Assiut Journal of Agricultural Sciences 55 (1) 2024 (38-44) Website: http://ajas.journals.ekb.eg/
ISSN: 1110-0486 / EISSN: 2356-9840 E-mail: ajas@aun.edu.eg

(Original Article) `

Inhibitory Effect of Propolis Extract on Some Pathogenic Fungi

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DOI: 10.21608/AJAS.2024.241740.1299

Abstract

There is considerable potential for propolis, a complex compound with antibacterial capabilities, to be used as a preservative in dairy manufacturing. This study aimed to study the antifungal effect against four pathogenic fungi including *Aspergillus flavus, Aspergillus niger, Penicillium corylophilum* and *Rhizopus stolinefer*. In order to determine antifungal properties, the ethanolic extract of propolis was provided and then added to potato dextrose agar media as 100, 200, 400 and 800 µg/ ml. The study's statistical findings demonstrated that pure propolis extracts work better at boosting concentrations against infections' mycelial development. Additionally, propolis ethanol extracts had an antifungal effect on the fungi that were being examined. Moreover, the tested fungi are very affected by all the tested concentrations. The concentration of 800 µg/ml was the inhibitoriest of three concentrations studied; it showed more than 50% inhibition against all tested fungi.

Keywords: *Propolis, antimicrobial, preservative, antifungal, ethanolic extracts*

Introduction

Worker honeybees gather propolis, a resinous material, from the buds and exudates of a range of plants (Pieta *et al*., 2002; Silici and Kutluca, 2005; Gómez-Caravaca, *et al*., 2006 and Quiroga *et al*., 2006). Honeybees generate propolis to adhere their colony to the beehive wall, cover the hive's wells to maintain sterile conditions, smoothen the interior walls of the hive, and prevent wind from entering the hive by plugging any openings (Matsushige *et al*., 1997; Pieta *et al*., 2002; Gómez-Caravaca *et al*., 2006 and Quiroga *et al*., 2006). Propolis' primary purpose is to defend the city, which is derived from the Greek words pro-, which means "in defense," and -polis, which means "city." As a result, propolis' name means "defense of the city." (Burdock, 1998). Propolis has a distinctive, pleasant perfume, and depending on the botanical source and time of collection, it can range in color from yellow to green to red and dark brown. When heated, it also becomes soft, flexible, gummy, and extremely sticky. As a result of variations in the plant source, timing, and location of the Propolis collection, more than 300 chemicals, including phenolic acids, cinnamic acid, caffeic acid, terpenes, flavonoids, and esters, are identified in propolis. Ethanol is the most popular and commonly used

Received: 15 October 2023/ Accepted: 31 December 2023/ Published online: 1 January 2024

solvent for propolis extraction. Propolis has fungicidal and fungistatic effects on a variety of fungus species, which accounts for its antifungal capabilities. Among other functions, propolis aids in the treatment of burns, ulcers, diabetes, and asthma (Przybyłek, 2019).

Because of the possible application of the chemical compounds from these biomaterials that have been found as antifungal agents for the treatment of plant diseases, some researchers have concentrated their interest on natural sources involving plants and their derivatives (Begum et al., 2007). Therefore, there was a decrease in post-harvest losses (Park *et al*., 2008).

The main objective of this study is to evaluate Propolis' antifungal potential against *Aspergillus niger*, *Aspergillus flavus*, *Penicillium* sp., and *Rhizopus stolinefer* via means of *in vitro* experiments.

Materials and Methods

Collection of Propolis

Propolis samples were collected from the Agriculture Research Center, Egypt. Samples were collected, weighed, and then kept separately in a cold storage unit until they were used as described by Pepeljnjak *et al*. (1982) and Muszynska *et al*. (1993).

Preparation of propolis

The propolis samples were chopped down into small pieces and dissolved in ethanol at a 1:10 (ten grams of propolis in one hundred milliliters of ethanol 70%) ratio. After keeping the propolis samples at room temperature and shaking for two days, the ethanolic extract of propolis (EEP) was filtered (Gonsales *et al*., 2006).

Cultures of fungi

The fungal species investigated were *Aspergillus flavus*, *Aspergillus niger*, *Penicillium corylophilum* and *Rhizopus stolinefer*. All fungal species were provided by Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Assiut Branch.

Determination of antifungal activities of propolis extract

The antifungal activity of propolis extract was determined by the well agar diffusion method according to the NCCLS (1993). Potato dextrose agar (PDA) media (200 g potato, 20 g dextrose, and 20 g agar) was thawed at 45 °C and put onto 9 cm petri dishes. The dishes were fertilized with homogeneous discs of the tested fungus after cooling at room temperature against the tested Propolis extract 100, 200, 400 and 800 µg/1 ml dimethylsulphoxid (DMSO). All of the plates were incubated at 25±3 °C for 7 days before the inhibitory zone was determined as described by McKee *et al*. (1990).

Inhibition Percent (IP%)

Propolis solutions were calculated using Equation 1.

$$\text{Inhibition (\%)} = \frac{\text{gc} - \text{gt}}{\text{gc}} \times 100$$

Where, gc: Mycelial colony diameter measured after the incubation period in the control set, slighting the inoculum disc diameter; gt: Mycelial colony diameter measured after the incubation period, ignoring the inoculum disc diameter Deans and Svoboda (1990).

Statistical analyses

The SAS software for Windows release 8.02 TS level 02M0, SAS Institute Inc., Cary, NC, USA was used to perform an analysis of variance in the data (SAS, 1999). Data are presented as means and standard errors of the means of three experiments.

Results and Discussion

The effect of a variety of propolis extracts on fungus growth:

The effect of varying concentrations of propolis extract on the growth of four different fungi in vitro is presented in Table 1. During the measure, the inhibition zone and Mycelial colony diameter inside the petri dishes were evaluated. The obtained results showed that all concentrations of propolis extract negatively affected the growth of all treated fungi clearly compared to the control; there was an increase in fungal inhibition and a decrease of fungi growth with an increase in the concentration of Propolis extract.

Table 1. Antifungal activity of different Propolis extracts against some fungus

Concentrations (EPDMSO**) (µg/ 1 ml)	Average fungus diameter (mm)			
	Aspergillus flavus	***Aspergillus niger***	***Pénicillium corylophilum***	***Rhizopus stolinefer***
Control	40±1.73^{b}	43±0.58^{c}	37±1.15^{b}	40±1.15^{b}
100	28±0.00^{a}	35±0.58^{b}	28±1.15^{a}	28±0.58^{a}
200	28±1.15^{a}	33±1.15^{b}	28±0.00^{a}	28±0.58^{a}
400	27±0.58^{a}	33±1.73^{b}	27±1.73^{a}	28±0.58^{a}
800	26±0.58^{a}	29±0.58^{a}	25±0.58^{a}	26±0.00^{a}

*a, b, c; means ± SE. (Standard Error) difference between averages with same letters in same column is not significant, but different letters are significant (p≤0.05). ** EPDMSO: DMSO extract of Propolis.

On the other hand, significant differences (P≤0.05) were discovered between the treatments and the control in all of the treated fungus, and no significant differences (P≤0.05) were found in the majority of the treatments. These findings are consistent with those reported by Gozdenur *et al*. (2022), where three different propolis solutions were tested for antifungal efficacy against the pathogen F. *solani.* Also, agreement with Quintero-Cerón (2014), who observed that these species' mycelial growing in these species (*A. niger, R. oryzae, Penicillium sp*. and *B. cinerea*) can be slowed to a lower level by using propolis. In general, found that the growth mycelial colony diameter in all treated fungi decreased with

increasing the concentration of Propolis extract inside dishes until the end incubation period, with non-significant differences between the studied fungi species. Similar results were reported by Ezazi and Davari, 2018; Gul, 2019; Ozyigit, 2020 and Gozdenur *et al*., 2022. In addition, our study revealed that there was an antifungal effect of Propolis extract, this was confirmed by Ugur and Aslan, 2004 and Curifuta *et al*., 2012, who revealed that Propolis extract had an antibacterial effect on the growth of C. albicans at rising concentrations when compared to propolis acetone extracts.

Data presented in Table 2 shows the percent inhibition zone with the statistical results. The inhibition percentages were determined to increase with the increase of propolis extract in the dishes.

Table 2. The inhibition incidence percentage of different concentrations of Propolis extract against some fungi.

Concentrations (EPDMSO) (µg/ 1 ml)	Inhibition (%)			
	Aspergillus flavus	***Aspergillus niger***	***Pénicillium corylophilum***	***Rhizopus stolinefer***
Control	0	0	0	0
100	30.0	18.6	24.3	30.0
200	30.0	23.3	24.3	30.0
400	32.5	23.3	27.0	30.0
800	35.0	32.5	32.4	35.0

In general, the results in the same Table show that there was no inhibition in the control samples, so the inhibition percentage was (0%), while there was an inhibition percentage in all treatments with the same trend in Table 1, which indicates that the Propolis extract is a negatively affecting in the growth of the studied fungi compared to the control samples. Similar results were reported by Gozdenur *et al*. (2022).

Conclusion

Propolis is a prominent honeybee product that has been studied for its antifungal properties. In this work, we obtained extraction at four different concentrations to determine the antifungal efficacy against *A. niger*, *A. flavus*, *P. corylophilum* and *R. stolinefer*. Based on the experimental and statistical results of four different concentrations, our findings revealed that 800 g/1 ml of Propolis is effective. Propolis can be used as a protection for dairy products against certain fungi.

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التأثير التثبيطي لمستخلص البروبوليس على بعض الفطريات الممرضة

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الملخص

البروبوليس هو مادة معقدة لها خصائص مضادة للميكروبات وتستخدم كمادة حافظة في الصناعات الغذائية، والهدف من هذه الدراسة هو دراسة التأثير المضاد للفطريات الأربعة الممرضة *Aspergillus flavus* *Aspergillus niger, Pénicillium corylophilum,* *Rhizopus stolinefer* من أجل تحديد الخواص المضادة للفطريات، تم تحضير المستخلص الإيثانولي للبروبوليس وتمت إضافته إلى وسط أجار دكستروز البطاطس بنسبة 100 ، 200 ، 400 ، 800 ميكروجرام / مل، وقد أظهرت النتائج الإحصائية للدراسة أن المستخلصات النقية من البروبوليس أكثر فاعلية في زيادة التأثير ضد النمو الفطري لمسببات الأمراض، وأظهرت نتائج الدراسة أن مستخلصات البروبوليس من الإيثانول له تأثير مضاد للفطريات المدروسة، وكان تركيز 800 ميكروجرام / مل هو الأكثر تأثيراً من التركيزات التي تمت دراستها، وأظهرت تثبيطاً أكثر من 50% ضد جميع الفطريات المختبرة.