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The biological effectiveness of crude propolis extract in inhibiting the growth of phytopathogens

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Abstract. Propolis is produced by honeybees (*Apis*) from a series of non-toxic, mucilage-based resinous and balsamic substances collected from the leaf buds of various tree species and mixed by the bees with their saliva secretions. It is used as an insulating, sealant, and disinfectant in the cell. Because of its antimicrobial properties, propolis has become a popular alternative biocide or food additive for health protection and disease prevention. It has been shown that the abundance of a huge number of flavonoids, essential oils, phenolic compounds, and antioxidants is responsible for most of the biological and pharmacological activities of propolis. This study aims to provide a critical analysis of various studies evaluating the activity of propolis against fungi and to identify the chemical components responsible for this activity. Discussion of the methodological approaches used, and results released is a key point of this review to highlight knowledge gaps. In this review, we will first learn about the chemical composition of propolis, and the contrast agents used in their ability to inhibit pathogenic fungi. The study showed that increasing the concentration 12.5, 25, 50, 100% of propolis extract led to an increase in the rate of fungal growth inhibition *Fusarium oxysporum, Pythium aphanidermatum, Rhizoctonia solani*, we find that the concentration of 100 ml/L was superior, which achieved the highest percentage of inhibition of the growth of the three fungi, *Fusarium oxysporum, Pythium aphanidermatum*, and *Rhizoctonia solani*. The average percentage of inhibition was 85.36, 85.77, and 83.14 respectively.

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Introduction

Propolis is a natural brownish resinous substance collected by honeybees (Apis mellifera), with a documented bioactivity against many microorganisms. Propolis is another important bee product, such as honey, royal jelly, and bee venom. Propolis is also an important source of natural chemical compounds [1]. Propolis has been used as a medicinal product since ancient times by the Greeks, Romans, and Egyptians, and it is still used present time [2]. Honeybees collect resinous substance from the buds and leaves of different types of plants, such as poplar, pine, pineapple, willow, and palm trees [3]. It is a solid substance when cold, soft, and sticky when hot, and has a distinctive smell, and its color varies from vellowish green to dark brown [4]. Propolis is of different types, as mentioned [5], including the green type and the red type. [6] has shown that the green type is more effective from a medical standpoint, and honeybees use propolis to fill the hexagonal gaps in the hive and to glue its parts together. They also use it to mummify dead bees inside the hive and some organisms that enter their hives and which they kill. Bees cannot carry it outside the hive to avoid its rotting, such as cockroaches, butterflies, and mice, so it remains inside the hive without decomposing [7]. Because of its distinctive properties, chemical composition, and biological effectiveness against many microorganisms, it is now used as a natural treatment in several countries of the world [8]. Propolis contains more than 300 different components, such as polyphenols (flavonoids, phenolic acids, and esters), phenolic aldehydes and ketones. The percentage of these materials is as follows: plant resins and balsams 50%, beeswax 30%, pollen grains 5%, essential and fragrant oils 10%, and some other materials that also include organic compounds [9]. The composition is affected by extraction methods; It is generally produced through ethanol extraction, although some steps (such as maceration) are variable [10]. Propolis plays a crucial role in immune defense, largely due to its antioxidant properties, which stem from its diverse bioactive phytochemical constituents. These compounds include phenolic acids, flavonoids, esters, diterpenes, sesquiterpenes, lignans, aromatic aldehydes, alcohols, amino acids, fatty acids, vitamins, and minerals [11]. The wide array of observed biological activities can be attributed to the chemical diversity of these phytonutrients [12, 13]. The composition of

propolis varies based on the local vegetation and the period of collection [14]. Despite these differences, all propolis types exhibit antimicrobial activity, suggesting that this function is influenced by overall composition rather than specific compounds [15]. Worldwide, propolis is used in supplements and as a food or beverage additive [16], although its approval as a drug or dietary supplement depends on thorough chemical, technological, and toxicological evaluation. To diminish the environmental impact of currently available fungicides, many researchers have been searching for naturally occurring bioactive compounds that act differently from commonly known antifungals. Propolis is a resinous product consisting of compounds that bees collect from the vegetation, e.g., phenolic acids, terpenoids, caffeic acids, and flavonoids [17]. The aim of the study was to determine the effective chemical compounds of propolis extract and the role of the aqueous extract of propolis seeds in inhibiting the growth of some fungi that are phytopathogenic, to find alternative biological methods that help farmers dispense with chemical pesticides that are harmful to the environment, and to reduce the accumulation of chemical substances in breeding, agricultural crops, and the production of pesticides, as propolis is environmentally friendly, harmless, low cost and easy to apply.

Materials and Methods

The method of Harbon (1984) was followed in preparing aqueous extracts of propolis. 50 grams of the dry weight of the natural propolis were sterilized with a 1 % sodium hypochlorite solution, dried using filter paper, then dried by placing them in an electric oven at a temperature of 50 degrees Celsius and ground with an electric grinder. The resulting powder was kept in sterile glass jars until used in preparing the water extract, and placed in a 500-ml glass beaker containing 200 ml of distilled water. Then the plant material was mixed with an electric mixer on a hotplate for 30 minutes via 48 hours, and the solution was left for 30 minutes. After that, it was filtered using filter paper to separate large plankton, and the filtrate was transferred to the centrifuge. The extract was centrifuged at 3000 rpm for 10 min to sediment the smallest phytoplankton and obtain a fine plant extract [18].

The fungi used in the experiment. *Fusarium oxysporum, Pythium aphanidermatum, and Rhizoctonia solani* sp. fungi were obtained from the Phytopathology Laboratory, Agrobiotechnology Department, RUDN University, grown on the nutrient medium Potato Dextrose Agar (PDA), and incubated at a temperature of 25 °C. This isolate is characterized by the growth of white mycelium with branched edges on the upper surface of the dish and light cream on the lower surface [19]. In the experiment, 5 mm-diameter discs were excised from 5-day-old colonies of pathogenic fungi. A single disc of each fungal species was placed at the center of Petri dishes containing potato dextrose agar (PDA) medium, supplemented with varying concentrations of the propolis extracts under investigation. The plates were incubated at 25 °C for one week, after which colony growth was measured. The fungi used in the experiment were classified according to established diagnostic categories [20], [21]. This setup enabled the assessment of the antifungal activity of the different propolis extracts.

Testing the inhibitory capacity of plant extract. To detect the inhibitory ability against pathogenic fungi, four concentrations 12.5, 25, 50, 100% of the plant extract were added to the culture medium in addition to the control concentration, and discs with a diameter of 5 mm were taken from the pathogenic fungal isolates to a Petri dish with a diameter of 90 mm containing 20 ml of the culture medium for each of the pathogenic fungi used. In the experiment, there were three replicates for each of them and the same number of Petri dishes, and the dishes were incubated at a temperature of 25 °C. After a week, the growth rates of the fungi were estimated after completion of growth of the control fungus for each of the pathogenic fungi, and then the percentage of the extract's efficiency in inhibiting the growth of the fungi was calculated using Abbott equation (1925) [22, 23].

Percentage of inhibition = Fungal growth rate in comparison_ Fungal growth rate by treatment/ Fungal growth rate in comparison*100

Statistical Analysis. The experiment was carried out according to a completely randomized design in four replicates based on the **CRD** factor to compare different fungi and four concentrations of aqueous extract of sumac. The recorded data to examine the antifungal extract were analyzed into percentages using a program (**Gene-STAT version 21**) [24].

Results and discussion

The current results are showed in Figure 1; the biologically active compounds present in natural propolis extract were studied using gas chromatography and mass spectrometry. They are expressed in terms of retention time (RT) and concentration (peak area%) and given in Table 1 and Figure 1, which show the presence of 8 bioactive phytochemical compounds in propolis extract belonging to specific groups of compounds. Among the plant compounds that were identified were a saturated **Pentadecane**, **Diethyl Phthalate**, **Docosane**, **Rosifoliol**, **Tricosane**, **Ethanone-1,2-diphenyl-2-[(trimethylsilyl)oxy]**, **3-Methylheneicosane**, **Alkanox 240**. The analysis results were similar to the results of 8 chemical compounds, which showed the presence of many chemical compounds revealed by GC-MS analysis [25].

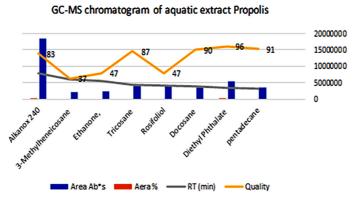


Figure 1. GC-MS analysis of aquatic extract of propolis

Source: compiled by M.H. Al-Mamoori, S.G. Okbagabir, E.N. Pakina, M. Zargar.

Figure 1 shows the spectroscopic GC-MS analysis of the chemical compounds of propolis extract. The diagram also shows the amount and peak area and quality of each chemical compound for extract of propolis, as detailed in Table 1.

Table 1 presents the results of the spectroscopic analysis (GC-MS) of the aquatic propolis extract which showed the following chemical compounds. At minute 18.719 at aera 3391986, the compound pentadecane appeared with a quality of 91 and the peak area was 7.85; at minute 19.694, at area 5400969, the compound Diethyl Phthalate appeared with a quality of 96, and the peak area was 12.49; at minute 22.813, at area 3579221, the compound Docosane appeared with a quality of 90, the peak area was 8.28; at minute 23.944, at area 4134337, the compound Rosifoliol appeared with a quality of 47, and the peak area was 9.56; at minute 26.471, at area 3857546, the Tricosane appeared with a quality of 87, the peak area was 8.92; at minute 32.417, at area 2248944, the compound Ethanone appeared with a quality of 47, the peak area was 5.20; at minute 35.452, at area 2113794, the compound 3-Methylheneicosane appeared with quality of 37, the peak area was 4.89; at minute 46.27, at the area 18504977, the compound Alkanox 240 was common in terms of peak area with a quality of 83, the peak area was 42.80. The mass spectrum was used to identify the name, molecular weight, and form of the components of crude propolis samples [26].

Table 1

No	RT, min	Area (Ab*s)	Peak Area%	Name	Quality	CAS Number
1	18.719	3391986	7.85	Pentadecane	91	000629-62-9
2	19.694	5400969	12.49	Diethyl Phthalate	96	000084-66-2
3	22.813	3579221	8.28	Docosane	90	000629-97-0
4	23.944	4134337	9.56	Rosifoliol	47	000000-00-0
5	26.471	3857546	8.92	Tricosane	87	000638-67-5
6	32.417	2248944	5.20	Ethanone, 1,2-diphenyl-2- [(trimethylsilyl)oxy]	47	026205-39-0
7	35.452	2113794	4.89	3-Methylheneicosane 37		006418-47-9
8	46.27	18504977	42.80	Alkanox 240 83 085454		085454-97-3

Chemical compounds by Spectral analysis results by GC–MS chromatogram of seeds aqueous extract of propolis

Source: compiled by M.H. Al-Mamoori, S.G. Okbagabir, E.N. Pakina, M. Zargar.

The results in Table 2, Figure 2 indicate that all concentrations of the propolis aqueous extract were superior to the control in inhibiting the growth of the fungus, as the average colony diameter was estimated at, respectively, 35.73, 27.14, 20.75, and 12.55 mm using the aquatic extract of crude propolis at concentrations of 12.5, 25, 50, and 100 ml/L compared to the control, which gave the highest value for the average diameter of the fungal colony. Comparing the different concentrations, we found that the concentration exceeded 100 ml/L, as it achieved the highest rate of inhibition of

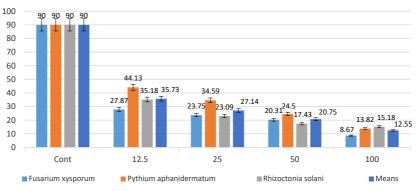
the growth of the three fungi (*Fusarium oxysporum, Pythium aphanidermatum*, and *Rhizoctonia solani*). The average diameter of the fungal colonies was 8.67, 13.82, and 15.18 mm, respectively. This is due to the role of the active compounds, and the volatile oil present in the extract contains compounds that are effective in the growth of fungi. This is consistent with Auriane Dudoit, et al. [27].

Table 2

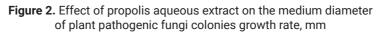
Effect of propolis aquatic extract on the medium diameter of plant pathogenic fungi colonies growth rate, compared with the control, mm

Fungus	Cont	12.5 %	25 %	50 %	100
Fusarium oxysporum	90.00	27.87	23.75	20.31	8.67
Pythium aphanidermatum	90.00	44.13	34.59	24.50	13.82
Rhizoctonia solani	90.00	35.18	23.09	17.43	15.18
Means	90.00	35.73	27.14	20.75	12.55
L.S. D	A = Extract 2.960		B = Fungi 2.564		A + B = 5.127

Source: compiled by M.H. Al-Mamoori, S.G. Okbagabir, E.N. Pakina, M. Zargar.



Fungal Growth Rate



Source: compiled by M.H. Al-Mamoori, S.G. Okbagabir, E.N. Pakina, M. Zargar.

The aquatic extract for crude propolis achieved a significant effect in inhibiting the growth of the fungus (35.73, 27.14, 20.75, and 12.55), as the averages (Table 3, Figure 3) were estimated, respectively, for the concentrations of the water extract **ml/L** compared to the control. Comparing the concentrations, we found that the concentration of 100 **ml/L** was superior, which achieved the highest percentage of inhibition of the growth of the three fungi, *Fusarium oxysporum, Pythium aphanidermatum*, and *Rhizoctonia solani*. The average percentage of inhibition was (85.36, 85.77, and 83.14%), respectively. By comparing the average percentage of inhibition of the fungus, we found a difference

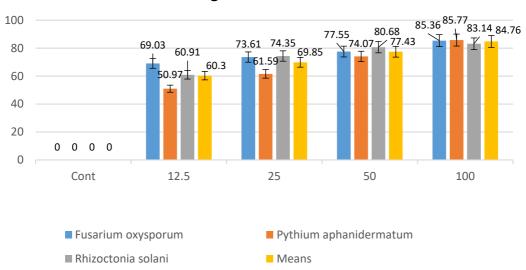
in sensitivity of the pathogenic fungi to the aqueous plant extract, as the extract achieved the highest percentage of inhibition of *Rhizoctonia solani*, followed by *Rhizoctonia solani*, then *Pythium aphanidermatum*; this is due to the role of the active compounds present in the extract and a small percentage of the essential oil, which contains active compounds soluble in water. A small amount of essential oil contains active compounds that affect the growth of fungi. This has been confirmed by previous studies. And this is consistent with Dudoit A. et al [27].

Table 3

Fungus	Cont	12.5%	25%	50%	100%
Fusarium oxysporum	0.00	69.03	73.61	77.55	85.36
Pythium aphanidermatum	0.00	50.97	61.59	74.07	85.77
Rhizoctonia solani	0.00	60.91	74.35	80.68	83.14
Means	0.00	60.30	69.85	77.43	84.76
LSD	A= Extract 3.828		B= Fungi 3.315		A+B 6.630

Effect of Rush coriaria L. on inhibition growth fungi, Percentage of inhibition rate (100%)

Source: compiled by M.H. Al-Mamoori, S.G. Okbagabir, E.N. Pakina, M. Zargar.



Precentage of Inhibition Growth

Figure 3. The inhibition ratio of Fusarium oxysporum, Pythium aphanidermatum, and Rhizoctonia solani. at various concentrations of propolis aqueous extract

Source: compiled by M.H. Al-Mamoori, S.G. Okbagabir, E.N. Pakina, M. Zargar.

Conclusion

This study investigates that the aqueous extract of propolis at four concentrations (12.5, 25, 50, and 100%) effectively inhibits the growth of pathogenic fungi *Fusarium oxysporum, Pythium aphanidermatum*, and *Rhizoctonia solani*. The highest inhibition rate was observed at the highest concentration (100%), where *Rhizoctonia solani*. exhibited the lowest growth rate (83.14%) and the highest inhibition rate (85.77%) for *Pythium aphanidermatum*. The presence of bioactive compounds such as Diethyl Phthalate, docosane, Alkanox 240, and Tricosane identified through GC-MS analysis underpins the antifungal properties of the extract. These compounds contribute to the antifungal activity, suggesting that propolis extract could be a viable bio-fungicide. Given its efficacy, low cost, and environmental friendliness, propolis extract presents a promising alternative to commercial chemical fungicides. Its application could reduce chemical fungicides' environmental and health impacts, supporting sustainable agricultural practices and enhancing crop protection naturally.

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Биологическая эффективность экстракта прополиса для замедления роста фитопатогенов

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Аннотация. Прополис производится медоносными пчелами (Apis) из ряда нетоксичных, муцилажных смолистых и бальзамических веществ, собранных с листовых почек различных видов деревьев и являющихся продуктом жизнедеятельности пчел. Он используется в качестве изоляционного, герметизирующего и дезинфицирующего средства. Благодаря своим антимикробным свойствам прополис стал популярным альтернативным биоцидом или пищевой добавкой для профилактики заболеваний. Показано, что наличие большого количества флавоноидов, эфирных масел, фенольных соединений и антиоксидантов отвечает за большинство биологических и фармакологических свойств прополиса. Целью исследования — критический анализ различных работ, оценивающих активность прополиса против грибов, и выявление химических компонентов, ответственных за эту активность. Обсуждение использованных методологических подходов и полученных результатов является ключевым моментом исследования. Представлен химический состав прополиса, а также информация о веществах, входящих в состав, и их способности ингибировать патогенные грибы. Исследование показало, что повышение концентрации (12,5, 25, 50, 100%) экстракта прополиса привело к увеличению скорости ингибирования роста грибов Fusarium oxysporum, Pythium aphanidermatum, Rhizoctonia solani. Обнаружено, что концентрация 100 мл/л была наиболее эффективной и позволила достичь самого высокого процента ингибирования роста грибов Fusarium oxysporum, Pythium aphanidermatum u Rhizoctonia solani. Средний процент ингибирования составил 85,36, 85,77 и 83,14 соответственно.

Ключевые слова: ГХ-МС анализ, химические соединения, Fusarium oxysporum, Pythium aphanidermatum, Rhizoctonia solani sp.

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