The seeds are also used in snakebite (Warrier *et al.,* 1996). The plant is used for the treatment of stomach tumors and cancer (Sivarajan and Balachandran, 1988). The alcoholic extract of *B. montanum* stimulates cell-mediated immunity by increasing neutrophil function (Wadekar *et al.,* 2008).

This commonly grown plant has a wide range of medicinal properties and has been found to be effective against some dermatophytes such as *Trichophyton rubrum*, *Epidermophyton floccosum*, *Microsporum annanum* and *Trichophytonus violaceum* (Mahmoud *et al.,* 2011). Therefore, the present study is based on the investigation of antidermatophytic activity of *Balliospermum montanum*. This is a natural product, cost-effective, environmentally friendly, and an alternative herbal treatment for dermatophytes (Sandeep and Prabhjot Kaur, 2023).

Materials and Methods:

Plant collection and preparation:

Plant materials were collected from various locations in Nanded District. The plant material was washed with water and air dried under shade and further dried in the air-drying oven at 50°C for 8hrs., then ground to a fine powder using a laboratory scale mill. Ten grams powder was macerated for 48h in 100mL of water, ethanol, chloroform and hexane. Each solvent extraction was repeated 3 times and the extracts were further filtered through qualitative filter paper and the solvent was then removed with a rotary evaporator, and stored at 4°C until tested and analysed. The extracts were redissolved in DMSO (dimethyl sulfoxide) at the concentration of 5%.

Microbial testing:

Fungal strains:

Trichophyton verrucosum, *Trichophyton rubrum*, *Epidermophyton floccosum* and *Microsporum canis* were cultured on Mycosel agar (Sabouraud's dextrose agar), then incubated at 27°C for 14-21 days. For preparation of spore suspensions, spores were harvested from fresh slants cultured, by adding 10 ml of

0.1% sterile non-ionic surfactant (0.01% of NSS-Tween 20) to the culture slant. The slant was vortexed for 5 min and the resulting suspension was filtered through four layers of sterile gauze.

Poison food technique:

Plant extracts were added in 20 ml of sterilized potato dextrose agar in petri plates. A 5mm diameter of the actively growing mycelium disc of the pathogen of 6-7 day old culture was placed in the center of the Petri dish. Plates without plant extract served as negative control. Plates were incubated at 270C. Radial growth of mycelium was measured after seven days of incubation. The results were compared with negative control. The percent inhibition of the fungus in treatments was calculated using following formula;

$$L = [(C-T)/C] \times 100$$

Where, L is the percent inhibition; C-Control and T is the colony radius in the presence of plant extracts.

Statistical analysis:

The results will be reported as mean ± standard error (SE), and the experiments will be replicated three times (n=3).

Results:

Antidermatophytic activity by poison food technique:

The antifungal potential of of *Baliospermum montanum* leaf and stem against four dermatophytic fungi i.e. *Trichophyton verrucosum*, *Trichophyton rubrum*, *Epidermophyton floccosum* and *Microsporum canis* was evaluated against fungal strains in water, ethanol, chloroform and hexane solvent by using poison food technique.

Table 1: The Antifungal potential of *Baliospermum montanum* leaf and stem extracts against *T. verrucosum*.

Sr. No.	Solvent extracts	Leaf		Stem	
		Zone of inhibition (mm)	Inhibition (%)	Zone of inhibition (mm)	Inhibition (%)
1.	Control	23±0.88	00±0.00	23±1.33	00±0.00
2.	Water	00±0.00	99±0.54	00±0.00	99±0.33
3.	Ethanol	05±0.57	78.26±0.61	20±1.20	13.04±0.66
4.	Chloroform	05±0.00	78.26±0.44	16±0.28	37.50±0.88
5.	Hexane	21±0.00	8.69±0.60	09.±0.44	60.86±0.45

Results presented here are the mean values from three independent experiments. Mean ± S.E.

Table 2: The Antifungal potential of *Baliospermum montanum* leaf and stem extracts against *T. rubrum*.

Sr. No.	Solvent extracts	Leaf		Stem	
		Zone of inhibition (mm)	Inhibition (%)	Zone of inhibition (mm)	Inhibition (%)
1.	Control	24±0.33	00	24±0.33	00
2.	Water	12±0.66	50.00±0.44	12±0.57	50.00±0.66
3.	Ethanol	23±0.57	04.16±0.44	18±0.88	33.33±0.60
4.	Chloroform	08±0.88	66.66±0.37	09±1.20	60.86±1.00
5.	Hexane	15±0.57	37.50±0.64	15±1.15	37.50±0.44

Results presented here are the mean values from three independent experiments. Mean ± S.E.

Table 3: The Antifungal potential of *Baliospermum montanum* leaf and stem extracts against *M. canis.*

Sr. No.	Solvent extracts	Leaf		Stem	
		Zone of inhibition (mm)	Inhibition (%)	Zone of inhibition (mm)	Inhibition (%)
1.	Control	23±0.44	0.0	23±1.04	0.0
2.	Water	20±0.33	13.04±0.31	19±0.76	17.39±0.75
3.	Ethanol	10±0.10	56.52±0.88	05±1.25	78.26±0.37
4.	Chloroform	21±0.57	08.69±0.41	17±0.66	26.08±0.28
5.	Hexane	22±1.20	04.34±0.60	20±0.44	13.04±0.37

Results presented here are the mean values from three independent experiments. Mean ± S.E.

Table 4: The Antifungal potential of *Baliospermum montanum* leaf and stem extracts against *E. floccosum.*

Sr. No.	Solvent extracts	Leaf		Stem	
		Zone of inhibition (mm)	Inhibition (%)	Zone of inhibition (mm)	Inhibition (%)
1.	Control	24±0.57	0.0	24±1.33	0.0
2.	Water	16±0.120	33.33±0.68	16±0.57	33.33±0.22
3.	Ethanol	12±0.88	50.00±0.66	13±0.88	45.83±0.44
4.	Chloroform	16±1.15	33.33±0.44	14±0.66	41.66±0.76
5.	Hexane	14±0.66	41.66±0.28	07±0.33	70.83±0.60

Results presented here are the mean values from three independent experiments. Mean ± S.E.

The in vitro antifungal activity of water, ethanol, chloroform and hexane extracts of *Baliospermum montanum* leaf and stem against dermatophytic fungi i.e. *Trichophyton verrucosum* was evaluated and presented in Table 1,2,3 and 4. The fungal strains were tested on all solvent extracts of leaves and stems, and the antifungal activity of the tested plant extracts was compared with the control. The percent inhibition growth is measured and recorded. Each test was performed three times and then the corresponding mean value was taken and tabulated in the above table. Zones of inhibition were measured in millimeters.

The water extracts of *Baliospermum montanum* leaf and stem showed no zone of inhibition against *Trichophyton verrucosum* and 99±0.54% inhibition of growth. It is followed by the ethanol and chloroform leaf extracts i.e. 05±0.57mm and 05±0.00mm zone of inhibition against *Trichophyton verrucosum* and showed 78.26±0.61% growth inhibition. The ethanol extract of the stem showed a 20±1.20 mm zone of inhibition against *Trichophyton verrucosum.* It is followed by the chloroform and hexane stem extracts i.e. 16±0.28mm and 09.±0.44mm zone of inhibition Most of the samples exhibited good antifungal activity. Antifungal potential of *Baliospermum montanum* leaf and stem extracts against *T. verrucosum* is depicted in Figure 51.

The hexane extracts of *Baliospermum montanum* leaf and stem showed maximum inhibition i.e. 22±1.20mm and 20±0.44mm zone of inhibition and 56.52±% and 78.26±0.37% inhibition of growth respectively against *M. canis*. It is followed by the chloroform extract of leaf and stem i.e. 21±0.57mm 17±0.66mm zone of inhibition against *M.*

canis and showed 08.69±0.41% and 26.08±0.28% growth inhibition respectively.

The ethanol extract of leaf and stem showed 23±0.57mm zone of inhibition and 04.16±0.44% growth inhibition, while stem showed 18±0.88mm zone of inhibition and 33.33±0.60% growth inhibition against *Trichophyton rubrum*. The chloroform extracts of *Baliospermum montanum* leaf and stem showed 08±0.88mm and 09±1.20mm zone of inhibition against *Trichophyton rubrum* and, 66.66±0.37% and 60.86±1.00% inhibition of growth respectively.

The water extracts of *Baliospermum montanum* leaf and stem showed 16±0.120mm and 16±0.57mm zones of inhibition, and 33.33±0.68 inhibition of growth respectively against *E. floccosum*. It is followed by the chloroform and hexane extract of leaf and stem against *E. floccosum*.

Discussion:

The aim of this study was to evaluate the antidermatophytic potential of *Balliospermum montanum*, focusing on its leaf and stem extracts. Dermatophytes are an important cause of skin infections, which often require antifungal treatments that can have varying degrees of efficacy and toxicity (Sardana *et al.*, 2018). It is of utmost importance to explore plant based remedies such as *Balliospermum montanum* to identify new, potentially safe antifungal agents (Hassan Alhazmi *et al.*, 2021).

The results of this study indicate that extracts of leaves and stems of *Balliospermum montanum* exhibited promising antifungal activity (Suresh *et al.*, 2018) against dermatophytes, including species such as *Trichophyton rubrum* and *Microsporum canis*. The ethanol extract of leaf and stem showed maximum 23±0.57mm zone of inhibition and 04.16±0.44% growth inhibition, while stem showed 18±0.88mm zone of inhibition and 33.33±0.60% growth inhibition against *Trichophyton rubrum* (Raveesha *et al.*, 2019). This observation supports the traditional use of this plant in folk medicine for the treatment of skin diseases including fungal infections (Shubha *et al.*, 2021). The antifungal efficacy observed in the study may be due to the presence of biologically active compounds such as flavonoids, alkaloids, saponins and tannins, which are known to exhibit antimicrobial properties investigated by Shamsudin *et al.*, 2022; Ullah *et al.*, 2020.

Interestingly, the leaf extract showed slightly higher fungicidal activity than the stem extract, which may be due to the higher concentration of active compounds in the leaves. The efficacy of the leaf extract is consistent with previous reports on other plants, where compounds obtained from the leaves exhibit stronger fungicidal properties than those obtained from other plant parts. This suggests that the leaves may be a more viable source for developing antifungal agents (Narasimharaju *et al.*, 2015).

However, the study also found that although the plant extracts were effective against dermatophytes, they were less effective than standard antifungal drugs such as Miconazole (for *Trichophyton verrucosum* and *Microsporum canis*) and Metronidazole (for *Trichophyton rubrum* and *Epidermophyton floccosum*). This highlights the need for further optimization of the extraction process and the possibility of combining *Balliospermum montanum* extracts with other antifungal agents to enhance their therapeutic efficacy (Cherian *et al.*, 2015). In addition, it would be beneficial to investigate the mechanism of action of these extracts to understand how they inhibit fungal growth, as well as any potential side effects or toxicity at high concentrations.

Further studies should explore the in vivo efficacy of leaf and stem extracts to confirm their therapeutic potential. Topical application of these extracts in clinical

settings will provide valuable insights into their practical utility. In addition, there is a need to standardize extract preparations to ensure consistency in terms of potency and reproducibility of results.

Conclusion:

In conclusion, extracts of the leaves and stems of *Balliospermum montanum* show significant antidermatophytic potential, which supports their use in traditional medicine. Despite showing less potency than conventional antifungal treatments, these extracts may serve as complementary or alternative treatments, especially in the context of emerging fungal resistance to standard drugs. Further research into pharmacological properties, optimal dosages, and formulations for human use is needed to fully explore their potential as antifungal agents.

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