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PRELIMINARY STUDY ON THE ANTIMICROBIAL POTENTIAL OF *PHALAENOPSIS* ORCHIDS METHANOLIC EXTRACTS

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Abstract

This paper aims to provide preliminary data about the antimicrobial potential of an ornamental flower, respectively Phalaenopsis orchid. Products from different parts of the orchids, like roots, leaves or flowers, are already in use for different purposes. The orchid plants used in the study were declared florists' waste, and they were carried out tests with methanolic extracts made of different parts of these orchids (roots, leaves, stems). Using the spot technique, it was tested the inhibitory activity of different methanolic extracts on potential pathogens, respectively four Candida spp and on four different Staphylococcus spp. Due to their high diversity, the bioactive compounds from plants are of great interest results have shown promising inhibitory activity of orchid extracts against Candida krusei.

Key words*: waste, orchids, antimicrobial, activity.*

INTRODUCTION

The *Orchidaceae* family, despite all beliefs, is one of the most widespread plant families in the world. The total number of species counts almost 28,484 worldwide (KewWCSP, 2017). In Romania, although that there are identified almost 58 species and hybrids, these flowers are mainly used for their ornamental purpose. Almost all the studies concerning the Romanian orchids aimed to identify the species if they need to be preserved and their threats. Most of them are spontaneous species. (Irimescu L. S. et al., 2019). The orchids species are mainly known as ornamental, but many of these are used for their healing properties, as well as a safe source for the food and perfume industry. Due to the fact that they are widespread, orchids have various uses in multiple cultures also because of the strong aphrodisiac effects, and, in other countries, they are being considered a treatment for gastrointestinal tract, diarrhea and bilious diseases (Singh A., Duggal S., 2009). The phytochemicals produced by orchids are alkaloids, anthocians, flavonoids, sterols and carotenoids, but it is still more to discover about their biological function (Irimescu L.S. et al., 2019). *Phalaenopsis* spp. is known to be the most commonly used orchid from a commercial and economic point of view, and some parts of the plant are often left unused, which can lead to waste management and further ecological problems. Until present times, reports on phytochemical analyses have been available especially for endangered orchids (Minh T.N. et al., 2016).

Although used for their remarkable beauty, orchids are found in cosmetics and have proven useful in creating herbal medicines. In the cosmetic industry, plants from the *Orchidaceae* family are evaluated for their potential anti-aging and skin depigmentation on Japanese female skin (Tadokoro T. et al., 2010). Also, orchids are used for their therapeutic properties. Most of them have been listed in traditional medicine.

Other medicinal properties of orchids have been reported, such as tonic in hysteria, spasm, insanity and epilepsy, rheumatic treatments, tuberculosis, body aches, eczema, headache and fever, aphrodisiac and heart, respiratory, and nervous disorders (Bijaya P., 2013). However, any new developed drug need to be tested before being marketed. Testing, in the beginning, takes the form of experiments performed to evaluate toxicity, and studies that follow the experiments

to evaluate important factors such as: efficacy and adverse effects.

The present study was performed on florists' waste of epiphytic orchids from the species *Phalaenopsis*.

Their phytochemical screening would help to discover new sources of economically important materials such as tannins, oils, gums, resins, quinones etc. In this study we have tried to highlight that the most beloved apartment plant, has important ethnomedicinal values, not only ornamental.

A first step in our research it was to test the antimicrobial potential of methanolic extracts made of orchid waste, respectively from dried leaves, stems and roots of *Phalaenopsis* species. The search of new antimicrobial agents in the field of ethnopharmacology, to isolate compounds with proven antimicrobial activity, that can be used for an effective treatment of the human diseases, with lowered therapeutic doses of antibiotics (Nicolcioiu M.B., et al., 2017)

MATERIALS AND METHODS

Collection of plant material

The flowers waste of *Phalaenopsis* orchids have been harvested from Tria's Flower Shop, Greenhouse Băneasa, Romania. The plants have been cultivated under special conditions and have been donated by the manufacturer.

Preparation of extracts

The leaves, stems and roots of *Phalaenopsis* orchid species have been chopped in small pieces and shredded. The roots were washed with water to remove bark or other impurities which could have contaminated the final sample. After cutting, the tissue was dried. The drying process was carried out in rooms with a constant temperature of 37°C for 7 days. For the preparation of the crude extract, the powdery vegetal material (leaves/roots/stems) was mixed in a stopper bottle (Erlenmeyer) with 80% methanol (ration 1/10) and placed in the microplate mixer at a controlled temperature for 60 minutes at 30°C at 150 rotations/ minute. The crude extracts obtained were then filtered through filter paper.

Test microorganisms used

To test the antimicrobial activity of *Phalaenopsis* extracts, four potentially pathogenic *Staphylococcus* strains and four *Candida* strains were used (Table 1). These pathogenic microorganisms were provided by the Faculty of Biotechnology U.S.A.M.V. of Bucharest.

Table 1. Microorganisms used to test the *Phalaenopsis* spp. antimicrobial activity

Pos.	Microorganisms	Origin
Yeast		
1.	*Candida albicans* ATCC 10231	American Type Culture Collection
2.	*Candida parapsilosis* ATCC20019	American Type Culture Collection
3.	*Candida guilliermondii* MI 40	Collection of Faculty of Biotechnologies, Bucharest, Romania
4.	*Candida krusei* 2016 MI 41	Collection of Faculty of Biotechnologies, Bucharest, Romania
Bacteria		
5.	*S. aureus* ATCC6538	American Type Culture Collection
6.	*S. aureus* ATCC43300 MRSA	American Type Culture Collection
7.	*S. epidermidis* ATCC 51625	American Type Culture Collection
8.	*S. epidermidis* ATCC12228	American Type Culture Collection

Culture media

The culture media used for *Candida* was PDA (Potato Dextrose Agar) and for *Staphylococcus* TSA (Tryptic Soy Agar).

The media were autoclaved at 121°C for 15 minutes.

Testing antimicrobial activity by spot method

The drop-diffusion test method, was used to measure the inhibition zones, in order to determinate the antimicrobial activity (Diguta C. et al., 2014). For the drop diffusion test, the inoculum was prepared in fresh culture, in liquid media. Strains in fresh culture were prepared by inoculation on liquid media, TSA for *Staphylococcus* bacteria, PDA for *Candida*.

For a period of 24-48 hours, the microorganisms were cultivated at 30°C for fungi and 37°C for bacterial strains; the final cells concentration was adjusted to 10^8 CFU/ml for *Staphylococcus* spp. and 10^6 CFU/ml for *Candida* spp.

According to the working method, the spread technique is used for inoculation of pathogenic strains on the surface of the culture media distributed in Petri dishes. After about 1 hour, 5 µL of each sample was added as a spot into the Petri dish. As control, has been used 5 µL of 80% Methanol solution and antibiotic. The next step is to incubate for 24-48 hours the cultures at temperatures of 30-37°C. By measuring the area of inhibition that appeared in the immediate proximity of the antagonist, the degree of sensitivity was determined (Figure 1).

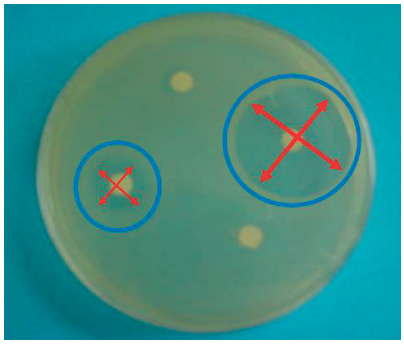

Figure 1. Spot method used to test antimicrobial activity of the extracts

Taking into account the size of the halos, 4 thresholds have been established indicating the level of antimicrobial activity as follows:

- = no antimicrobial activity (no inhibition reaction is observed)

+ = low activity: 0.1-0.9 mm (indicates possible reactivity, halo size close to unobserved)

++ = average activity: 1.0-1.9 mm (visible reactivity, small halo size)

+++ = increased activity: > 2 mm (large hall size, indicates increased reactivity).

RESULTS AND DISCUSSIONS

The microbial cultures can be examined using a variety of techniques; in our case the simplest was the measurement of formed halos diameters.

After incubation, the inhibitory effect on the test organism is indicated by a clear area (halo) around the test substance, in this case, the extracts obtained from different parts of the *Phalaenopsis* orchids. The general image of the inhibitory activity registered by spot method is presented in Table 2.

Clear inhibition halo (medium inhibitory activity) was observed in the case of methanolic stem extract on *C. parapsilosis* and *C. guilermondi*. In the case of methanolic root extract, high inhibitory activity has been noticed against *C. krusei. Staphyloccus aureus* has been inhibited in different degrees by the stem extract, while *Staphyloccus epidermidis* has been moderately inhibited by the root extract table 2. An important finding is that the methanolical extract obtained from the stem has high inhibitory activity on the MRSA (methicillin resistant) strain ATCC43300 of *Staphyloccus aureus*.

Such inhibitory activity may be linked to reports that revealed the presence of phytochemicals such as phenols, tannins, flavonoids, saponins, glycosides, steroids, terpenoids and alkaloids in the orchid extracts (Kumari R. et al., 2017).

Table 2. The inhibitory activity of *Phalaenopsis* extracts on *Candida* and *Staphylococcus* pathogens

Pathogen	Inhibitory effect		
	Leafs - 1	Stems - 2	Roots - 3
Candida albicans	-	-	-
Candida quilliermondii	-	++	-
Candida parapsilosis	-	++	-
Candida krusei	-	-	+++
Staphylococcus aureus 43300 MRSA	-	+++	-
Staphylococcus aureus 6538	-	+	-
Staphylococcus epidermidis 12228	-	-	++
Staphylococcus epidermidis 51625	-	-	-

Legend: -: non inhibitory halo; +: low inhibitory activity (0.1-0.9 mm); ++: moderate inhibitory activity (1.0-1.9 mm); +++: high inhibitory activity (: > 2 mm).

Tannins (commonly called tannic acid) are polyphenols, known as antimicrobial agents that precipitate proteins and prevent the development and growth of microorganisms, in this way, making nutritional protein unavailable to microbes. The growth of large numbers of fungi, yeasts, bacteria and viruses has been inhibited by tannins (Stahl, E., 1988).

The results may suggest a new valuable resource to replace the use of antibacterial drugs. Because of the abuse of antibiotics on a very large scale, as a panaceum, infections caused by multidrug-resistant strains are gaining resistance to antimicrobial drugs, due to their natural mechanisms, therefore antimicrobial agents of plant origin are needed to be use for the treatment of infections caused by multidrug-resistant strains. Considering that nowadays among the florists the orchids' waste, especially of *Phalaenopsis*, are registering increased quantities, their use to develop added-value pharmaceutical or cosmetics new products with antimicrobial activity can be a viable solution in an economy context.

CONCLUSIONS

This preliminary study has revealed that methanolic extracts made of stems and roots of *Phalaenopsis* wastes have potential antimicrobial activity, with an inhibitory effect on pathogens like methicillin resistant *Staphylococcus aureus* and different *Candida* species.

The antimicrobial activity of leafs extract against all tested microorganisms was very low, while in the case of stems and roots extracts, further antimicrobial activity should be performed.

In addition, other tests should be performed to prove any antioxidant potential of such extracts for a higher value of new products.

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