Antifungal activity of medicinal herbs aqueous extracts: common wormwood (Artemisia absinthium L.), greater burdock (Arctium lappa L.), common thyme (Thymus vulgaris L.) and pot marigold (Calendula officinalis L.) on yeast Saccharomyces cerevisiae

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Abstract. **One of the modern approaches to identifying alternative broad-spectrum against microorganisms that cause human diseases raises an urgent need to search for bioactive compounds with antifungal activity from medicinal plants. One of the main problems of antibiotic therapy for diseases is the development of resistance to opportunistic diseases of relatively low virulence, which are caused by yeast. In this regard, the search for natural medicinal herbs is relevant. The present study was undertaken to evaluate the effectiveness of the antifungal activity of different medicinal plant aqueous extracts: greater burdock (*Arctium lappa* L.), common wormwood (*Artemisia absinthium* L.), common thyme (*Thymus vulgaris* L.), and pot marigold (*Calendula officinalis* L.) on the growth of yeast: *Saccharomyces cerevisiae*. Aqueous extracts were prepared from leaves, stems, inflorescences, and roots of ready-made medicinal mix, and their antifungal activity against yeast was tested using the Kirby-Bauer standard disk diffusion method. Disks were soaked in aqueous extracts of medicinal plants and were placed on an agar medium previously inoculated with yeast *Saccharomyces cerevisiae* as a test object. The prepared agar plates were cultured in the dark at 30°C for 3-5 days. The results obtained showed that the aqueous extracts of medicinal plants demonstrated different antifungal activity concerning test culture, the level of which variable by the plant species. The aquatic extract of medicinal plants: common wormwood (*Artemisia absinthium* L.), and pot marigold (*Calendula officinalis* L.) had the most noticeable antifungal activity. The results obtained during the preliminary suggest that the studied aqueous extracts of medicinal plants of natural origin can be used as antifungal agents with a specific mechanism of action.**

Keywords: antifungal activity, disk diffusion method, medicinal plants, Saccharomyces cerevisiae, SEM, yeast.

I. Introduction

Higher plants are highly biodiverse which can provide a huge range of various active compounds with different biological activities. These chemical compounds produced by plants can serve as attractants for pollinators and as chemical defenses against insects, herbivores, and microorganisms. Medicinal plants are rich in a variety of complex and structurally diverse chemical compounds that

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have antimicrobial and antifungal activities [1]. Of an estimated 250000 higher plants in the world, only 5-15% have been studied for therapeutic potential [2]. Extracts isolated from medicinal plants exhibit various biological activities such as antifungal, antibacterial, antioxidative, anti-inflammatory, antiviral, and insecticidal activities which may inhibit the growth of bacteria, fungi, viruses, and protozoa as well as may have a significant clinical value in the treatment of resistant strains of microbes [3]. These antimicrobial and antifungal compounds produced by plants are active against plant and human pathogenic microorganisms. Recently, there has been a growing interest in investigations of new potential antimicrobial and antifungal agents that focus on plant and microbial extracts, essential oils, pure secondary metabolites, and newly synthesized molecules [4], [5].

The World Health Organization (WHO) has revealed that approximately 80% of the developing world's population continues to benefit from using traditional medicines obtained from medicinal plants [6]. The WHO has also recorded the names of more than 20000 species of medicinal plants and has described medicinal plants as one of the potentially cheaper and safer alternative sources of new drugs [1]. Antimicrobials of medicinal plant extracts are natural, safer than synthetic alternatives, available in local communities, cheaper to purchase and they can offer profound therapeutic benefits and more affordable treatment. Thus medicinal plants can be regarded as the richest bio-resource of drugs of modern medicine, folk medicine, and chemical entities for synthetic drugs. Natural antifungals can act alone or in combination with antibiotics to enhance antifungal activity against a wide range of microorganisms.

Saccharomyces cerevisiae is one of the most intensively studied versatile eukaryotic model organisms in molecular and cellular biology. *Saccharomyces cerevisiae* is a single-celled fungus that reproduces by budding from an existing cell representing the main components of a typical eukaryotic cell. Its cell wall is a dynamic structure, relatively rigid, which protects cells, and osmotic support and determines the shape of cells. Cells of *Saccharomyces cerevisiae* are round or egg-shaped and have a diameter of approximately 5–10 micrometers [7].

Under certain conditions, for example, under reduced immunity, *Saccharomyces cerevisiae* can cause various infectious diseases in a person. There are also known cases of infections of the oral cavity and pharynx caused by *Saccharomyces cerevisiae* [8]. There are known cases where the fungus *Saccharomyces cerevisiae* was discovered in people whose professional activities were associated with regular stays in various bakeries and caused lung disease. It is believed that the source of infection in this case was inhalation of dry yeast powder [9]. In rare cases, *Saccharomyces cerevisiae* causes invasive infections infecting the main bloodstream or other body fluids that should normally be sterile, or internal organs, such as the lungs, liver, and spleen. Such an infection can become systemic, that is, affect several organs. Invasive mycoses caused by *Saccharomyces cerevisiae* are very dangerous – the mortality rate is more than 30% even with treatment [10].

The present study was undertaken to evaluate the effectiveness of the antifungal activity of different medicinal plant extracts: greater burdock (*Arctium Lappa* L.), common wormwood (*Artemisia absinthium* L.), common thyme (*Thymus vulgaris* L.), and pot marigold (*Calendula officinalis* L.) on the growth of yeast.

II. MATERIALS AND METHODS

Plant material and preparation of aqueous extracts

The dried samples from the pharmacy of greater burdock (*Arctium Lappa* L.), common wormwood (*Artemisia absinthium*), common thyme (*Thymus vulgaris*), and pot marigold (*Calendula officinalis*) were used. To prepare freshly prepared aqueous extracts of plants, 5 g of dried plant materials of each plant were pulverized (~5 μm) by using CryoMill (Retsch GmbH, Germany). The crushed raw materials of each sample were poured into flasks and 100 mL of distillated water was added. The mixtures were sterilized for 5 minutes at 100°C (HSP Laboklav Steriltechnik AG, Germany) and then cooled to room temperature for 30 minutes.

Fungal test-culture, preparation of culture-media and scanning electron microscopy (SEM) of yeast

A test yeast culture of *Saccharomyces cerevisiae* was used for the experimental study. This is a type of unicellular microscopic (5-10 microns in diameter) fungi (yeast) from the class of *Saccharomycetes*, widely used in scientific research. Nutrient agar powder Mueller–Hinton agar (Thermo Fisher Scientific, US) 9.5 g was suspended in 250 mL of cold, distilled water, the mixture was stirred and boiled to dissolve the agar powder.

The nutrient media was sterilized by autoclaving at 121°C (15psi) for 15 minutes. After autoclaving the agar was cooled and poured into sterile Petri plates (90 mm diameter). Mueller-Hinton agar is primarily used for antimicrobial susceptibility testing (AST) and it has become the standard medium for the Bauer-Kirby method as more suitable for yeast culture comparing with others [11] – [13].

For scanning electron microscopy (SEM), isolated colonies of *Saccharomyces cerevisiae* from Petri plates were slowly air-dried (1 h) at room temperature (20 °C ±2°C) and transferred to observation chamber of SEM TM 1000 (Hitachi Tabletop Microscope, Japan), the colonies were located on the temperature-controlled SEM cooling-stage (Deben, UK) according to simplified procedure [14].

The yeast colonies surface structure was observed at x10k magnification without digital zooming, DPI=130.05, accelerating voltage 15kV, working distance=9620 μm, emission current=76000nA and under high vacuum conditions. The uncoloured (SEM) of *Saccharomyces cerevisiae* colonies during experiment represented in Fig.1.

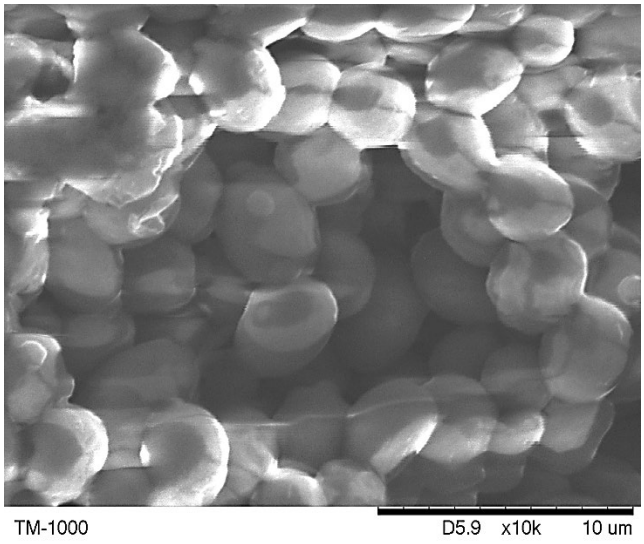

Fig.1. Image of *Saccharomyces cerevisiae* cells gained by SEM.

Disk diffusion test and antifungal activity assay

Antifungal activities of the plant extracts of four selected plants were tested on nutrient agar Mueller–Hinton agar (Thermo Fisher Scientific, US) by disk diffusion method [15] with some modifications. 1 g of *Saccharomyces cerevisiae* was inoculated into 9 mL of Brain Heart Infusion Broth (BioMaxima, Poland). Test tubes were placed in a thermostat for 24 hours at a temperature of +25°C. Using a sterile dry cotton (150mm) swabs (Aptaca SPA, Italy) the culture was applied to the surface filled with nutrient agar medium Mueller–Hinton agar (Thermo Fisher Scientific, US) by surface method and cultivated at 25°C within 48 hours in Refrigerated Thermostat Incubator FTC 90E (Velp Scientifica, Italy) [16].

Inoculated plates were allowed to dry for ten minutes at room temperature in a secure box. Filter–paper discs having a diameter of 5 mm were prepared, sterilized, and impregnated with extracts. Paper disks were placed on the previously inoculated agar plates with yeast using sterile forceps. Four filter–paper disks were placed on each plate and were placed at the distance from each other and the edge, to prevent overlapping of inhibition zones.

The plates were incubated within 48 hours at 25°C on nutrient agar. After 48 hours of incubation, the inhibition zone of *Saccharomyces cerevisiae* around the disks immersed in plant extracts was determined. For measuring distances of zones of yeast growth inhibition, the software: Acquisition & analysis Ver. 8.20 (Vision Works, US) was used. Results were documented by measuring and calculating the zone of inhibition in millimeters (mm), including the diameter of the disk. The experiment was repeated three times for each extract and the mean diameter was taken.

Statistical analysis

All the data are reported as mean ± standard deviation (SD) and ± error mean (SE). Each value of 4 different plant extracts was the mean (n=48) of 3 replicates and performed with Microsoft Excel Ver. 14.0.7214.5000.

III. RESULTS AND DISCUSSION

Saccharomyces cerevisiae is an opportunistic pathogen of relatively low virulence [17] and under certain conditions, for example, when immunity is reduced, it can cause infectious diseases in humans. Obtaining nutrients from natural food sources is the best option, which is necessary for the construction and continuous renewal of cells and tissues, the supply of energy necessary to replenish the body's energy however, in case of illness, widely available dietary supplements are commonly used which offer the potential to improve health.

The analysis of the antifungal parameter values shows that the aqueous extracts of medicinal plants, namely common thyme (*Thymus vulgaris* L.), pot marigold (*Calendula officinalis* L.), and common wormwood (*Artemisia absinthium* L.) had the most noticeable antifungal potency against the test organism *Saccharomyces cerevisiae*. The growth inhibition zones were 8.71±0.156 mm for common thyme (*Thymus vulgaris* L.), 10.23 ±0.176 mm for pot marigold (*Calendula officinalis* L.), and 10.45 ± 0.235 mm for common wormwood (*Artemisia absinthium* L.) respectively. The results of the research are represented in Fig. 2. The antifungal effects of aqueous extracts of these tested plants can be attributed to the presence of bioactive compounds that can act alone or in synergy, as demonstrated by other studies [18], [19].

In addition to this, the tannins contained in these medicinal plants have noticeable toxic activity against bacteria and fungi, which may have pharmacological importance. Furthermore, the studied plants contain saponins, which are a special class of glycosides with soapy properties and are considered active antifungal agents [20]. In turn, the flavonoid artemisinin contained in wormwood, considered one of the most powerful, can also kill pathogens of fungal diseases [21].

It is known that the contents of active ingredients in plant materials fluctuate constantly with the genetic heterogeneity of a plant species, differences in soil condition, variations in a seasonal cycle, climatic influences, age of the plant, and alterations in weather, sun, and shade fluctuations [22]. The variability of their efficiency would be not only connected to various secondary metabolites content (alkaloids, flavonoids, anthocyanins, lignans, terpenoids, amines, polyphenols, quinones, peptides, coumarins) that plants produce but also to the toxic power of these biomolecules to microorganisms [23].

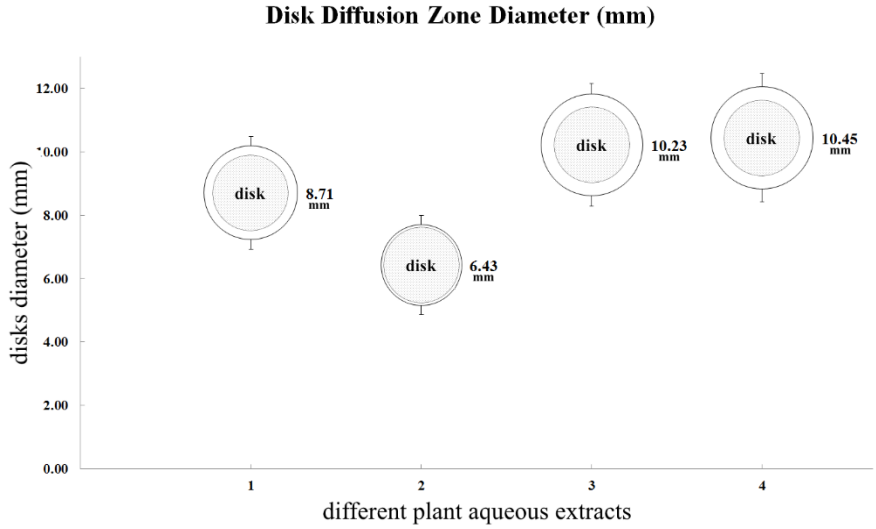

Fig.2. Zones of suppression of yeast activity by various medicinal plant aqueous extracts: 1- common thyme (*Thymus vulgaris* L.) 2- greater burdock (*Arctium Lappa* L.), 3- pot marigold (*Calendula officinalis* L.) 4- common wormwood (*Artemisia absinthium* L.).

The obtained results show that the tested plant aqueous extract of greater burdock (*Arctium Lappa* L.) possesses some very low levels of antifungal activity 6.43 ±0.208 mm. It is well known that plant extracts with good antifungal activity usually have high levels of total polyphenols and titratable acidity, as well as low pH values [24].

However, burdock roots contain insignificant content of phenolic acids and flavonoids, particularly quercetin and luteolin, which are antioxidants promoting health through cytotoxic, anti-inflammatory, and antioxidant effects [25].

At the same time, the results of previous investigations revealed that the hydroethanolic extracts concentrating a greater proportion of active principles are more active than their equivalent aqueous extracts [26].

Aqueous extracts contain a great content of macromolecules (polysaccharides, proteins, and glycoproteins) and also a few species of polar lipids of small size, whose structures are simple [27].

This high content of polysaccharides, glycoproteins, and proteins may explain why aqueous extracts are always less active. Many authors explained that most plants synthesize various secondary metabolites that are useful for their normal biology and to fight pathogenic microorganisms (viruses, bacteria, fungi, and various parasites) attacks which in hydroethanolic extracts concentrate a greater proportion of active principles than their aqueous equivalents [27] – [29]. It has been pointed out that there is a relationship between the antifungal activity in various extracts and their bioactive compounds, for example, fatty acids play an important role in fungal resistance. All bioactive compounds present in plant extracts act synergistically on fungi either by inactivating enzyme production, inhibiting, or reducing the ergosterol content in filamentous fungi, which enhances the overall antifungal activity [30].

Commonly using medical antifungal agents has varied toxicity, efficacy, and cost differences, as well as low biological digestibility, and its repeated usage leads to the emergence of resistant strains that cannot be treated with normal antifungal drugs [31] which represents a serious barrier for using them in the therapy. In addition, biologically active compounds of common wormwood (*Artemisia absinthium* L.) can neutralize individual determinants of antibiotic resistance and thereby restore the sensitivity of resistant strains to the appropriate drugs [32].

Taking into account the growing demand for new remedies to overcome various infections caused by antibiotic-resistant microorganisms, the practice of using plant-derived bioactive products as secondary metabolites has been widely studied in various scientific communities all over the world for more than many centuries, and the considerable practical achievement in this regard could be the creation of the new effective agents with fungicidal and antimicrobial properties from medicinal plants which could be outperforming the best traditional methods of infectious disease treatment.

IV. CONCLUSIONS

On the whole, our current study revealed that the aqueous extracts of traditional medicinal plants: common thyme (*Thymus vulgaris* L.), pot marigold (*Calendula officinalis* L.), and common wormwood (*Artemisia absinthium* L.) that produce various types of secondary metabolites have noticeable antifungal effect, but aqueous extract of greater burdock (*Arctium lappa* L.) showed insignificant antifungal effect against yeast. For this reason, these tested medical plant species with antifungal properties can be used as a remedy with a specific mechanism of action and are recommended to treat yeast infections as an auxiliary tool.

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