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Research Article

Isolation of Extracts from Spices and Determination of their Antimicrobial Effects against *Saccharomyces Cerevisiae*

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Abstract

Saccharomyces cerevisiae is commonly known yeast for its role as "baker's yeast", but recently, some strains of it have been reported as an emerging food born pathogen. It can lead to spread of infections in specific organs like lung and blood, especially in immune-compromised patients. The main cause is the multi-drug resistance of the species to the drugs including amphotericin B and azole derivatives. To control the antibiotic resistance of this food born pathogen, different ways have to be explored. There has been a surge in interest in evaluating plants with antibacterial and antifungal activity against variety of common contaminating agents. The biologically active compounds found in various plant parts in the form of spices have evolved the interest of scientists working in this field. The spices like clove, oregano, thyme, cinnamom and cumin possess certain antibacterial and antifungal activities against organisms. This study is conducted to investigate the antimicrobial activity of the extracts of five common spices- black pepper (Piper nigrum), black cardamom (*Amomum subulatum*), cloves (*Syzygium aromaticum*), red chilli (*Capsicum annum*), and cinnamon (*Cinnamom umverum*) against *Saccharomyces cerevisiae*. Out of the five extracts, Piper nigrum showed maximum zone of inhibition of 22 mm diameter against the strain of *Saccharomyces cerevisiae*. So, the black pepper extract can be considered as one of the best antimicrobial agent and an alternative against the diseases caused by *Saccharomyces cerevisiae*. This can be applied further in various medicines and food products to prevent the diseases caused by this yeast.

Keywords: Antimicrobials; antifungal; multi-drug resistance; plant extracts*; Saccharomyces cerevisae*

Introduction

Saccharomyces cerevisiae is a unicellular fungus, possessing nuclear genomic DNA of size 12068 kilobase (kb) organized in 16 chromosomes [1]. There are certain infections caused by *Saccharomyces cerevisiae* investigated in contaminated foods including fungemia, endocarditis, pneumonia, peritonitis, urinary tract infections, skin infections, esophagitis, as well as chronic illness, cancer, and immunosuppression. The multi-drug resistance to the drugs like amphotericin B and azole derivatives in *Saccharomyces cerevisiae* [2] has led to decrease in the treatment of these diseases. The

cause identified for multi-drug resistance in the isolates of *Saccharomyces cerevisiae* is due to changes in the activity of transcription factors that causes overproduction of several target genes [3]. The increased multi drug resistance in the fungal species has led the researchers to think about exploring various plant extracts to be used as antimicrobials.

Literature survey

Plants have become a source of medicinal agents for thousands of years, which have resulted in increased interest in the investigation of various plant extracts as potential sources of new antimicrobial agents. The antimicrobial properties can be found in all parts of the plant, including stem, root, flower, bark and leaves [4,5]. The growing interest in traditional ethnomedicine and antimicrobial capabilities could lead to the identification of new therapeutics and microorganism inhibitors. Many plant species that are endowed with phytochemicals and strong antimicrobial properties have been documented pharmacologically and therapeutically throughout the world [6-8]. One such example is the category of spices.

The spices have been used for food preservation since prehistoric days because they have antiseptic and disinfectant properties [9,10]. The spices like clove, oregano, thyme, cinnamom and cumin possess certain antibacterial and antifungal activities against food spoilage organisms like *Bacillus subtilis*, *Pseudomomas fluorescens*, *Staphylococcus aureus*, *Vibrio parahaemolyticus* and *Aspergillus flavus* [11]. Similarly, the compound allicin present in garlic has shown antimicrobial effects against *Saccharomyces cerevisiae* [12].

In the present study, the antimicrobial activity of aqueous extracts of five different spices including black pepper (*Piper nigrum*), black cardamom (*Amomum subulatum*), cloves (*Syzygium aromaticum*), red chilli (*Capsicum annum*) and cinnamon (*Cinnamom umverum*) against the yeast *Saaccharomyces cerevisiae* has been done.

Material and Methods

Collection of plant material

The fresh samples of plant parts of the spices *Piper nigrum*, *Amomum subulatum*, *Syzygium aromaticum*, *Capsicum annum and Cinnamom umverum* (Table 1) were collected from botanical garden, Noida. These were thoroughly washed with tap water and then rinsed with distilled water. The samples were dried in an oven for 48 hours. After 48 hours, the samples were grounded into fine powder with a pestle and mortar. The powdered material was kept in airtight jars in the refrigerator at 4˚C [13].

Botanical Name	Common Name	Family	Commercial Part Taken
Piper nigrum	Pepper	Piperaceae	Fruit
Ammomum subulatum	Black Cardamom	Zingiberaceae	Fruit/Seed
Syzygium aromaticum	Clove	Myrtaceae	Unopened flower bud
Capsicum annum	Red Chili	Solanaceae	Dried Pod
Cinnamom umverum	Cinnamon	Lauraceae	Bark

Table 1: Collection of Plant parts of the Spices.

Reference culture

Reference culture of *Saccharomyces cerevisiae* (MTCC Y-11857) in liquid form was bought from Microbial Type Culture Collection, IMTECH, Chandigarh to compare the results obtained from the test organism.

Preparation of test organism

Dehydrated yeast powder of *Saccharomyces cerevisiae* (test organism) was bought from Reliance Fresh store, Noida. 0.01 g of powder was scattered on the surface of 50 ml sterile double distilled water in a sanitized jar and kept it for mixing for 15 minutes. The jar was completely sealed with parafilm and was kept in a clean, cool (10-20˚C) and dark place for 72 hours to allow the culture to grow.

Extraction of antimicrobial components from ground plant spices

For extraction of the antimicrobial components from the ground spices, 50 ml of total volume of solvents- formaldehyde, alcohol and diethyl ether was used at the concentration of 60:20:40 respectively in five sterile containers, properly covered to prevent evaporation. 5 grams of each powdered spice was then mixed with the solvents within the sterile containers. The mixture was kept at room temperature (30-35˚C), undisturbed for 24 hours. After 24 hours, the mixture was filtered through sterilized Whatman No.1 filter paper.

Purification and characterization of the filtrate

The obtained filtrate was purified using membrane filter of pore size 0.22 µm. The purified extract from the five ground spices was in different colours. The colours of the extracts were compared with the studies conducted by [15] for the antimicrobial compounds. To confirm the presence of antimicrobial components within the extract, the pH and total soluble solids (TSS) of all the extracts was measured and recorded. The TSS was measured using a hand refractometer (ThermoFischer Scientific, Hyderabad). Then, they were stored in a refrigerator till further use.

Antimicrobial assay of the extract against *saccharomyces cerevisiae*

The antimicrobial assay was carried out using agar well diffusion method [14]. To test the antifungal activity of the prepared extracts, 0.6 ml of standardized fungal stock suspension along with the reference culture was mixed thoroughly with 60 ml of sterile Potato Dextrose Agar (PDA) under sterile conditions. 20ml of the inoculated PDA was distributed in sterile petridishes under sterile conditions. The plates were allowed to dry for 15 minutes. Within every plate, extract wells of 10 mm diameter were created using a sterile cork borer No. 4. To prevent the extracts from diffusing within the agar, the lower parts of the wells were sealed with one drop of sterile PDA. The five different extracts of the spices were poured into three of four wells and the fourth was kept as control with no extract.

Results and Discussion

Purification of the filtrate and characterization of the extract

After purification with the membrane filter, the purified extract from the five ground spices showed different colours. The coloured extracts and their components as compared with [15] are presented in the table 2. To confirm for the presence of antimicrobial components within the extract, the pH and the total soluble solids of the five extracts were measured which are also shown in the table 2.

Antimicrobial assay of the extract against *saccharomyces cerevisiae*

The fungicidal properties of five spice extracts on the test isolate and reference culture of *Saccharomyces cerevisiae* were found positive as mentioned in the table 3 and shown in the figure 1. The values given are the averages of three replicates. Out of the aqueous extracts of five spices, the extract of *Piper nigrum* showed maximum inhibition against *Saccharomyces cerevisiae* with a zone of inhibition of diameter 22 mm, followed by *Capsicum annum*- 21mm, *Syzygium aromaticum*- 20 mm, *Amomum subulatum*- 20 mm and *Cinnamom umverum*- 15mm.

Common Name	Extract colour	pH	TSS (%)	Active spice constituent
Pepper	Yellow green	6.4	28.5	Piperine
Black Cardamom	Colour less	5.5	20.2	alpha and beta-pinene
Clove	Pale yellow	6.8	30.1	Eugenol
Red Chili	Pink	5.1	15.0	Capsaicin
Cinnamon	Dark brown	6.5	30.0	Cinnamomaldehyde

Table 2: Characterization of the extracts.

In a study, it is shown that *Piper nigrum* has showed the activity at 40% aqueous extraction against pathogenic organisms. The inhibitory action of natural products causes the fungistatic or fungicidal effect of spices, and the mechanisms involved are cytoplasm granulation, cytoplasmic membrane rupture, and inactivation and/or inhibition of intracellular and extracellular enzymes [16]. These biological events may occur separately or concurrently, culminating in mycelium germination inhibition [17].

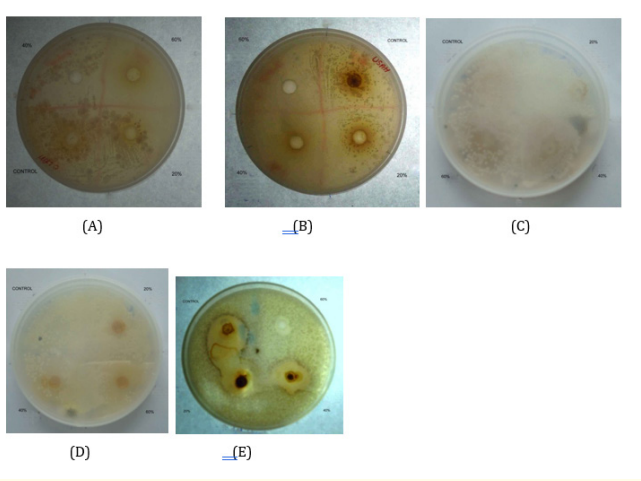

Figure 1: Inhibitory effects of the extracts of (A) *Piper nigrum* fruit; (B) *Amomum subulatum* fruit capsule; (c) *Syzygium aromaticum* unopened flower bud; (D) *Capsicum annum* dried pod; and (E) *Cinnamom umverum* bark against *Saccharomyces cerevisiae.*

Spices	Control	Zone of Inhibition (mm)
Piper nigrum	0	22
Ammomum subulatum	0	20
Syzygium aromaticum	0	20
Capsicum annum	0	21
Cinnamom umverum	0	15

Table 3: Antifungal activity of aqueous extracts of different spices against *Saccharomyces cerevisiae*.

Conclusion and Future Scope

The present research concluded that all of the extracts can be used as a potential source of natural antimicrobial compounds when applied to food products. Before it can be brought to market as food preservatives, additives, or nutraceutical foods, more research is needed to identify the bioactive molecules present in the extracts and their in- vivo efficacy against microorganisms. The current study can be expanded to characterize the active component within the aqueous extract, allowing for the creation of efficient medical formulations. According to a study, the plant extracts and phytochemicals, both having antimicrobial characteristics can play a significant role in pharmacological therapies [18]. Further, it will also be fascinating to analyze the influence of mixing two or more of these spice extracts for an anti-diabetic activity and their activity can be checked against other multi-drug resistant microorganism.

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