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Original scientific paper

The Antifungal Properties of a Fresh Extract Derived from *Allium Sativum*

Vesna Kalaba,[1] Dragana Kalaba,[2] Dragica Đurđević Milošević[3], Tanja Ilić[4], Željka Marjanović Balaban[5]
[1]*School of Applied Medical Sciences Prijedor, Republic of Srpska, Bosnia and Herzegovina, vesna.kalaba@virs-vb.com*
[2]*AU "Benu", Novi Sad, Serbia*
[3]*Institute of Chemistry, Technology and Microbiology, Belgrade, Serbia*
[4]*PI Veterinary Institute Republic of Srpska „Dr Vaso Butozan"Banja Luka, Banja Luka, Bosnia and Herzegovina*
[5]*Faculty of Forestry, University of Banja Luka, Republic of Srpska, Bosnia and Hercegovina*

ABSTRACT: Many fungal species are opportunistic and rarely pathogenic to healthy individuals unless dealing with an immunocompromised host. There are numerous reasons why a host might be immunocompromised (HIV, AIDS, TB, leukemia, diabetes, and many other causes), as well as therapies with antimicrobial drugs, corticosteroids, immunosuppressants, etc., which act immunocompromising on the host's immune system. Such conditions favor opportunistic systemic fungi that will seize the opportunity to colonize the weakened organism, which is not prepared and strong enough to fight off the infection. Aspergillosis, candidiasis, and other fungal diseases are typical examples of opportunistic systemic fungal infections. This study aims to assess the antifungal potential of fresh *Allium sativum* extract originating from the Republic of Srpska in comparison with clotrimazole against two isolates of *Candida albicans*, one reference strain of *Candida albicans* WDCM00053, *Saccharomyces cerevisiae* WDCM3058 and *Aspergillus brasiliensis* WDCM00054. The results of the study showed that fresh *Allium sativum* extract has higher antifungal activity against *Candida albicans*, *Saccharomyces cerevisiae*, and *Aspergillus brasiliensis* compared to clotrimazole, a standard antifungal drug.

Keywords: *Allium sativum*, antifungal properties, clotrimazole

INTRODUCTION

Fungal infections represent a significant health problem and most commonly occur as a result of weakened immunity. Their frequency depends on various factors, including the individual's immune status, the presence of other underlying health issues, and environmental factors. The most common fungal infections include those affecting the skin, nails, and mucous membranes. Serious systemic fungal infections often occur in individuals with compromised immune systems, such as those with HIV, organ transplants, serious illnesses, or after antibiotic therapy, leading to various pathological conditions (Yang et al., 2006; Nantz et al., 2011; Lockhart et al., 2019).

Candida albicans is naturally present in the human body, and commonly found in the mouth, intestines, and genital area. However, when the balance of microorganisms is disrupted, *Candida albicans* can cause infection. It most commonly presents as vaginal candidiasis in women but can also affect other parts of the body, including the mouth and skin. These infections often occur in individuals with weakened immune systems, diabetes, or after antibiotic use (Pfaller & Diekema, 2007; Castanheira et al., 2016).

Saccharomyces cerevisiae is the most useful type of yeast, better known as baker's yeast, used in traditional or industrial bread, beer, or wine production. It can be found as a harmless and transient commensal and colonizer of mucosal surfaces in healthy individuals. It is often present in the human gastrointestinal tract and is usually beneficial, aiding in digestion. However, in individuals with weakened immune systems, such as those with serious illnesses, it can cause infections, although such cases are rare (de Llanos et al., 2011; Pérez-Torrado & Querol, 2016; Lockhart et al., 2019).

The genus *Aspergillus* is responsible for about 75% of cases of otomycosis, with *Aspergillus brasiliensis* being the most common causative agent. *Aspergillus brasiliensis* is an opportunistic pathogen that becomes pathogenic when the first line of defense is breached, such as in individuals with weakened immune systems (Cairns et al., 2018; Cornelia Lass-Flörl, 2019). It is used in industry to produce enzymes and metabolites.

In the treatment of fungal diseases, only a few classes of antifungal drugs are available, making treatment difficult due to multidrug resistance. Since fungi are eukaryotes, like humans, manipulating fungi and treating them is more challenging. Eukaryotic organisms, unlike prokaryotic organisms, have much more complex molecular processes and cell structures, which are the main reasons why there are no broadly effective antifungals, and why each fungal infection requires aggressive and prolonged therapy (Perlin et al., 2017).

The antifungal nature of plant products has been determined by the presence of flavonoids, phenols, saponins, tannins, and terpenoids and the mechanism of action of such molecules involves inhibiting the fungal cell membrane and hyphal progression (Suurbaar et al., 2017; Silva et al., 2010)

Allium sativum has wide applications in human nutrition and scientific research due to its biological properties. In traditional medicine, it is used as a remedy for treating bacterial, fungal and viral diseases (Martins et al., 2016; El-Saber Batiha et al., 2020; Genatrika et al., 2020). It helps maintain healthy microbiomes in the gastrointestinal tract by removing harmful bacteria while supporting beneficial bacteria. *Allium sativum* stimulates the liver and colon, providing a strong additional effect in the body's detoxification process, improving lymphatic system function, encouraging the body to eliminate waste materials more competently (Rafe, 2014; Shang et al., 2019; Jacob, 2019; Genatrika et al., 2020).

Allium sativum bulbs are rich in phytochemicals (phenolics, flavonoids, alkaloids, terpenoids, and fatty acids), and the antimicrobial activity is attributed to the sulfide compound isolated from freshly ground pulp known as allicin (Cavallito et al., 1944). However, some researchers disagree and believe that allicin itself is very unstable and quickly degrades, arguing that some smaller metabolic breakdown products also have strong antimicrobial effects (Haris et al., 2001).

Various factors (e.g., temperature, pressure, extraction method, solvent type, size, and territorial origin of the plant) influence the quantity and type of bioactive compounds obtained from *Allium sativum* and the content of bioactive compounds correlates with the biological activity of the extracts.

This study aims to assess the antifungal potential of fresh *Allium sativum* extract originating from the Republic of Srpska in comparison with clotrimazole against two isolates of *Candida albicans*, one reference strain of *Candida albicans* WDCM00053, *Saccharomyces cerevisiae* WDCM3058 and *Aspergillus brasiliensis* WDCM00054.

MATERIALS AND METHODS

The *Allium sativum* used in this study was of the domestic variety, matured, and purchased at the city market in Banja Luka. Fresh bulbs were divided into cloves, each clove was peeled and then crushed in a sterile mortar until juice or solution was obtained without any additives (such as water, etc.). The obtained juice was filtered through sterile gauze, and the filtrate (100% aqueous solution of *Allium sativum* extract) was used on the same day in the antifungal testing.

Preparation of disks:

Twenty microliters (20 µL) of *Allium sativum* filtrate were applied to commercial sterile paper disks with a diameter of 9 mm, and 20 µL of clotrimazole solution (10 mg/ml) was used as a positive control. Disks saturated with fresh *Allium sativum* extract (100%) were used as the test group.

Preparation of fungal strains:

The fungal strains used in this study were two isolates of *Candida albicans* from the collection of isolates of the Laboratory for Microbiology of food, feed, and water from PI Veterinary Institute Dr Vaso Butozan, Banja Luka and one reference strain *C. albicans* WDCM 00053, *Saccharomyces cerevisiae* WDCM3058, and *Aspergillus brasiliensis* WDCM 00054. The strains were cultured on Sabouraud dextrose agar for 48-72 hours. They were examined microscopically and subcultured on Sabouraud dextrose broth for 48 hours at 25°C. Broth cultures were kept in the refrigerator until use.

Susceptibility testing to antifungal drugs:

The antifungal activity of fresh *Allium sativum* extract was determined by the disk diffusion method (Kirby-Bauer, 1996). One hundred microliters (100 µL) of each fungal strain from the broth culture was spread on the surface of sterile Petri dishes with Sabouraud dextrose agar using a Drigalski spatula. Disks saturated with fresh garlic juice and clotrimazole disks were placed on agar plates inoculated with the strains and incubated at 25°C for 42-72 hours. After incubation, the diameter of the clear inhibition zone around the disks was measured in millimeters (mm). The test was repeated three times.

Analysis of results:

Microsoft® Excel 2013 was used for the calculations of average values, standard deviations, histograms, and the one-way analysis of variance (ANOVA). ANOVA was used to determine whether there were any statistically significant differences between the similar mean values of inhibition zones.

RESULTS AND DISCUSSION

The tested fungal strains (*Candida albicans*, *Saccharomyces cerevisiae*, and *Aspergillus brasiliensis*) were highly sensitive to *Allium sativum* extract compared to the commercial antifungal drug. The results of the antifungal activity of the tested fungi are shown in Table 1. and the average values of the inhibition zones are presented in Figure 1.

Table 1. The antifungal Activity of *Allium sativum* extract compared to Clotrimazole.

Strain	Inhibition zone (mm ±)	
	***A.sativum* extract**	**Clotrimazole**
Candida albicans 1	40	20
	43	19
	45	19
Candida albicans 2	4	25
	45	24
	42	25
Candida albicans WDCM 00054	45	20
	45	21
	40	19
Saccharomyces cerevisiae WDCM 3058	37	35
	41	31
	45	33
Aspergilus brasiliensis WDCM 00053	7	0
	8	0
	7	0

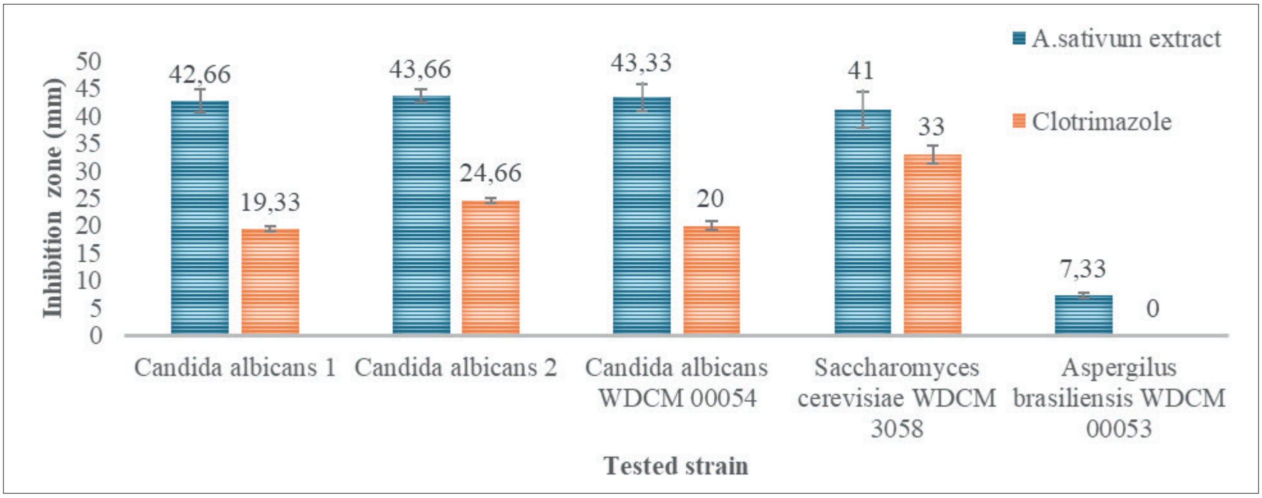

Figure 1. The average values of inhibition zones

The antifungal activity of *Allium sativum* extract, with a prominent mean inhibition zone ranging from 42.66 mm to 43.66 mm, was observed against isolates and the reference strain *Candida albicans*, and 41.00 mm for *Saccharomyces cerevisiae*. The level of statistical significance was set at $p \leq 0.05$ and based on one-way ANOVA there are no differences in the mean value of inhibition zones of yeasts, regardless used strain.

Weaker antifungal activity of *Allium sativum* was shown towards the reference strain *Aspergillus brasiliensis* with a mean inhibition zone of 7.33 mm. The antifungal activity of the commercial antifungal drug for isolates and the reference strain *Candida albicans* ranged from a mean inhibition zone of 19.33 mm to 20.00 mm, and for *Saccharomyces cerevisiae*, it was 33.00 mm. However, it did not affect the reference strain *Aspergillus brasiliensis*, unlike fresh *Allium sativum* extract (mean inhibition zone 7.33 mm). These results have shown that fresh *Allium sativum* extract is more effective than the commercial antifungal drug (clotrimazole) against the tested fungi. Clotrimazole is an antifungal drug commonly used to treat fungal infections, but it is not always effective against all fungi, and it is not always the first choice for treating infections caused by *Aspergillus brasiliensis*.

Many studies investigating the antifungal activity of different concentrations of *Allium sativum* extract indicate that higher concentrations give larger inhibition zones for fungi (Irkin and Korukluoglu, 2007; Agarwal et al., 2010; Kumar et al., 2012; Agustatina & Soekartono, 2021). Additionally, in a study conducted by Agustantina and Soekartono (2021), the effect of different concentrations of *Allium sativum* (5%, 25%, 50%, and 75%) on *Candida albicans* was examined. *Allium sativum* extract exhibited the greatest antifungal effect at the highest concentration (75%) on the tested fungi. In an investigation of the antifungal activity of water and alcohol extracts of six different spices against *Aspergillus niger*, *Candida albicans*, and *Trichophiton rubrum*, Ikegbunam et al. (2016) found that *Allium sativum* extract had the highest antifungal activity against all three tested strains. Ankri and Mirelman (1999) discovered that allicin in its pure form, extracted from fresh bulbs of *Allium sativum*, inhibits the growth of *Aspergillus niger*, *Aspergillus ustus*, and *Penicillium spp*. Allicin isolated from Allium sativum demonstrates potent activity against Candida primarily by inhibiting amino acids and proteins containing thiol, disrupting cell metabolism (Ankri and Mirelman 1999). Human cells contain glutathione, which can bind to allicin, preventing cell damage, while glutathione is lacking in Candida, making allicin a selective and effective candidate in Candida therapy (Davis, 2005).

Cavalcant et al. (2021) emphasize that the extraction method, with particular attention to the solvent used in isolating active substances from *Allium sativum* has impact on the composition and biological activity of extracts, and his great importance. The use of water for extraction has significant advantages primarily because it is non-toxic to humans and the environment, provides the possibility of clean processing, prevents contamination, and enables the avoidance of extracting unwanted components (Filly et al., 2016; Castro-Puyana et al., 2017; Lefebvre et al., 2021). In addition to water, organic solvents such as acetone, ethyl acetate, hexane, heptane, dichloromethane, methanol, ethanol, tetrahydrofuran, acetonitrile, dimethylformamide, toluene, and dimethyl sulfoxide are used. The use of these solvents achieves a high level of extraction of individual compounds, but the main drawback of these solvents is the health hazards associated with ingestion, inhalation, and skin irritation, as well as possible damage to the central nervous system and other parts of the body (Joshi & Adhikari, 2019). Additionally, a review of the literature has shown that water extracts are best for allicin extraction, while methanol- and ethanol-based extraction methods are best for isolating polyphenols. Water and ethanol extracts have numerous health-beneficial properties (antibacterial, anticancer, antidiabetic, antifungal, anti-hypercholesterolemic, anti-hypertensive, anti-inflammatory, antioxidant, antiparasitic, antiviral, and immunostimulatory effects) and can be used as adjuncts in conventional cancer therapy, but further research in this area is needed. In addition to culinary use, *Allium sativum* extract can be used as an adjunct in conventional therapies for various skin infections and pathologies due to its high concentrations.

CONCLUSION

Fresh extract of *Allium sativum* prepared in a simple manner, without modern extraction methods, was found to be more effective, as revealed by its inhibitory effects with highly significant antifungal efficacy compared to clotrimazole against *Candida albicans*, *Aspergillus brasiliensis*, and *Saccharomyces cerevisiae*.

Based on one-way ANOVA there are no differences in the mean value of inhibition zones of used yeast strains (*Candida albicans* and *Saccharomyces cerevisiae)*.

Since *Allium sativum* is readily available and inexpensive with minimal adverse effects compared to commercial antifungal drugs, further investigations using different concentrations and preparation methods of *Allium sativum* extract are needed to confirm its antifungal efficacy alongside its actual antimicrobial benefits.

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