

EVALUATION OF ANTIFUNGAL ACTIVITY OF AQUEOUS AND ALCOHOLIC  
EXTRACT OF *BILWADI AGAD* AGAINST SOME FUNGAL STRAINSKumari Anupama\*<sup>1</sup>, Tiwari R. C.<sup>2</sup>, Sharma Ved Bhushan<sup>3</sup>, Tiwari Shashikant<sup>4</sup><sup>1</sup>MD Scholar at UAU in Agadtantra. Rishikul campus Haridwar (UK).<sup>2</sup>Professor and H.O.D at UAU in Agadtantra.<sup>3</sup>Assistant Professor at UAU in Agadtantra.<sup>4</sup>Assistant Professor at UAU in Rognidan.

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## ABSTRACT

This study was carried out with an objective to investigate the antifungal potentials of aqueous and alcoholic extract of bilwadi agad. The aim of the study is to assess the antifungal activity and to determine the zone of inhibition of extracts on some fungal strains. In the present study, the antifungal activity of aqueous and alcoholic extracts of bilwadi agad was evaluated against medically important fungal strains. The antifungal activity was determined in the extracts using well diffusion method. The antifungal activities of aqueous and alcoholic extracts (5, 10, 15 % concentration) of bilwadi agad were tested against *Candida albicans*, *Microsporum canis*, *Microsporum fulvum*, *Trichophyton rubrum*. Zone of inhibition of extracts were compared with Ketoconazole. The results showed that the remarkable inhibition of the fungal growth was shown against the tested organisms. We conclude from this that these extracts exhibit amazing fungicidal properties that support their traditional use as antiseptics. Hence, bilwadi agad can be used as natural products to fight with fungal infections.

**KEYWORDS:** *Bilwadi Agad*, antifungal activity, aqueous extract, alcoholic extract, well diffusion method, zone of inhibition.

## INTRODUCTION

Fungi are universal in the environment, and infection due to fungal pathogens has become more common. In the past few decades, a dramatic increase in fungal infections occurrence has been noted across the world due to the presence of resistant fungi to different fungicides used in medicinal practice.<sup>[1]</sup> Also Very few antifungal agents are known till now, and their continuous use have run to the development of resistance by fungal species, some shows ineffectiveness toward fungal disease.<sup>[2]</sup> The spread of multidrug-resistant strains of fungus and the reduced number of drugs available make it necessary to discover new classes of antifungals from natural products including medicinal plants. The World Health Organization estimates that plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population.<sup>[3]</sup> Ayurvedic System of Medicine has its long history of healing potential. *Ayurveda* is already well accepted and used since thousand years. There are many ayurvedic formulations which are claimed to be antifungal in nature. These preparations contains many plants in it so that the effect of the preparation becomes faster and more effective. And here is the formulation that can be used as a natural antifungal agent named bilwadi agad.

Bilwadi agad is an ayurvedic formulation mentioned in *Asthang Hridayam*,<sup>[4]</sup> and *Asthang Sangrah*.<sup>[5]</sup> Bilwadi agad contains 13 ingredients - Bilwa moola, sursa pushp, karanj phal, tagar moola, suraha twak (devdaru), vyosh (sunthi, maricha, pippli), nisha dwya (haridra, daruharidra), phal triya (bibhitaki, haritaki, amalki) and aja mutra as bhavana dravya.

बिल्वस्यमूलंसुरसस्यपुष्पफलंकरंजस्य नतंसुराहम् |

फलत्रयं व्योषनिशाद्वयंच बस्तस्य मूत्रेण सुसूक्ष्मपिष्टम् ||

भुजंगलूतोन्दुरुवृश्चिकाद्यै विषुचिकाजीर्णगरज्वरैश्च |

आर्तान् नरान् भूतविधार्षितांश्च

स्वस्थीकरोत्यन्जनपाननस्यैः || (अ.स.42/87-88)

(अ.ह.36/84-85)

Most of the ingredients of bilwadi agad shows *kitaghana* property, such as *bilwa*, *devdaru*, *haridra*, *pippli*, *maricha* etc. so further detailed study of antifungal property of *bilwadi agad* was required. hence present study was done to evaluate the antifungal activity of *bilwadi agad* using well diffusion method.

## AIMS AND OBJECTIVES

To evaluate the antifungal effect of *Bilwadi agad* on standard fungal strains.

## MATERIAL AND METHOD

### Collection of drug

All the ingredients of *bilwadi agad* (100gm) were collected each in pure form from their natural habitat. The main ingredient of *bilwadi agad* like *bilwamoola*, *sursapusphmanjari* were collected from Rishikul campus Haridwar in winter season. *Karan phal*, *triphala*, *sunthi*, *maricha*, *pippli* and *tagar* were bought from pannalal store Haridwar. *Suraha* and *Daru haridra* were collected from Dhanaulty in Uttarakand. *Haridra* was collected from Himachal Pradesh, 7 litres of goat urine was collected from farmer house in Haridwar. All the ingredients of *bilwadi agad* were identified and verified by imminent experts of *Dravyaguna* Dept. at Rishikul Campus-Haridwar Uttarakhand Ayurved University.

### Preparation of drug

Powder of ingredients of *bilwadi agad* was prepared at Hans Pharmacy Sidcul Haridwar. The powder of all these ingredients were mixed thoroughly, the process of *bhavana* with *ajamutra* were carried out in *Rasashastra* Department at Rishikul campus Haridwar. Total 7 *bhavana* was given to prepare *bilwadi agad*. The obtained *bilwadi agad* was black brownish in colour with smell of *ajamutra*.

## ANALYTICAL STUDY

### Preparation of Extract

Test sample: Aqueous and Alcoholic Extract of *Bilwadi Agad* had been prepared by reflex extraction method. For aqueous extraction - 20 gram of *bilwadi agad* was added to 200 ml of solvent (distilled water) and kept in reflex extractor for 1 hour. Filter it with filter paper and residue was kept in water bath until it reaches to dry form. From this process 3.6 gram dry extract was obtained.

Now from the dry extract, we took 500 mg, 1 gram, 1.5 gram of dry extract and 10 ml of water is added in each from which 5%, 10% and 15% concentration of aqueous extract of *bilwadi agad* was obtained.

For alcoholic extraction -20 gram of *bilwadi agad* was added to 200 ml of solvent (methanol) and put it in reflex extractor for 1 hour. Filter it with filter paper and residue was kept in water bath until it reaches to dry form .from this process 3 gram extract was obtained.

10 ml of methanol was added to 500 mg, 1 gram, 1.5 gram of dry extract respectively from which 5%, 10% and 15% concentration of alcoholic extract of *bilwadi agad* was obtained.

## Antifungal Study

### Methods

#### (1) Selection and collection of pathogens

For the present study a four strains i.e *Candia albicans* (MTCC: 227), *Microsporium canis*(ATCC: 36299), *Microsporium fulvum* (MTCC: 2837), *Trichophyton rubrum* (MTCC: 3272) were selected. The pathogenic strains of different species of bacteria were procured from 'Institute of Microbial Technology' (IMTECH), Chandigarh, ATCC and the stock cultures maintenance & antibacterial study were done at 'Analytical Division of Bilwal Medchem and Research Laboratory Pvt Ltd. Jaipur (Rajasthan) with registration no. 2005/PO/RcBt/S/18/CPSEA.

### Groups design

#### Negative Control

- Distilled water
- Alcohol

#### Positive control

Ketoconazole (10mg/ml) for *Candia albicans*, *Microsporium canis*, *Microsporium fulvum*, *Trichophyton rubrum*.

#### Test groups

- 5 % solution of Aqueous Extract
- 10 % solution of Aqueous Extract
- 15 % solution of Aqueous Extract
- 5 % solution of Alcoholic Extract
- 10 % solution of Alcoholic Extract
- 15 % solution of Alcoholic Extract

Well diffusion method (Murray et al., 1995 later modified by Olurinola, 1996) was used to screen the antifungal activities of different solvent extracts. Muller Hilton agar solution was prepared and poured into sterile petri dishes. Upon solidification bacteria were spread in zig zag motion with cotton bud. After that, wells were made using a sterile borer (4mm in diameter) into agar plates. Then 5%, 10%, 15 % of aqueous and alcoholic extract along with positive control (ketoconazole) was added to wells respectively. The plates were incubated at 37°C for 18 hours. Antifungal activity was detected by measuring the zone of inhibition appeared after the incubation period. Finally the mean of the above findings was calculated

### Determination of the activity index

The activity index of the sample was calculated as Activity index (AI) =Zone of inhibition of the sample/ Zone of inhibition obtained for standard antifungal drug.

## OBSERVATIONS

The results of various antifungal activity of fungal strains are shown in table 1 while table 2 shows the activity index of fungal strains and fig. 1 shows zone of inhibition (mm) with different fungal strains.

### Interpretation of Antifungal Study

- Antifungal activity of aqueous and alcoholic extract of *Bilwadi agad* on different strains (*Candida albicans*, *Microsporum canis*, *Microsporum fulvum* and *Trichophyton rubrum*) was found at 5%, 10%, 15% concentration :
- Inhibition was absent on 5% concentration of aqueous extract of *Bilwadi agad*.
- Alcoholic extract of *Bilwadi agad* at 5% concentration was found 11, 14, 13 mm zone of inhibition and it was biological active because the activity index of this sample found more than 0.5 i.e., *Microsporum canis*(0.50), *Microsporum fulvum*(0.50) and *Trichophyton rubrum*(0.50) . But this was non active against *Candida albicans*(0.39)
- Aqueous extract of *Bilwadi agad* at 10% concentration was found biologically active against all the fungal strains i.e *Candida albicans*(0.50), *Microsporum fulvum* (0.60), *Trichophyton rubrum* (0.64) and non active against *Microsporum canis* (0.48)
- Alcoholic extract of *Bilwadi agad* at 10% concentration of was found biological active against all the fungal strains. The activity index was *Candida albicans* (0.57), *Microsporum canis*(0.59), *Microsporum fulvum*(0.68), *Trichophyton rubrum* (0.77).
- Aqueous extract of *Bilwadi agad* at 15 % concentration showed activity index of *Candida albicans*(0.57), *Microsporum canis*(0.52), *Microsporum fulvum*(0.52), *Trichophyton rubrum*(0.77) , which means *Bilwadi agad* at 15% aqueous concentration was active against all the fungal strains and showed significant result.
- Alcoholic extract of *Bilwadi agad* at 15% concentration was highly active against all the fungal strains. i.e *Candida albicans*(0.71), *Microsporum canis*(0.66), *Microsporum fulvum* (0.76), *Trichophyton rubrum*(0.86)

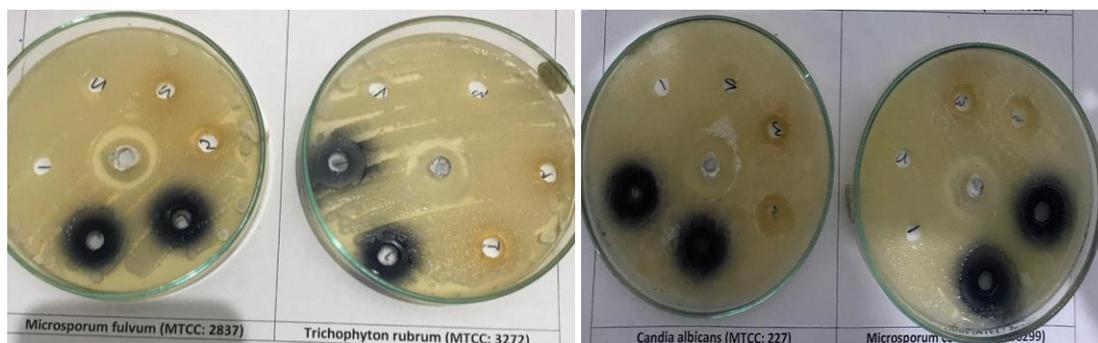


Fig. 1: Showing Zone of Inhibition (mm) with different Fungal strains.

Table 1: Antimicrobial activity of water and alcohol extract of *Bilwadi agad* on different strains Zone Of Inhibition (mm).

Microorganisms	-ve Control		Aqueous Extract			Alcoholic Extract			+ve control (ketakenazole)
	Water	Alcohol	5%	10%	15%	5%	10 %	15 %	
<i>Candida albicans</i>	5	5	12	14	16	11	16	20	28
<i>Microsporum canis</i>	5	5	12	14	15	14	17	19	29
<i>Microsporum fulvum</i>	5	5	13	15	13	14	17	19	28
<i>Trichophyton rubrum</i>	5	6	11	14	17	13	17	19	26

Table 2: Activity Index of Antimicrobial activity of water and alcohol extract of *Bilwadi agad* on different strains.

Microorganism	Aqueous extract			Alcoholic extract		
	5 %	10 %	15 %	5 %	10 %	15 %
<i>Candida albicans</i>	0.43	0.50	0.57	0.39	0.57	0.71
<i>Microsporum canis</i>	0.41	0.48	0.52	0.48	0.59	0.66
<i>Microsporum fulvum</i>	0.46	0.60	0.52	0.50	0.68	0.76
<i>Trichophyton rubrum</i>	0.42	0.64	0.77	0.50	0.77	0.86

### DISCUSSION

*Bilwadi agad* showed potent antifungal activity, most of the ingredients of *Bilwadi agad* have antifungal properties. *Cedrus deodera*,<sup>[6]</sup> *Terminalia chebula*,<sup>[7]</sup> *Zingiber Officinale*,<sup>[8]</sup> *Piper longum* Linn,<sup>[9]</sup> *Ocimum*

*sanctum* Linn,<sup>[10]</sup> all are well known for their antifungal activity. Other study revealed that *Terminalia chebula* also has antifungal action against a number of dermatophytes,<sup>[11]</sup> Also in another study Water extract of *cedrus* showed sensitive inhibition against *Candida*

albicans and *Aspergillus niger*,<sup>[12]</sup> Majority of the drugs of bilwadi agad are tikta (bitter), katu (pungent) rasa pradhan which acts as kapha-vatahara (pacifies kapha & vata). All drugs are ushna veerya (hot potency), majority are katu vipaka hence can act as vishgna and keetaghna.

## CONCLUSION

The present study affirms the *in vitro* antifungal potential of aqueous and alcoholic extract of the *bilwadi agad* with results comparable to ketoconazole. In this study 5,10 and 15% concentration of aqueous and alcoholic extract of *bilwadi agad* was evaluated for its antifungal activity against following human pathogenic strains- *candida albicans*, *microsporium canis*, *microsporium fulvum* and *trichophyton rubrum*. Both type of extract of *bilwadi agad* have shown a dose dependent antifungal effect. Alcoholic extract of *bilwadi agad* have shown better result in comparison to aqueous extract. Aqueous extract with 10% concentration have shown positive result against all fungal strains except *microsporium canis*, while 15% concentration of aqueous extract of *bilwadi agad* have shown positive results against all the fungal strains used in this study. Alcoholic extract with 5% concentration showed positive results in case of both *microsporium fulvum* and *trichophyton rubrum*. Alcoholic extract with 10% and 15% concentration of *bilwadi agad* have shown positive result against all the fungal strains. Present study has confirmed the antifungal potentials of our preparation supporting its application may be as preventive remedy for various fungal diseases. This study will help us to understand the importance of traditional medicines in the treatment of different fungal disease. Also Further studies are needed to determine the chemical identity of the bioactive compounds responsible for the observed antifungal activity. Natural plant-derived fungicides may be a source of new alternative active compounds.

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