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Therapeutic Effect of Propolis against Biofilm Gene Expression in *Candida albicans*

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ABSTRACT

The sticky substance called propolis is made from plants and is produced by honeybees. It has been used as a folk remedy since ancient times, and it has numerous pharmaceutical benefits, such as antibacterial and antifungal. The objective of this work was to determine the impact of propolis on the expression of three genes (*Ece1*, *Sap5*, and *Als3*) known to be implicated in the development of *C.albicans* biofilms and define the minimum inhibitory concentration of propolis required for this purpose. The XTT test was used to assess the anti-biofilm activity of propolis in order to ascertain the formation of biofilm on 100 *C. albicans* isolates from stool samples and calculate the minimum inhibitory concentration of propolis that inhibits the biofilm of *C. albicans* during 24 and 48 h. Finally, the impact of propolis on the expression of the *Ece1*, *Sap5*, and *Als3* genes in *C. albicans* was examined using a real-time polymerase chain reaction and compared with the results that appeared in the gene expression of the biofilm *C. albicans* untreated propolis during 24 and 48 h, and it was considered a control. Through comparison, biofilm formation was found to decrease as propolis concentration and time increased. Accordingly, the MIC of propolis was 40% w/v, and its minimum fungicidal concentration (MFC) was 50% (w/v) in biofilm-forming *C .albicans*. Additionally, gene expression level analysis revealed a decrease in *Ece1*, *Sap5*, and *Als3* expression levels with propolis treatment during 24 and 48h.

Keywords: Expression; Ece1; Sap5; Als3; genes.

INTRODUCTION

A healthy human body with a functioning immune system naturally contains *C. albicans* in its microbiome (1). However, several factors combine to create a balance disorder that facilitates the fast growth of this fungus and infection (2, 3). *C. albicans* infection can be caused by a number of factors, including when the immune system is suppressed or there is a disturbance in the host environment. *C. albicans* can rapidly transition to pathogens that can cause various infections. For example, it is well recognized that *C. albicans*' virulence factors, particularly its involvement in biofilm formation, account for the fungus' resistance to both the immune and antifungal systems (4). *C. albicans* biofilm formation starts in the lab when round yeast cells stick to a solid surface. The subsequent phase of biofilm formation involves cells' growth and the attached cells' initial filamentation. The next step is biofilm maturation, which produces a complex network of multiple layers of polymorphic cells encased in an extracellular matrix. These cells include round yeast cells, pseudohyphal cells, ellipsoidal cells joined end to end, hyphal cells, and chains of cylindrical cells. The extracellular matrix gives the biofilm a thick, structured appearance and protects it from physical and chemical damage (5, 6). Mature biofilms usually form in less than 24 hours, and when viewed under a microscope, they appear as an ordered collection of various cell types. To the unaided eye, they appear as a cloudy surface structure on top of a solid surface. The least researched stage of *C. albicans* biofilm development is the dispersal stage,

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which is the last stage of biofilm development, where some round yeast cells scatter from the biofilm to seed new sites (7, 8).

Propolis is one of the natural product's alternative therapies. Honeybees naturally produce a substance called propolis (9). Brazilian propolis breaks the extracellular polymeric substance down, which also causes cell death and prevents *C. albicans* from forming biofilms in vulvovaginal candidiasis (10). A flavonoid is one of the substances in propolis that has been shown to have antibiofilm properties, such as pinocembrin, apigenin, chrysin, and kaempferol (11). However, the kind of plant the bees live in, when the propolis is collected, and where the propolis is from can all affect its composition (12).

C. albicans possesses a particular class of proteins called adhesins, which help the organism adhere to host cells, other microbes, abiotic surfaces, and other *C. albicans* cells. There are eight members of the *C. albicans* adhesions family, which are agglutinin-like sequence (Als) proteins (Als1–7 and Als9) (13). *Candida* species produce two main families of histolytic enzymes: phospholipases (PL) and secretory aspartyl proteinases (Sap) (14). Among the virulence factors in *C. albicans* that have been studied the most are the secreted aspartic proteases (Saps) (15).

Numerous biofilm genes code for predicted or known cell wall proteins. These proteins are particularly interesting because heterologous expression studies suggest that *Als1* and *Als3* have roles in cell-substrate or cell-cell adhesion (16). Previous research has shown that the development of biofilms on mucosal surfaces is linked to the expression of genes from the *Als* and *Sap* gene families, and the hyphae-specific protein *Ece1*(17). *C.albicans*' ability to form hyphae is a crucial component of its virulence because it allows the fungus to adhere to host cells, invade them, and eventually spread throughout deep tissues (18).

The purpose of this study was to ascertain the effect of propolis on the expression of three genes (*Ece1*, *Sap5*, and *Als3*) that are involved in *C. albicans* biofilm formation. Also, this experiment was to find out whether propolis could prevent the growth of *Candida* biofilms and the minimum concentration of propolis needed to accomplish this.

MATERIALS AND METHODS

Sample collection

A total of 100 stool samples were examined for isolated *C. albicans*. Samples were taken from gastrointestinal patients, ranging in age from 1 to 50 years, who visited the pediatric teaching hospital in Samawa City from October 2021 to May 2023. The Al-Muthanna University ethics committee gave its approval for this study.

Candida albicans isolation

Sterile phosphate-buffered saline (PBS) was used to dilute small samples of freshly provided stool specimens, which were then thoroughly vortexed in a 1.5 ml Eppendorf tube. About 100 microliters of this suspension were distributed across a minimum of three solid culture media, which were always Potato Dextrose Agar (PDA), Yeast Peptone Dextrose Agar supplemented with 0·05 g/L chloramphenicol, and Sabouraud Dextrose Agar with 0·05 g/L chloramphenicol. The plates were then incubated for up to 60 hours at 37°C in an aerobic incubator. Once microbial growth was detected, each plate was sealed and kept at 4°C until it was time for additional processing.

Serum is added to promote the growth of *C. albicans*, and when the yeast cells are examined under a light microscope, they resemble large cocci and develop into germ tubes. When *C. albicans* was isolated and identified using Sabouraud dextrose agar and CHROM agar, the colony of the fungus looked green. API (Analytical Profile Index) Candida (HIMDIA/India) employed the biochemical test for *C. albicans* detection to validate the identification of the morphological, cultural, and biochemical characteristics required to differentiate between *Candida* species and to demonstrate that *Candida* can utilize different types of sugar.

Propolis collection

Propolis was taken directly from honeybee hives in Samawah city from 21 March to 21 June 2022. These hives

reliant on apricot, apple, and grape trees for their sustenance. The models were kept in 200 ml sterile, tightly sealed bottles. Each bottle was then labeled with specific information, such as the model type and the collection date until use (19).

Minimum Inhibitory Concentration (MIC) determination

The macro broth dilution assay was used to calculate the MICs of natural propolis against the biofilm of *C. albicans*. Propolis was diluted in YPD (Yeast extract Peptone Dextrose) broth in steps of 80–5% w/v. Under vigorous shaking (120 revolutions per minute), The cultures were incubated at 35 °C for 36 hours. Using the broth from the incubation period, 10^3 CFU (Colony Forming Unit) of *Candida* were aseptically injected into Petri dishes that contained Sabouraud dextrose agar. Following a 48-hour period, the colonies' growth was evaluated, and the MIC of propolis (w/v) was found to be the one that prevented *C. albicans* from growing significantly (20).

Formation of Biofilms

The XTT(2,3-bis(2-metoxy-4-nitro-5-sulphophenyl)-2H-tetrazolium-5-carboxanilide inner salt) reduction assay was used to assess the biofilms' growth in 96-well flat-bottomed plates. Utilizing a technique derived from Lal *et al*. (21) and polystyrene microtiter plates. The kinetics of biofilm inhibition were studied using various MIC dilutions of propolis in YPD broth (5% w/v, 10% w/v, 20% w/v, 40% w/v, and 80% w/v) in order to ascertain whether propolis could prevent the formation of *Candida* biofilms and to find the minimum concentration of propolis that could do so. Every MIC dilution in a microtiter plate was analyzed in at least 7 wells. The microtiter plate wells were filled with aliquots of each dilution, each holding 190 milliliters. 10 milliliters of YPD broth containing 5×10^8 CFU/ml were used to cultivate *C. albicans* for 48 hours. To ensure uniform distribution and adherence to the well surfaces, 10 microliters of this 48-hour culture were added to each well and incubated for 1.5 hours at 37°C in an orbital shaker operating at 75 rpm. In order to remove non-adherent cells, adherent cells were carefully washed twice in sterile phosphate buffered saline (PBS) with a pH of 7.4, being cautious not to disturb the adherent cells. This process was done after 1.5 hours. Following washing, 200 ml of a second aliquot of the same propolis dilution in sterile YPD broth was added to each plate well. After that, the plates were kept in the same incubator for 48 hours so that the biofilms could settle in and grow. As a control, 200 ml of autoclaved YPD broth was added to each of the seven micro-titer plate wells. The broth could be positive (containing *Candida*) or negative (not containing *Candida*). The plate was then incubated at 37°C for 48 hours (21).

The purpose of the experiment was to determine whether propolis could disrupt established *C. albicans* biofilms. Ten milliliters of 5 x 108 CFU/ml were used to cultivate biofilms in 96-well microtiter plates. Place C. albicans in YPD onto the microtiter plate. To ensure even dispersion and adherence to the well surfaces, the plate was put in an orbital shaker set to run at 75 rpm for 1.5 hours at 37° C. After an hour and a half, non-adherent cells were extracted by gently washing them twice in sterile PBS (phosphate buffered saline), which had a pH of 7.4. To promote appropriate adhesion and the formation of biofilms in the absence of propolis, 150 milliliters of sterilized YPD broth was added to each well. The plate was then incubated again for 24 to 48 hours at 37 degrees Celsius. 200 ml final volumes of various propolis concentrations (5% w/v, 10% w/v, 20% w/v, 40% w/v, and 80% w/v) were added to each well in the YPD broth. After that, the plate was stored for 48 hours at 37°C. The XTT reduction assay was used for quantification in each triplicate experiment (22).

Biofilm Evaluation with the XTT Reduction Assay

The cells in the biofilms were measured using the XTT assay following propolis treatment. Used a filter with a pore size of 0.22 mm to filter and sterilize after making the XTT solution (1 mg/ml in PBS). Prior to the commencement of every assay, the menadione solution and XTT solution were

mixed at a 5:1 (v/v). To stop the adherent biofilms from growing further, 2.5% glutaraldehyde was applied for five minutes to the microtiter plate wells after three PBS cleanings. Following the removal of the fixative, two PBS washes were applied to the wells. In all but the control well, which had no biofilm, 1 mL of PBS was added to the XTT-menadione solution. After that, the MTPs (Metyrapone) were maintained in the dark for two hours at 37 °C. After the incubation period, 75 ml of XTT-menadione solution was added to each well on a fresh microtiter plate, and spectrophotometry was used to measure the absorbance at 492 nm (22).

RNA isolation and RT-PCR

The PrestoTM Mini gDNA Yeast Kit was used to extract total RNA from *C. albicans* culture cells and co-culture them with propolis in accordance with the manufacturer's instructions. The concentration and degree of RNA presence were determined using a Nanodrop-1000 UV-VIS spectrophotometer. For the agarose gel electrophoresis, RNA templates were employed. The cDNA Synthesis SuperMix Kit and EasyScript One-Step Gdna Removal were used to perform the reverse transcription PCR cDNA synthesis. One cycle at 42°C for 30 minutes and 85°C for 5 seconds was the RT-PCR setup (23).

mRNA transcripts were detected using real-time PCR with the Mx3000p (Agilent Technologies, USA). 10 mM primers were used to prepare the PCR components, which were then amplified by the genes in **Table 1** (24). In this experiment, the housekeeping gene beta-actin is amplified in the forward direction. 5'-CGTCGGTAGACCAAGACACC-3' and the opposite direction 5'-CCCAGTTGGAGACAATACCGT-3', A final volume of 20 µl was achieved by adding nuclease-free water, 200 ng of cDNA, and 10 µl of Luna universal qPCR master mix. The following parameters were used to run the PCR: thirty seconds at 94°C, 45 cycles of 94°C for five seconds, and thirty seconds at 60°C. Then, a dissociation curve was employed, with a single cycle lasting 1.0 minute at 95°C, 30 seconds at 55°C, and 30 seconds at 95°C (24).

Table (1): The PCR Primers for Real-Time Reverse Transcriptase

Genes	primer	Product size
Sap5	Forward 5'-CCAGCATCTTCCCGCACTT- 3' Reverse 5'-GCGTAAGAACCGTCACCATATTTAA- 3'	71bp
EceI	Forward 5'-CCAGAAATTGTTGCTCGTGTTG-3' Reverse 5'-CAGGACGCCATCAAAAACG-3'	138bp
Als3	Forward 5'-CAACTTGGGTTATTGAAACAAAAACA-3' Reverese 5'-AGAAACAGAAACCCAAGAACAACCT-3'	80bp

Statistical Analysis

Different means of biofilm biomass (absorbance) were compared using the ANOVA and t test. Data analysis was performed using GraphPad software.

RESULTS

MIC determination

Propolis exhibited concentration-dependent inhibition of *C. albicans* growth. Propolis's MIC was 40% (w/v), and its minimum fungicidal concentration (MFC) was 50% (w/v) in biofilm-forming *C .albicans*. The MFC required to eliminate 99.9% of the inoculum. In most cases, the MFC value exceeds the MIC value. Growth curves for yeast exposed to 40% (w/v) propolis for 24 hours revealed a reduction in both the total number of cells and the growth rate when compared to cell growth without propolis (Figures 1 and 2). There was no evidence of *C. albicans* growth in the growth assays using 50% (w/v) propolis. Biofilms were allowed to establish for a full day before being incubated for varying periods of time with and without 40% w/v propolis. The biofilm biomass was then measured in order to track the efficacy of propolis over

time. During this study, a decrease in the biofilm biomass values of *C. albicans* was observed when propolis was added to it, in contrast to the values when propolis was not added and was considered a control, especially at 24 to 48 hours.The results of the one-way ANOVA test indicated a significant difference between the treatment group and the control group at p< 0.01.

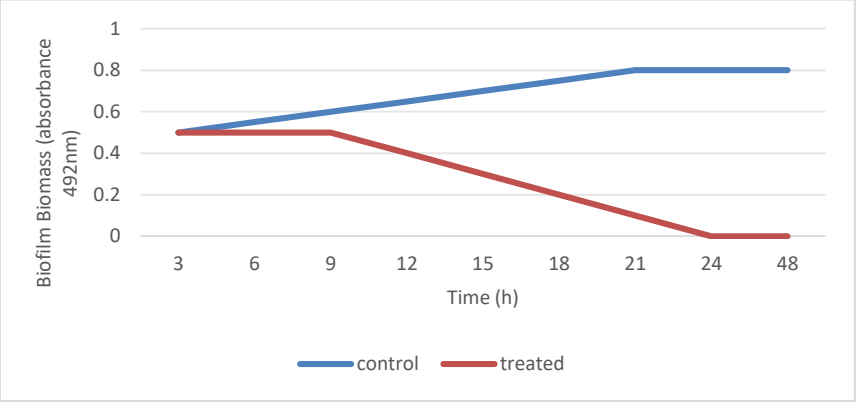

Figure (1): Growth evaluations of propolis-treated established *Candida albicans* biofilms during 24 to 48 h. (F=25.71291, P< 0.01)

Reducing the Formation of Biofilms

It achieved this by using propolis in YPD broth at varying concentrations (5% w/v, 10% w/v, 20% w/v, 40% w/v, and 80% w/v). It was found that the concentration of propolis affected the inhibition of biofilm formation. propolis concentrations less than 10% w/v were shown to neither inhibit nor promote the growth of biofilm (Figure 2). Biofilm formation was considerably inhibited by concentrations greater than 10% w/v. The control and treatment groups differed significantly at p< 0.01, according to the one-way ANOVA test analysis.

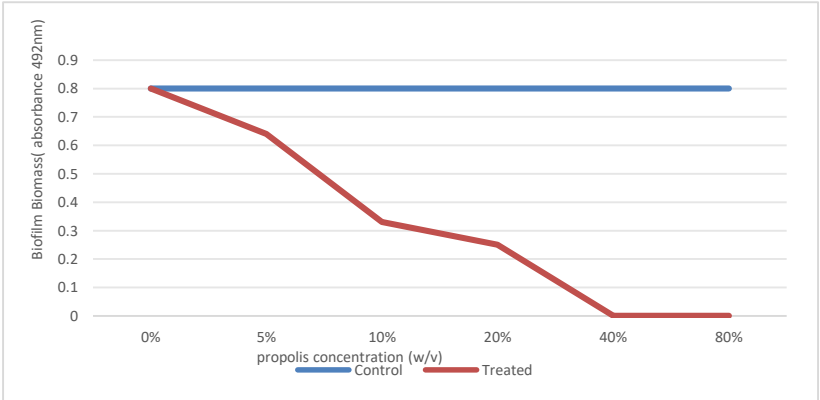

Figure (2): Propolis's impact on the development of *C. albican* biofilms. Propolis's MIC was 40% (w/v), and its minimum fungicidal concentration (MFC) was 50% (w/v) in biofilm-forming *C .albicans* during 24 to 48h. (F=11.90597, P< 0.01)

Transcriptase real-time PCR

Reverse transcription real-time PCR was used to measure the relative expression of the implicated genes (*Ece1*, *Sap5*, and *Als3*) in *C. albicans* throughout the biofilm experiment for 24 and 48 hours.

The expression of the biofilm genes developed by *Candida albicans* was evaluated by quantitative reverse transcriptase PCR in this study. Biofilm genes (*Sap5*, *EceI*, and *Als3*) increased dramatically in expression over time without the treatment of propolis, and It is considered a measure of control when compared with the genes encoding the biofilm treated with propolis (Figure 3). According to the t-test analysis, the biofilm genes expression was a $p>0.05$, considered statistically nonsignificant.

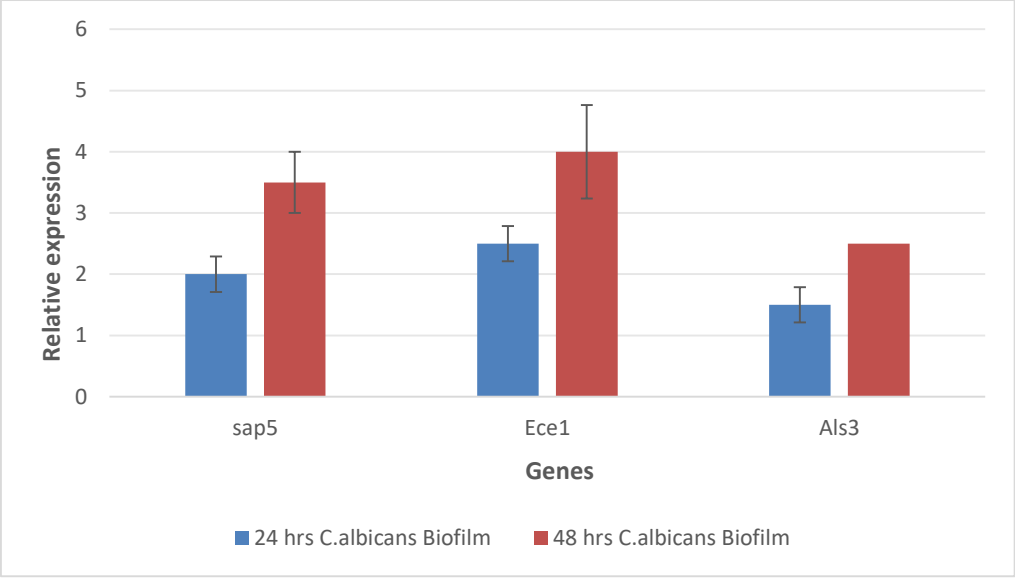

Figure (3): the relative expression of the implicated genes (*Ece1, Sap5, and Als3*) in *C. albicans* during the biofilm experiment without treated propolis and it is considered as control for 24 and 48 hours using reverse transcription real-time PCR. (t= 2.5298, df=4, standard error of difference = 0.527) p>0.05 was considered statistically nonsignificant

C. albicans cells exposed to propolis had less biofilm formation for 24 hours, according to results from a real-time PCR using the specific primers *Ece1*, *Sap5*, and *Als3*. Figure 4 shows that the *Als3* (0.5), *Sap5* (1), and *Ece1* (1.5) genes were treated with propolis, respectively. This indicates the inhibitory role of propolis on the *C. albicans* biofilm within 24 hours compared to the control gene expression. The expression of the biofilm genes, according to the t-test analysis, was a $p>0.05$, considered statistically nonsignificant.

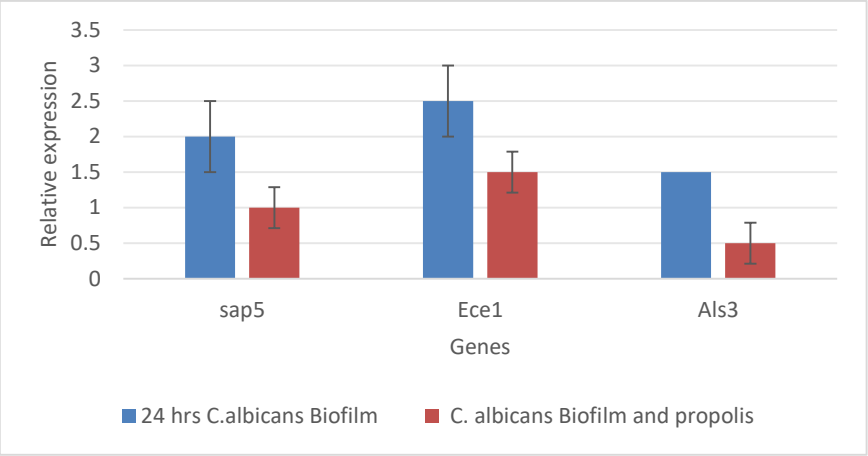

Figure (4): the relative expression of the implicated genes (*Ece1, Sap5, and Als3*) in *C. albicans* during the biofilm experiment for 24 and *C. albicans* biofilm with propolis using reverse transcription real-time PCR. (t=2.4495, df=4, standard error of difference= 0.408) p>0.05 was considered statistically nonsignificant

Results from a real-time PCR using the particular primers *Ece1*, *Sap5*, and *Als3* showed that *C. albicans* cells exposed to propolis had less biofilm formation for 48 hours. Figure 5 illustrates that propolis was applied to the *Als3* (1), *Sap5* (1.5), and *Ece1* (2) genes, respectively.

These results are conclusive evidence thatpropolis is a highly efficient inhibitor of the biofilm of *C. albicans.* It can be used as therapeutic alternatives for many diseases caused by *C. albicans*. These results have an effective effect on the genes responsible for the formation of the biofilm of *C. albicans* (**Figure 5**). The expression of the biofilm genes according to the t-test analysis was a p<0.05, considered statistically significant.

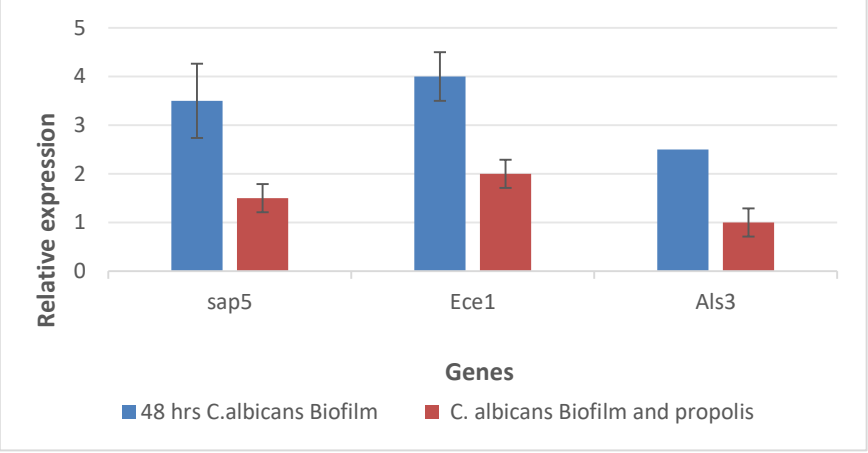

Figure (5): the relative expression of the implicated genes (*Ece1, Sap5, and Als3*) in *C. albicans* during the biofilm experiment for 48 and *C. albicans* biofilm with propolis using reverse transcription real-time PCR. (t= 3.4785, df= 4, standard error of difference= 0.527) p<0.05 was considered statistically significant

DISCUSSION

Propolis is a great natural food item with antioxidants, minerals, and simple sugars. Propolis can inhibit oxidative food deterioration processes, such as lipid oxidation in meat, fruit, and vegetable browning. Propolis inhibits the growth of microorganisms and foodborne pathogens that cause food spoilage (25, 26). Numerous studies have demonstrated the antimicrobial properties of propolis, which enable it to help treat a range of oral infections, including mucositis, ulcers, and periodontal disorders (27,28,29,30).

Studies have shown its value in treating fungal infections brought on by *Candida* species with natural remedies like propolis (31, 32). Propolis's antimicrobial activity has been studied recently as a potential substitute for traditional therapeutic approaches; however, its antifungal activity is still underappreciated, necessitating further research to fully understand its potential therapeutic application. With a MIC range of 0.185 to 3 lg/mL, an ethanolic extract of Turkish propolis exhibited the strongest antifungal activity against 76 *Candida* isolates that were isolated from the blood cultures of patients in intensive care units (33, 34, 35). On the other hand, little knowledge exists regarding propolis and its impact on *C. albicans* biofilms. This study's main objective was to ascertain whether propolis could disrupt or stop the formation of *C. albicans* biofilms. We chose to use propolis for our investigation because a variety of infections and illnesses are frequently treated with it as a folk remedy. *C. albicans* cultures were found to be successfully inhibited by a few different types of propolis, the most effective of which came from different plant origins and geographical locations. The literature emphasizes the critical role that geographic location plays in the types of propolis, primarily due to regional variations in climate and ethnobotanical flora (36). The best propolis can be found in poplar, alder, willow, elm, birch, horse chestnut, beech, and conifer tree species (37). Poplars are widely distributed throughout Europe, North America, Asia, and New Zealand. Unlike poplar propolis, which is derived from Betula verrucosa, Russian birch propolis contains both flavonols and flavones (38). Red propolis, which is found in large quantities in Brazil, Cuba, Mexico, China, and Venezuela, is derived from the plants *Dalbergia ecastophyllum, Clusia scrobiculata, Clusia minor, Clusia major*, and *Clusia rosea*. Its active phytochemicals are polyisoprenylated benzophenones (39). Similarly, the collection of Brazilian propolis is accounted for by the leaf resin of Baccharis dracunculifolia, which contains a range of phytochemicals such as higher concentrations of artepillin C, lignans, p-coumaric acid, acetophenone, and flavonoids (40). Sesquiterpenoid compounds, such as spatulenol, ledol, and germacren D, are phytochemicals that are exclusive to tropical climates. Furthermore, a type of propolis typical of the Mediterranean region can be found in Greece, Cyprus, Croatia, Egypt, Algeria, Morocco, and Malta. Its primary constituents are diterpenes, which are most likely derived from conifers in the Cupressaceae family (41, 42, 43). It was decided that propolis could be tested against *C. albicans* biofilms one more time. The development of new *C. albicans* biofilms were found to be inhibited, and existing ones were prevented by the MIC of propolis.

This study is the first to identify propolis as an inhibitor of Candida albicans biofilm genes using quantitative reverse transcriptase PCR through study propolis's MIC was 40% (w/v), and its minimum fungicidal concentration (MFC) was 50% (w/v) in biofilm-forming *C .albicans* during 24 to 48h.

The development of biofilms on the surface of yeast-form cells appears to be dependent upon adhesion genes, such as *Als3*, and their direct interaction with one another during the process (44, 45). In addition to the *Sap5* gene being found in biofilm adhered to mucosal surfaces, *Ece1* expression was associated with *Candida* hyphal elongation (46, 47, 48).

Previous research has shown that biofilm drug resistance is a complex phenomenon that is influenced by

a number of variables, including the amount of matrix formation, the makeup of the biofilm, and the simultaneous presence of bacteria in the biofilm. These variables can cause clinical problems when antifungal therapies are used (49, 50). The necessity of researching novel antifungal compounds is demonstrated by treatment failure (51). The most promising antifungal treatments seem to be natural goods like medicinal herbs (52, 53).

Propolis damages cells through a number of different mechanisms. Propolis has a reputation for harming cytoplasm and cell walls (54, 55). Nevertheless, the integrity of the cell wall is crucial for cell division. Then, the flavonoid in propolis has the ability to inhibit the process of oxidative phosphorylation, which in turn inhibits the formation of ATP. It may contribute to the inadequate growth of energy. Cell viability will thus decline (56, 57).

Limitation of the study

We limited the genes encoding the biofilm of *C. albicans* because they play a more significant role in encoding the biofilm than other genes. Also, the type of propolis used in the study was not specified, as this is the first study to determine the minimum inhibitory concentration and its effect on the genes encoding the biofilm of *C. albicans*.

CONCLUSION

According to the results, propolis's MIC was 40% (w/v), and its MFC was 50% (w/v) in biofilm-forming *C .albicans* and it can inhibit the gene expression (*Ece1*, *Sap5*, and *Als3*) *C. albicans* biofilms. Propolis has been shown to have strong antifungal properties, which implies that it may contain compounds with therapeutic value for treating infections caused by *Candida*. Whether these results can be used to treat biofilm-associated candidiasis requires additional in vivo testing.

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Conflicts of interest:

The authors declare no conflict of interest.

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التأثير العلاجي للبروبوليس ضد التعبير الجيني للأغشية الحيوية في المبيضات البيضاء

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ملخص

المادة اللزجة المسماة البروبوليس مصنوعة من النباتات وتنتجها نحل العسل .وقد استخدمت كعلاج شعبي منذ العصور القديمة، ولها فوائد صيدلانية عديدة، مثل كونها مضادة للبكتيريا والفطريات .كان الهدف من هذا العمل هو تحديد تأثير البروبوليس على التعبير عن ثلاثة جينات (Ece1و Sap5و Als3)معروفة بتورطها في تطوير الأغشية الحيوية للمبيضات البيضاء وتحديد الحد الأدنى من تركيز البروبوليس المثبط المطلوب لهذا الغرض .تم استخدام اختبار XTT لتقييم النشاط المضاد للأغشية الحيوية للبروبوليس من أجل التأكد من تكوين الأغشية الحيوية على 100عزلة من *C. albicans*من عينات البراز وحساب الحد الأدنى لتركيز البروبوليس المثبط الذي يثبط الأغشية الحيوية لـ C. albicans خلال 24و 48ساعة .أخيرًا، تم فحص تأثير البروبوليس على التعبير عن جينات Ece1و Sap5و Als3في *C. albicans*باستخدام تفاعل البوليميراز المتسلسل في الوقت الحقيقي ومقارنته بالنتائج التي ظهرت في التعبير الجيني للأغشية الحيوية *C. albicans*البروبوليس غير المعالج خلال 24و 48ساعة، وتم اعتباره عنصر تحكم. من خلال المقارنة، وجد أن تكوين الأغشية الحيوية ينخفض مع زيادة تركيز البروبوليس والوقت .وعليه، كان الحد الأدنى لتركيز البروبوليس المثبط 40٪ وزن /حجم، وكان الحد الأدنى لتركيزه المبيد للفطريات 50 (MFC)٪ وزن /حجم في *C. albicans*المكونة للأغشية الحيوية .بالإضافة إلى ذلك، كشف تحليل مستوى التعبير الجيني عن انخفاض في مستويات التعبير عن Ece1و Sap5و Als3مع معالجة البروبوليس خلال 24و 48ساعة.

الكلمات الدالة: التعبير، Ece1، Sap5، Als3، الجينات.

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