ETHANOL EXTRACTS OF *SALVIA OFFICINALIS* EXHIBIT ANTIFUNGAL PROPERTIES AGAINST *SACCHAROMYCES CEREVISIAE* CELLS

Ileana C. Farcasanu * **and Eliza Oprea**

abstract: The baker's yeast *Saccharomyces cerevisiae* was tested for modifications in sensitivity to ethanol extracts from *Salvia officinalis* leaves. The extracts obtained with aqueous ethanol of various concentrations showed different antifungal effect against the yeast cells. The strongest growth inhibitory capacity was noted for the extracts obtained in 90% ethanol demonstrating that under these conditions the yeast cells were more susceptible to metabolic or structural damage.

Introduction

Plant oils and extracts have been used for a wide variety of purposes for many thousands of years. In particular, the antimicrobial activity of plant oils and extracts has formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies.

In recent years, the interest in the possible use of natural alternatives to food additives to prevent bacterial and fungal growth has notably increased. Plants and plant products can represent a source of natural alternatives to improve the shelf life and the safety of food. Also, they are characterized by a wide range of volatile compounds, some of which are important flavor quality factors [1]. Recently, the interest in the application of essential oils to control plant and postharvest pathogens has increased and their potential role in food preservation has been exploited [2, 3]. Plant essential oils have been studied for their antimicrobial activity against many microorganisms including several pathogens [4, 5].

Compound*s* from *Salvia officinalis* essential oil have been shown to exhibit high antibacterial activity against *Staphyloccocus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, cytotoxic activity against Vero cells and virucidal activity against herpes simplex virus 1 and vesicular stomatitis virus [6, 7]. *Salvia officinalis* essential oil is applied in the treatment of a large range of diseases such as nervous system, heart and blood circulation, respiratory, digestive, metabolic and endocrine diseases, while the *Salvia officinalis* infusion is commonly used for the haemostatic, estrogenic, anti-perspiration, anti-neuralgic, antiseptic, hypoglycemic and many other therapeutic effects [8].

* Bucharest University, Faculty of Chemistry, Department of Organic Chemistry and Biochemistry, Sos. Panduri 90-92, Bucharest, Romania. Tel.: 40-21-4103178/135.
Corresponding author. E-mail: farcasanu.ileana@unibuc.ro.

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In this work we investigated the antifungal effect of ethanol extracts from *Salvia officinalis* leaves upon *Saccharomyces cerevisiae* cells.

Materials and Methods

Yeast strain and growth conditions. The laboratory *Saccharomyces cerevisiae* strain W303-1A was used throughout our experiments. Yeast growth and manipulation was done as described [9]. Briefly, cells were cultured in YPD medium (pH 5.5) containing 1 g yeast extract, 2 g peptone and 2 g glucose per liter of distilled water. For solid medium, 2% agar was added. Liquid cultures (10mL per 100mL culture flasks) incubated at 25ºC for 16 h under constant agitation served as inocula for all tests. Ethanol plant extracts were added to autoclaved media after cooling to 55ºC.

Preparation of plant extracts. Air-dried *Salvia officinalis* leaves were frozen with liquid nitrogen and ground to fine powder. The powder was suspended in 0, 50, 70 and 90% aqueous ethanol (5% w/v) and extracted with light agitation for two days. The extracts were separated from the plant material by centrifugation and subsequently filter-sterilized (pore size: 45 μm).

Determination of the antifungal activity of plant extracts upon yeast cells.

The antimicrobial activity of the extracts was tested on *Saccharomyces cerevisiae* cells inoculated in liquid or solid medium. To evaluate the antimicrobial effect in solid medium, two approaches were used. In a first approach, serial dilutions of yeast cells were placed onto YPD agar plates that contained ethanol extracts of *Salvia officinalis* leaves, then the plates were incubated at 25ºC for two days. In a second approach, the yeast cells were suspended at concentration 1 x 10^5 cells/mL in 5 mL of molten YPD soft agar (0.7% agar, 50ºC), vortexed briefly and spread onto YPD plates. The extracts to be tested were applied on the surface of the top agar [10], then the plates were incubated at 25ºC for two days, before being photographed.

For growth tests in liquid media, cells from an overnight pre-culture were inoculated into fresh YPD that contained ethanol plant extracts, at cell density 1 x 10^6 cells/mL. Cells were incubated with shaking at 25ºC and the cell growth was assessed after 24 hours by measuring optical absorbance at 600 nm. Data presented represent means of data collected from three different experiments, each performed in triplicate.

Results and Discussion

We monitored the influence of ethanol extracts of *Salvia officinalis* leaves on the growth of the *Saccharomyces cerevisiae* cells, by testing extracts obtained in various concentrations of ethanol. We found that the extracts obtained in 50% and 70% ethanol did not impair the growth of the yeast cells significantly when added in volumes at which the blind solvent had no effect on growth. However, we noticed that when cells were exposed to extracts obtained in 90% ethanol, the growth of yeast cells was seriously impaired (Fig. 1).

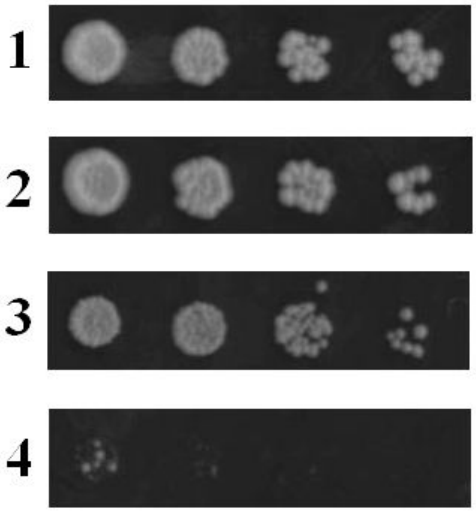

Fig. 1 *The effect of ethanol extracts of Salvia officinalis leaves on the growth of Saccharomyces cerevisiae cells. Yeast cells were stamped on YPD agar plates as 10-fold serial dilutions, starting from 1x10^6 cells/mL (left).*

The plates were photographed after two days incubation at 25°C. **1**: blind solvent (90% ethanol); **2**: extract in 50% ethanol; **3**: extract in 70% ethanol; **4**: extract in 90% ethanol. The amount of blind solvent or leaves extract used was 100 μL/mL medium. Experiments were carried out three times and the results were similar.

To determine the effect of ethanol extracts of Salvia officinalis leaves on the sensitivity of *Saccharomyces cerevisiae* cells, we also assessed the growth of the yeast cells using a spot approach. To do so, extract of *Salvia officinalis* leaves obtained in 90% ethanol was spotted onto a suspension of yeast cells in YPD-soft agar. This test has the advantage that smaller amounts of plant extracts are used. As expected, we found that the extracts obtained with 90% ethanol exhibited anti-fungal effect, apparently in a dose-dependent manner (Fig. 2).

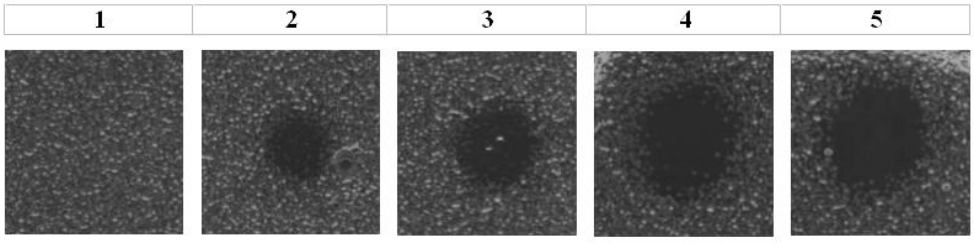

Fig. 2 *The effect of extract obtained in 90% ethanol from Salvia officinalis leaves upon the growth of Saccharomyces cerevisiae cells.*

The suspension of yeast cells (10^5 cells/mL) in 5 mL molten soft agar YPD medium (0.7% agar, 50ºC) was overlaid on solid YPD. Blind solvent or extract obtained in 90% ethanol were spotted directly on the surface of the top agar to assess the anti-fungal effect. **1**: 20 μL 90% ethanol (blind solvent); **2**, **3**, **4**, **5**: 5 μL, 10 μL, 15 μL, and 20 μL extracts in 90% ethanol, respectively. The plates were photographed after two days incubation at 25°C.

Since the *Saccharomyces cerevisiae* cells exhibited highest sensitivity to extracts obtained in 90% ethanol, we also determined the effects of ethanol extracts of *Salvia officinalis*

leaves on the ability of yeast cells to grow in liquid media containing various concentrations of these extracts. To do so, cells from an overnight pre-culture were inoculated into fresh YPD that contained or not the leaves extracts. We found that the yeast cells could grow in the presence of extracts obtained in 0, 50, and 70% ethanol (data not shown), while growth was inhibited in a dose-dependent manner when using extracts obtained in 90% ethanol (Fig. 3). We found that the growth of yeast cells was reduced to more than 50% when the extract was present in the incubation medium at 50 μL/mL, while doubling the extract concentration, inhibited cell growth almost completely (Fig. 3).

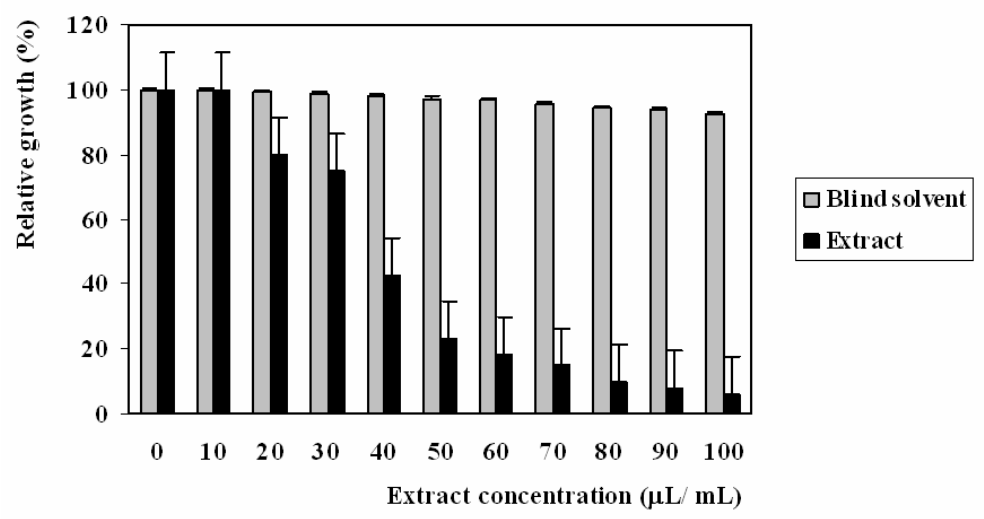

Fig. 3 *Antifungal effect of extracts from Salvia officinalis leaves obtained in 90% ethanol.*

Saccharomyces cerevisiae cells were inoculated from an overnight culture into fresh YPD at cell density 1 x 10^6 cells/mL. Following inoculation, leaves extract obtained in 90% ethanol was added at various concentrations, and then cells were further incubated with shaking at 25ºC. Cell growth was assessed after 24 hours by measuring optical absorbance at 600 nm. Relative growth was calculated as percentage relatively to the growth in the absence of any added solvent (only YPD). Data represent triplicate determinations and are expressed as mean standard deviations.

The composition of essential oil from *Salvia officinalis* leaves was determined recently [11], while the composition of extracts from *Salvia officinalis* leaves obtained in aqueous ethanol of various concentrations is under investigation.

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