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## Antimitotic activity of *Piper nigrum* on clinical isolates of candida

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### ABSTRACT

The objective of this study is to analyse the antimycotic activity of pepper on the clinical isolates of *Candida*. The extracts were prepared in the following concentrations in sterile water. 5mg/ml and 10mg/ml and 20mg/ml. 100µl of an extract of different concentrations were loaded on sterile filter paper discs measuring 6mm in diameter, so that the concentration of the extract on each disc was 500µg, 1000 µg and 2000µg respectively. The discs were dried and kept aseptically. Screening of antifungal activity [disc diffusion technique]. The ethanolic extract of *Piper nigrum* was screened for antifungal activity by the disc diffusion method. Effect of three different concentrations (2000, 1000, 500, µg /disc) of the ethanolic extract of *Piper nigrum* was tested against *Candida albicans* using disc diffusion technique. All the concentrations of the test solution inhibited the fungal species with varying degree of sensitivity. The inhibitory zone was measured with respect to the different concentration of the extracts which include 2000 µg/disc and 500 µg/disc at both room temperature and cold temperature (4-8°C). The inhibition zones were evaluated at 24 and 48 hrs. The result of this study revealed that the pepper oil has pronounced inhibitory activities against *Candida albicans*. This result is comparable with studies which have shown that *Piper nigrum* has a broad antibacterial activity. Since *Piper nigrum* is easily available and well-tolerated, it can be incorporated into medication for topical antifungal therapy as well.



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### INTRODUCTION

Due to increased prevalence of drug-resistant microorganisms, there is great need to search for new effective drugs having a natural or synthetic origin. Over the past decade, herbal medicine which has become a topic of augmented global

importance has impacts on both world health and international trade (Khalil Abdullah, Mohamed Soliman *et al.*, 2013). *Candida albicans*, a natural flora, can be found in various body parts of an individual (mouth, gastrointestinal tract and vagina) that grows into a deleterious infection depending upon the medical condition of an individual. Most commonly found infections are the oro-pharyngeal thrush and vaginal yeast infections in women. Patients having AIDS, extensive burn, pregnancy, birth control pills, long-term antibiotic therapy, steroid treatment, organ transplant, immunosuppressant, cancer treatments, genetic disorders, heart surgery, diseases due to endocrine deficiency, tuberculosis infections, diabetes and surgery (involving use of unsterilized needles or catheters) contribute greatly as predisposing factors for candidiasis (Hardeep Kaur, R.R. Goyal *et al.*).

The resistance of pathogenic fungi, including *C. albicans* and non-*C. albicans* species isolated from patient against anti-fungal agents has increased (Khalil Abdullah, Mohamed Soliman *et al.*, 2013). However, since many of the available antifungal drugs have undesirable side effects or are very toxic, produce recurrence, show drug-drug interactions or lead to the development of resistance, some shows ineffectiveness and have become therefore less successful in therapeutic strategies (Rekha Sharanappa and Vidyasagar G.M). *Candida* species are members of the normal microbiota with high prevalence in the normal population and can invade tissue and cause oral Candidiasis or life-threatening disease in patients whose immune defences have been altered by old age, disease or iatrogenic intervention. (Reimer LG, Wilson ML, Weinstein MP. 1997)

Therefore, it has become a necessity to search for alternatives that overcome the disadvantages of antifungal drugs. A number of researchers have reported the antimicrobial effects of various plant extracts against certain pathogens. (Apadopoulos C, Soulti K, Roussis IG. 2005) Wide varieties of plant extracts have antimicrobial and antimycotic effects and also anti-inflammatory (Chaieb K, Zmantar T, Ksouri R, *et al.*, 2007), (Saikia D, Khanuja Sps, *et al.*, 2001). Medicinal plants are renewable, unlike the synthetic drugs that are obtained from non-renewable sources of basic raw materials such as fossil sources and petrochemicals (Samanta, M.K., Mukherjee *et al.*, 2001). Herbal medicines are in great demand in the developed countries primarily for their cost-effectiveness and no side effects. (Phani Kumari Uddandapul, K. Chandrasekhara Naidu *et al.*, 2016). In recent years, the antimicrobial properties of medicinal plants have been increasingly reported in different parts of the world. It is expected that plant extracts demonstrating target sites other than those used by currently available antimicrobials will be active against drug-resistant microbial pathogens (Susana Johann1; Moacir G. Pizzolatti *et al.*, 2007).

Black pepper (*Piper nigrum* L) is a flowering vine of the Piperaceae family that is cultivated for its fruit, which is usually dried and used as a spice and seasoning. In dried form, the fruit is referred to as peppercorns. It is a native of south India and popularly known as "King of Spices. It has been found that *P. nigrum* leaf extract inhibits the growth of *Pseudomonas aeruginosa* describing the antimicrobial activity of volatile oils of black pepper. (S.K. Shiva Rani, Neeti Saxena and Udaysree. 2011) Piperine is a component of pepper which exhibits diverse pharmacological activities like antihypertensive and antiplatelets,

antioxidant, antitumor, antiasthmatics, antipyretic, analgesic, anti-inflammatory, anti-diarrheal, antispasmodic, anxiolytic, antidepressants, hepato-protective, immunomodulatory, antibacterial, antifungal, anti-thyroids, antiapoptotic, anti-metastatic, antimutagenic, anti-spermatogenic, anti-Colon toxin, insecticidal and larvicidal activity (Zoheir A Damanhour1, and Aftab Ahmad 2014). Phytochemicals present in plants having a solubility in ethanol include tannins, polyphenols, polyacetylenes, flavonol, sterols and alkaloids. The antimicrobial activity of black pepper is due to the presence of essential oil (3%), whose aroma is dominated by monoterpenes hydrocarbons: sabinene,  $\beta$ -pinene and limonene. Furthermore, terpinene,  $\alpha$ -pinene, myrcene, and monoterpene derivatives like borneol, carvone, carvacrol, 1, 8-cineol and linalool are also present. The mechanism of action of terpene is not fully understood but is speculated to involve membrane disruption by the lipophilic compounds (Ram Kumar Pundir1, and Pranay Jain. 2010).

The aim of the study is to analyse the antimycotic activity of *Piper nigrum* on clinical isolates of candida.

## MATERIALS AND METHODS

*Candida albicans* was isolated from clinical samples and cultured and maintained on Sabouraud's dextrose agar medium at 30°C. The ethanolic extract of *Piper nigrum* was obtained commercially and used for the study

## METHODOLOGY

The extracts were prepared in the following concentrations in sterile water. 5mg/ml and 10mg/ml and 20mg/ml. 100 $\mu$ l of an extract of different concentrations were loaded on sterile filter paper discs measuring 6mm in diameter, so that the concentration of the extract on each disc was 500 $\mu$ g, 1000  $\mu$ g and 2000 $\mu$ g respectively. The discs were dried and kept aseptically.

### Screening of antifungal activity [this diffusion technique]

The ethanolic extract of *Piper nigrum* was screened for antifungal activity by the disc diffusion method. Activated cultures of *Candida albicans* in Sabouraud's broth was adjusted to 0.5 McFarland standards [108 cfu/ml]. 100  $\mu$ l of the inoculum was introduced to molten Sabouraud's dextrose agar and poured in the sterile Petri plates and allowed to set. Sterile filter paper discs (6.0 mm diameter) impregnated with 2000 $\mu$ g/disc, 1000  $\mu$ g /disc and 500  $\mu$ g /disc of the plant extract dissolved in sterile water were placed on fungal seeded plates and incubated at 28°C for 48 hrs. As a positive control,

Fluconazole (10 mcg /disc) and Amphotericin B (100 units /disc) were used. Following an incubation period of 48 hr, plates were removed from the incubator, and antifungal activity was evaluated by measuring zones of inhibition of fungal growth. Clear zones within which fungal growth was absent were measured and recorded as the diameter (mm) of complete growth inhibition. The whole experiment was performed three times to minimize error.

## RESULTS

Effect of three different concentrations (2000, 1000, 500, µg /disc) of the ethanolic extract Of *Piper nigrum* was tested against *Candida albicans* using disc diffusion technique. All the concentrations of the test solution inhibited the fungal species with varying degree of sensitivity. The antifungal activity of the extract against the fungal strains is shown in Table 1. The extract showed good antifungal activity at different concentrations with a maximum zone of inhibition of 22 mm at concentration 2000µg/ml.

**Table 1: Anti-mycotic activity of *Piper nigrum* against *Candida albicans***

The concentration of the extracts	Zone of inhibition [in mm diameter]
500µg/ml	10
1000µg/ml	16
2000µg/ml	22
Fluconazole (10 mcg /disc)	24
Amphotericin B (100 units /disc)	27

## DISCUSSION

Fungicidal effect of spices may be due to the lysis of fungal cell wall and cytoplasmic membrane due the liberation of antimicrobial products and it was also reported that plant lytic enzymes act on the fungal cell wall causing breakage of B-1,3 glycan, B-1,6, glycan and chitin polymer similar to the study conducted by (Nayak et al) suggesting that the phytochemical properties of the plants are an indication that such a plant has medicinal and physiological activities against microbes. In our study ginger extract was effective in inhibiting the growth of *Candida albicans*. The positive control (Fluconazole, Amphotericin) produced significantly sized inhibition zones with *Candida albicans*. The inhibitory zone was measured with respect to different concentrations of the extracts which included 50microlitres and 100 microlitres and at both room temperature and cold temperature (4-8°C). The inhibition zones were evaluated at 24 and 48 hrs. The results of this study displayed in table1 revealed that the pepper

extract had pronounced inhibitory activities against *Candida albicans*.

The spread of drug-resistant pathogens is one of the most serious threats to successful treatment of microbial diseases (Faazila Fathima, Vishnu Priya V. and Geetha R. V 2011). Medicinal plants represent rich sources of antimicrobial agents used medicinally in different countries (Jannathul ferdioz1, Anitha Roy. 2017). The term spices refer to aromatic or pungent vegetable substances used for flavouring foods and have several commercial uses according to (ISO). Since ancient times people used spices for preventing food deterioration and pathogenic diseases. Spices have become today as an integral part of our daily diet, and many of the spices are widely used to flavour food and beverages, for food preservations, medicinal preparations, cosmetics, perfumery, bakery goods and various other products (1 S.K. Shiva Rani, Neeti Saxena and Udaysreem 2013). Black pepper (*Piper nigrum* L) is a flowering vine. The *Piperaceae* family that is cultivated for its fruit, food which is usually dried and used as a spice and seasoning. In dried form, the fruit is referred to as peppercorns. It is a native of south India and popularly known as "King of Spices". Pepper is most commonly used in curry recipes, as masalas and also included in the prescriptions of ayurvedic and other traditional medicinal systems. Pepper is also used in folk medicine as aphrodisiac, carminative, stomachic, antiseptic diuretic and for the treatment of cough, rheumatoid arthritis, peripheral neuropathy, melanoderma and leprosy due to the presence of volatile compounds, tannins, phenols and other unknown substances (Algothary, M.E.M., B.M. Mahmoud, H.M. *et al.*, 1994; Chiranjib, B., V.S. Narayn, et al 1990; Ali, W.E.M.M., 1995; Park, J.E., H.J. Choi, *et al.*, 2004) *P. nigrum* leaf extract inhibits the growth of *Pseudomonas aeruginosa* describing the antimicrobial activity of volatile oils of black pepper against *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Aspergillus niger*, *Candida albicans* and *Saccharomyces cerevisiae* which was established by the study conducted by (Larhsini *et al.*; Sasidhran, I et al)

This result of our study is comparable with other studies which have shown that pepper has a broad antimicrobial activity. The widespread use of herbal remedies and healthcare preparations, such as those described in ancient texts like the Vedas and the Bible, has been traced to the occurrence of natural products with medicinal properties. Plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. Plants with possible antimicrobial activity should be tested against an appropriate microbial model to confirm the activity and to ascertain the parameters associated with it (M.

Abbas Ali, Noor Mahbub Alam *et al.*, 2007). Since pepper is a commodity that is easily available and extracts also be made easily, it can be incorporated into medications for topical antifungal therapy.

## CONCLUSION

In the study, pepper extract was effective in inhibiting the growth of *Candida albicans*. The positive control (fluconazole, amphotericin B) produced significantly sized inhibition zones with *Candida albicans*. The inhibitory zone was measured with respect to the different concentration of the extracts which include 2000 µg/disc and 500 µg/disc at both room temperature and cold temperature (4-8°C). The inhibition zones were evaluated at 24 and 48 hrs. The result of this study revealed that the pepper oil has pronounced inhibitory activities against *Candida albicans*. This result is comparable with studies which have shown that *Piper nigrum* has a broad antibacterial activity. Since *Piper nigrum* is easily available and well-tolerated, it can be incorporated into medication for topical anti-fungal therapy as well.

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