



## Antifungal activity of Spices against *Candida albicans*

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### ABSTRACT

*Candida* species are normal commensals and are isolated intra-orally in 17% - 75% of healthy individuals and all debilitated people. Eradication of candidiasis is complicated by the emergence of *Candida* strains that are resistant to the currently used antifungal agents. Spices as remedies are gaining popularity in the world today. Although many species have already been investigated against *Candida albicans*, the search is still to find long-term prevention or cure for candidiasis. The objective of this study was to evaluate the antifungal activity of cinnamon, and clove against *C. albicans*. Spices obtained from the local market were dried and powdered. Solvent extracts were obtained by adding ethanol followed by filtration and evaporation. The study aims to counter the antifungal action of different flavors/spices against *Candida albicans*, by applying the comparative technique for both the agar well diffusion method and the Disc-diffusion method. The entire research project was carried out at the Microbiology Laboratory of D.A.V. College from January 2022 to April 2022, under the guidance of a supervisor. Extracts retrieved from a total of 5 spices - Bay leaf (*Laurus nobilis*), Black pepper (*Piper nigrum*), Cinnamon (*Cinnamomum verum*), Clove (*Syzygium aromaticum*), and Cumin (*Cuminum cyminum*) showed remarkable antifungal activity against *C. albicans*. The antifungal activity of Clove was even determined the best against pathogenic *Candida albicans* even when compared with the most used antifungal drug i.e., Fluconazole. While analyzing the comparative techniques, the Disc diffusion method seemed to be more useful due to its absorbing capacity. Thus, the study concluded results exhibit the antifungal activity of the spice extracts against *Candida albicans*, which may be useful in the treatment of candidiasis.

### KEYWORDS

Spices, Agar Well Diffusion Method, Disc-Diffusion Method, Antifungal Agents, Candidiasis

### HOW TO CITE THIS ARTICLE

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In today's world, microorganisms have frequently been much of the reason for causing various prevailing diseases that present a serious general medical problem in a critical fragment of the population. The financial crisis, the significant expense of industrialized prescribed drugs and inefficient community access to clinical and drug administrations, notwithstanding the secondary effects brought about by synthetic drugs, are some of the factors contributing to the central role of medicinal plants in health care. *Candida* species are liable for many fundamental as well as superficial opportunistic infections (candidiasis) happening most often in the vaginal or oral mucosa.

High levels of antifungal resistance have been accounted for in *Candida* species, and these have exhibited primary resistance patterns toward medications like nystatin, clotrimazole, fluconazole, itraconazole, and amphotericin B. In the current situation, the rise of numerous medication obstructions in human pathogenic growths and the modest number of antifungal classes of medications accessible have stimulated research directed toward the discovery of novel antifungal agents from other sources, such as traditionally used spices.

For millennia, different regular products have been utilized in conventional medication all around the world and originated before the presentation of anti-microbials and other current medications. As indicated by the WHO, medicinal spices would be the best source for getting an assortment of medications.

The present study aimed to investigate the antifungal properties of different extracts such as Bay leaf (*Laurus nobilis*), Black pepper (*Piper nigrum*), Cinnamon (*Cinnamomum verum*), Clove (*Syzygium aromaticum*), and Cumin (*Cuminum cyminum*) using both Agar Well Diffusion Method and Agar Disc-Diffusion Method against the most common species of *Candida* i.e. *Candida albicans*. Spices not just decrease the possibilities of growth of *Candida albicans* causing disease yet additionally assist the body with emerging from these invulnerable compromised states and lessen the side effects of these sicknesses which consequently restrains the development of *Candida albicans*. A comparative examination of the antifungal properties of these flavors with that of the most used antifungal drugs will give significant data in regards to the antifungal action of spices and their restorative potential focusing on the substitution of normal regular medications.

## Materials and Methods

### Study Site and Duration

The study site is Lalitpur district of Bagmati Province, Nepal. The study was conducted from January 2022 to April 2022.

### Collection of Sample

Seven different varieties of spices named Bay leaf (*Laurus nobilis*), Black pepper (*Piper nigrum*), Cinnamon (*Cinnamomum verum*), Clove (*Syzygium aromaticum*), and Cumin (*Cuminum cyminum*) were collected from the local market of Lalitpur in the month of January.

### Source of Microorganisms

The microorganism used in this study consists of Gram-positive (*C. albicans*). Microorganisms were obtained from the culture collection of Teaching Hospital, Department of Microbiology.

## Study Site

The study was conducted in the Microbiological Laboratory, Department of Microbiology, D. A. V. College from 4<sup>th</sup> January 2022 to 12<sup>th</sup> April 2022.

## Antifungal Susceptibility Assay

Firstly, the stock solution of spices extract was prepared (1000 mg of the spice extract was dissolved in 1ml of 10% DMSO). This concentration of the spice extract was taken to determine the antifungal property. The antifungal property of spices was determined by the agar well diffusion method, disc diffusion method, and 10% DMSO was kept as a control. After proper incubation, the zone of inhibition was noted. After all the above-mentioned procedure was repeated thrice to obtain the concurrent value

## Results and Discussion

Antifungal activity of spice extracts against *Candida albicans* by Agar Well Diffusion Method and Disc Diffusion on SDA and MHA media. Comparatively, ZOI in MHA showed more effective and adequate results than SDA media as the highest ZOI was shown by Clove with 30mm in MHA while in SDA it was only 15mm. Following this Cinnamon and bay leaf with clear ZOI about 32mm and 30mm on MHA media whereas in SDA showing 20mm and 10mm respectively. The least ZOI was shown by black pepper and cumin on both MHA and SDA having 10mm or with complete no ZOI. Comparative antifungal effects shown by spice extract on MHA and SDA are in the table 1 and table 2 shown below:

**Table 1. Antifungal activity of spice extracts against *Candida albicans* by Agar Well Diffusion Methods on SDA and MHA media**

Extracts	SDA		MHA	
	10µl	50µl	10µl	50µl
Clove	9mm	15mm	20mm	36mm
Cinnamon	NZ	20mm	10mm	31mm
Cumin	NZ	NZ	NZ	NZ
Bay Leaf	NZ	NZ	10mm	30mm
Black Pepper	NZ	NZ	NZ	10mm

**Table 2. Antifungal activity of spice extracts against *Candida albicans* by Disc Diffusion Methods on SDA and MHA media**

Extracts	SDA (10µl)	MHA (10µl)
Clove	10mm	20mm
Cinnamon	NZ	10mm
Cumin	9mm	NZ
Bay Leaf	10mm	10mm
Black Pepper	10mm	10mm

**Antibiotic Susceptibility Pattern of *C. albicans***

Antibiotic Susceptibility testing of Fluconazole on MHA and SDA plates both resulted in sensitivity against *Candida albicans* showing ZOI 30mm and 20mm respectively. Antibiotic Susceptibility testing shown by antibiotics is in table 2 shown below:

**Table 3. Antibiotic susceptibility pattern of *C. albicans***

S. N.	Antibiotics	Media used	Diameter of ZOI (mm)	Inference
1.	Fluconazole	MHA	30	S
2.	Fluconazole	SDA	20	S

**Comparison of Antifungal Activity of Spice Extract with Fluconazole**

Fluconazole being the most widely used antibiotic for fungal infection its ZOI is compared with the extracts that showed better and clear ZOI. As Fluconazole showed clear ZOI of 30mm on MHA which stated that it was sensitive against *C.albicans*. While Clove, and Cinnamon showed better ZOI than Fluconazole having 36mm and 32mm respectively. Also, bay leaf shows sensitive ZOI with 30mm as Fluconazole, stating that these spices are effective against *C. albicans*. Comparative antifungal effects shown by antibiotic Fluconazole and spice extract are in table 3 shown below.

**Table 4. Comparison of antifungal activity of spice extract with Fluconazole**

SN	Spice extract	Diameter of ZOI	Antibiotic (mm)	Diameter of ZOI
Cinnamon (50µl)		32	Fluconazole	30
Clove (50µl)		36	Fluconazole	30
Bay leaf (50µl)		30	Fluconazole	30

**Discussion**

Antifungal disease has been arising public health issue in both developed and developing countries. Spice extracts have been used for many thousands of years, in food preservation, pharmaceuticals, alternative medicine, and natural therapies. Thus, it is necessary to investigate those spices scientifically which have been used in traditional medicine to improve the quality of healthcare. Several investigations have been directed towards their antibacterial properties but not many studies investigating the antifungal efficacy of spice extracts have been reported. The present study gives an account of the antifungal activity of solvent extracts of clove, cinnamon, black pepper, cumin, and bay leaf against *C. albicans*. Organic solvent (ethanol) rather than distilled water was used in the preparation of the extracts. The polarity of antimicrobial compounds makes them more readily extracted by organic solvents, and using organic solvents does not negatively affect their bioactivity against microbial species.

Clove demonstrated excellent antifungal activity against *C. albicans*. The antifungal activity of cinnamon may be attributed to the presence of its various phytochemical compounds which inhibits the growth of micro-organisms. Between the two media used Clove extract showed the best result showing 36mm of ZOI in MHA media containing 50µl of the extract by the agar well diffusion method and 15mm ZOI in SDA media containing 50µl of clove extract. Other studies confirmed that among the various powders tested clove has a good range of susceptibility (Kaung, *et al.*, 2011). Dilek Keskin and Sevil Toroglu (2011), Shan, *et al.*, (2009) and Ziwei, *et al.* (2003) reported their results with ethanol extracts of clove showed considerable differences in susceptibility patterns against various bacteria and fungi tested.

Cinnamon also showed great effectiveness against *C. albicans* with 32mm and 20mm ZOI in MHA and SDA media respectively containing 50µl of the cinnamon extract by agar well diffusion method. Whereas ZOI was comparatively low on both the SDA and MHA media having only 10µl of the cinnamon extract by both disc diffusion and agar well diffusion.

Results are in agreement with the studies conducted by (Aneja, *et al.*, 2009) and (Khan, *et al.*, 2009). The activity of cinnamon is due to the presence of cinnamaldehyde, an aromatic aldehyde that inhibits amino acid decarboxylase activity (Wendakoon and Sakaguchi, 1995), and has been proven to be active against many pathogenic bacteria (Suresh, *et al.*, 1992). Black pepper extract showed very little antifungal activity against *C. albicans* having a maximum of 10mm ZOI in MHA media containing 50µl of its extracts by agar well diffusion method and 10mm ZOI on both MHA and SDA media with only 10µl of black pepper extract. The results are consistent with previous research conducted by (Johann *et al.*, 2007, Joe *et al.*, 2009; Sasidharan and Menon, 2010). This concludes that black pepper showed very mild antifungal activity against *C. albicans*. The extracts of *Cuminum cyminum* (jeera) have evoked interest as sources of natural products for their potential uses as alternative remedies to heal many infectious diseases as they contain essential oils.

In the present study, the cumin extracts demonstrated antifungal activity but to a lesser extent than other commonly used spices. However, most of the studies concerning *Cuminum* showed that they hardly show any antifungal activity, which is consistent with the data of the present study. The results were similar to studies conducted by (Pai *et al.*, 2010 and Swapna *et al.*, 2018). The antifungal activity of Bay leaf may be attributed to the presence of certain volatile oil components such as cinnamic aldehyde and eugenol. Bay leaf showed effective ZOI of 30mm in MHA media containing 50µl of its extract by agar well diffusion method but comparatively less ZOI on the other methods used. The results of our study are consistent with the studies conducted by (Yilmaz *et al.*, 2013), which states that bay leaves do have moderate use of antifungal activity against *C. albicans*. In order to determine whether the spices showed considerable anti-fungicidal activity or not, they were compared with the standard chart of antibiotics. Fluconazole was chosen to compare the obtained result as fluconazole is an antifungal medication used for a number of fungal infections. According to the zone diameter of fluconazole, fungi are said to be resistant if ZOI is less than or equal to 14mm and sensitive if ZOI is more than or equal to 19mm.

During this study, Antibiotic Susceptibility testing of Fluconazole on MHA and SDA plates both resulted in sensitivity against *Candida albicans* showing ZOI 30mm and 20mm respectively. The results are consistent with the chart compared and previous research conducted by (Berkow and Lockhart, 2017). Also, no ZOI was seen on both MHA and SDA plates containing DMSO as it was only used to dissolve phytochemical compounds present on spice extract and for better diffusion on the agar medium for which it was referred to as a control. The overall results obtained, showed that the disc diffusion method is more effective as compared with the Agar well diffusion method because the Disc diffusion technique was able to show the zone of inhibition even at low concentration on both MHA and SDA plates, alongside side with less possibility of spilling of concentrate throughout the media and less time consumed for dissemination.

In previous studies, it was also found that both the technique i.e. disc diffusion and agar well diffusion were applied to get the right results yet the fact that majority of the research was done on the disc diffusion method. Comparing the two methods both showed an adequate amount of effectivity, however, the sterilized 6mm filter paper was able to absorb the extract showing productive results may be due to the above-mentioned reason. As SDA media is used as culture media and MHA media is specifically used for Antibiotic susceptibility testing, herein we had added 2% glucose and methylene blue to MHA because glucose serves as the energy source for fungal growth whereas methylene blue inhibits the growth of bacteria also additionally resulting in showing the adequate amount of zone of inhibition. But no other ingredient was added to SDA media due to which MHA media showed a sufficient amount of effectiveness of the spices and organism test could cultivate the right amount of results. In a conclusion, we were able to interpret the fact that adding significant extra fixings to the media improves the zone of inhibition on bringing about legitimate adequacy of spices. Fluconazole being the most widely used antibiotic for fungal infection its ZOI is compared with the extracts that showed better and clear ZOI. As Fluconazole showed clear ZOI of 30mm on MHA which stated that it was sensitive against *C. albicans*.

While Clove and Cinnamon showed better ZOI than Fluconazole having 36mm and 32mm respectively. Also, bay leaf shows sensitive ZOI with 30mm as Fluconazole stating that these spices are effective against *C. albicans*. This study showed that spices are and can be used as an alternative to antibiotics. Hence, the present study represents an alternative source of natural antifungal substances for use in food systems to prevent the growth of infectious diseases also suggesting that further research on the effects of spices on microorganisms can be rewarding to pursue in the search for new broad spectrum antimicrobial agents.

## Conclusion

The present study provides an important basis for the antifungal activity of these 5 spices i.e. Bay leaf (*Laurus nobilis*), Black pepper (*Piper nigrum*), Cinnamon (*Cinnamomum verum*), Clove (*Syzygium aromaticum*), and Cumin (*Cuminum cyminum*). This guided the more pure form of these extracts can be a more effective agent and can be used as an alternative for the treatments of infectious diseases associated with *Candida albicans*.

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## Conflicts of Interest

The authors declare that there is no conflict of interest

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