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Assessing the antimicrobial strength of Moroccan propolis from four regions on human infection-causing bacteria and yeast strains

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ABSTRACT

This study aims to explore the potential of ethanolic extracts of propolis from different regions in Morocco as a means of combatting microbial infections. Specifically, we investigate the antimicrobial activity of these extracts against five distinct microbial strains and analyze the correlation between this activity and the polyphenol and flavonoid content of the extracts. The inhibitory effects of the extracts on *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Candida albicans* were evaluated by measuring the inhibition diameters, followed by determining the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and the minimal fungicidal concentration (MFC). The study revealed that the ethanolic extract of Moroccan propolis displayed potent antibacterial and antifungal activity, with a greater sensitivity towards *Staphylococcus aureus*. The extracts' antimicrobial activities were correlated with the concentration of flavonoids and polyphenols present in them. The results also suggest that propolis collected from Rabat and Agadir exhibited more substantial antimicrobial potential than that collected from Settat or Marrakech. Overall, the findings of this study provide valuable insights into the possibility of Moroccan propolis as an antimicrobial agent.

Keywords: Moroccan propolis, polyphenols, flavonoids, antibacterial activity

INTRODUCTION

Various microorganisms, including *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Candida albicans*, are known to cause microbial infections and are responsible for fatal diseases and common epidemics (Pollack et al., 2015). While some of these microorganisms are harmless or beneficial in certain circumstances, they can all threaten human health if they enter the body or are present in food or water (Lyczak et al., 2002). For instance, *Staphylococcus aureus* can cause skin infections and more serious infections such as pneumonia and sepsis, while *Klebsiella pneumoniae* can cause pneumonia, urinary tract infections, and bloodstream infections (Chang et al., 2021). Also, *Escherichia coli* can cause a range of health problems in humans, from mild gastroenteritis to severe infections such as sepsis and meningitis (Allocati et al., 2013). *Pseudomonas aeruginosa* can cause various infections in humans, including respiratory tract infections, urinary tract infections, and wound infections, and is a common cause of hospital-acquired infections (Neuhauser et al., 2003). Concerning *Candida albicans*, a fungus commonly found in the human body, can cause a range of infections, including oral thrush, vaginal yeast infections, and systemic candidiasis in immunocompromised individuals (Wilson, 2019).

While antibiotics and antifungal substances have been developed to treat such conditions, their overuse has led to multi-microbial resistance, making it critical to identify natural alternatives that can serve as antibiotics to provide effective treatment against microbial infections while minimizing the risk of resistance (Anand et al., 2019).

Bees collect and modify resins from various plants in the region to obtain propolis, a natural resinous substance. Propolis has been observed to be a potent chemical weapon against microorganisms and is even utilized by bees to prevent the decomposition of intruding and dead animals (Trembecká et al., 2016; Zulhendri et al., 2021). Traditional medicine has utilized propolis for a long time due to its various biological and pharmacological activities, including antioxidant, antifungal, antibacterial, antiviral, anti-inflammatory, and even anti-tumor properties (Salatino, 2022). Propolis typically contains around 50% resins, rich in polyphenolic compounds, 30% waxes and fatty acids, 10% essential oils, 5% pollen, and 5% various organic and mineral materials, though its composition can vary significantly depending on the local vegetation and climate conditions (Haščík et al., 2014; Huang et al., 2014). Therefore, exploring the antimicrobial properties of natural products is essential to identify potential natural alternatives to antibiotic and antifungal treatments.

Morocco is one of the leading producers of these products in Africa and the Arab world. However, the production and quality of these products are influenced by several factors, such as climate, region, bee species, floral sources, and harvesting methods (Oyerinde et al., 2014). Consequently, the primary objective of this investigation was to examine the potential antimicrobial efficacy of ethanolic extracts of propolis (EEP) obtained from four distinct regions in Morocco (EEP1: from Agadir, EEP2: from Marrakech, EEP3: from Rabat, and EEP4: from Settat) against a range of microorganisms, including *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Candida albicans*.

MATERIALS AND METHODS

Origins of Propolis

The propolis samples used in this study were collected from four regions in Morocco between July and August of 2014. the propolis was harvested through the metal grid These regions were Agadir, Marrakech, Rabat, and Settat. After collection, the propolis was kept in a dry, dark location until it was utilized for the study. The regions of sampling, weather conditions, and the predominant vegetation in each region are presented in Table 1.

Extract preparation

To obtain the ethanolic extract of propolis (EEP: EEP1: Agadir, EEP2: Marrakesh, EEP3: Rabat, EEP4: Settat), the collected propolis samples from each region were first cut into small pieces. Then, a 10 g mass of propolis was extracted with 100 ml of 70% ethanol, with constant agitation (150 rpm) at room temperature in the dark, for seven days. The resulting solution was left to settle, and the supernatant was then centrifuged for 10 minutes at 2550×g. The obtained solution was then restored to its initial volume of 100 ml with 70% ethanol and stored in a clear glass beaker at + 4 °C until needed.

Evaluation of antibacterial activity

Preparation of bacterial strains

A set of bacterial strains, including *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* were obtained from human infections at the Hassan II Hospital Settat in Morocco. These strains were inoculated onto Petri dishes containing Müller Hinton agar (oxoid, Britain) and then incubated at 35 °C ± 2 °C for 24 hours to allow young cultures with well-isolated colonies to grow. Subsequently, the isolated colonies were utilized to prepare the inoculum for the study.

The Kirby-Bauer disk diffusion susceptibility test

The first step of the disk diffusion method was performed on Mueller-Hinton agar to demonstrate antibacterial activity.

Table 1. The regions of sampling, weather conditions, and the predominant vegetation

Region	Weather Conditions	Predominant Vegetation
Agadir	Mediterranean	*Citrus, Avocado, Amande, Argan, thymus, cactus, jujube*
Marrakesh	Semi-arid	*walnut, Amande, thymus, Cactus, jujube, Cistus, Olivea*
Rabat	Mediterranean	*Ceratonia, Cistus pine, Oak, jujube*
Settat	Semi-arid	*Eucalyptus, Cactus.*

This method enables the determination of bacterial growth inhibition by measuring the diameter of inhibition around a disk (Sharififar et al., 2007). To evaluate the bacterial concentration, a suspension of each bacterium was prepared using sterile physiological water at 0.9% and adjusted to 0.5 Mc-Farland (108 CFU/mL) from a young 24-hour bacterial culture grown on Mueller-Hinton agar with CFU = Colony Forming Units. The 90 mm diameter Muller-Hinton agar surfaces in Petri dishes were inoculated using a sterile swab well-soaked in the adjusted microbial suspension. Next, pure Wattman No. 04 paper disks of 6 mm diameter, washed in 20 x 10^{-6} mL of each propolis extract (corresponding to 100 µg/ml), were placed on the surface of the inoculated medium. For each extract, three repetitions were carried out (three disks of the same extract and concentration per dish). Negative control disks were soaked in 20 x 10^{-6} mL of 70% ethanol while positive control disks were standard gentamicin (oxoid, Britain) (10 µg/disk). The Petri dishes were incubated at 35 °C ± 2 °C for 24 hours, and the activity of the extracts was recorded by measuring the diameters of the inhibition zones around the disks at the end of the incubation.

Evaluation of Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) of Propolis

After demonstrating the antibacterial activity of propolis extracts from four different regions using the diffusion method, their effectiveness was further investigated by determining their MIC and MBC. A range of extract concentrations from 100 µg/mL to 400 µg/mL was prepared by diluting them in test tubes. Then, 0.2 mL of ethanolic extracts of propolis were added to 8.7 mL of Mueller-Hinton broth in each test tube, followed by inoculation with 0.1 mL of bacterial inoculum adjusted to 0.5 McFarland turbidity, reducing it to 106 CFU. Negative controls with no bacteria and positive controls without propolis extracts were also included.

After incubating the inoculated test tubes and control tubes at 35 °C ± 2 °C for 24 hours, bacterial growth was evaluated in each tube by assessing turbidity. The MIC was defined as the lowest concentration of propolis extracts that inhibited bacterial growth, while the MBC was the lowest concentration that resulted in a 99.99% bactericidal effect (i.e., 0.01% survivors). The MBC values of the propolis extracts were evaluated at concentrations equal to or greater than the MIC.

Antifungal activities

In this study, the antifungal activity of ethanolic extracts of propolis from four studied regions was tested against strains of human-origin *Candida albicans* (isolated and identified at Hassan II Hospital in Settat), and the results were compared with those of Itraconazole, an antifungal drug used as a positive control. The activity was evaluated by determining the diameters of the inhibition zones evaluated by the disk diffusion method and the MIC and MFC. The activity was analyzed using the standards (CLSI = Clinical and Laboratory Standards Institute).

Strains culture media

Human-origin strains of *Candida albicans* were employed for our experiments. The identification of all strains was carried out using standard methods, which involved the assessment of the macroscopic and microscopic characteristics of the culture strain.

To determine the Minimum Inhibitory Concentration (MIC) in a Sabouraud broth, the dilution technique in Sabouraud broth was adopted. On the other hand, the disk diffusion method with Sabouraud-Dextrose Agar was used to determine the Minimum Fungicidal Concentration (MFC).

Sensitivity tests

The antifungal activity of propolis samples was studied by dilution and diffusion methods on a solid medium following standard guideline from the National Committee for Clinical Laboratory Standards.

Disk Diffusion Method

Antifungal activity was determined by the disk diffusion method. A volume of 10 x 10^{-6} mL of suspension containing 106 CFU/mL of microbial cells (*Candida albicans* yeasts) was spread onto Petri dishes containing Sabouraud-dextrose agar. Sterile disks (6 mm in diameter) were separately impregnated with 20 x 10^{-6} mL of various extracts at a final 100 µg/mL concentration and placed onto the agar already inoculated with *Candida albicans* yeasts. An appropriate reference antibiotic disk (Itraconazole (8 µg/disk)) was applied to each Petri dish as a positive control. 70% ethanol disks were used as negative controls. The plates were kept at 4 °C for 1 hour, then incubated for 48 hours at 25 °C. Antifungal activity was evaluated by measuring the diameter of the growth inhibition diameter zone in millimeters (including the diameter of the 6 mm disk). Three repetitions were performed for each extract.

Determination of Minimum Inhibitory Concentration

The antifungal activity of different extracts was studied using the broth dilution method (Cosentino et al., 1999). The microbial culture was adjusted to 0.5 McFarland (i.e., 106 CFU/mL) and then diluted in peptone water (0.1% w/v) to 104 CFU/mL. Then,100 x 10^{-6} mL of each culture was suspended in Sabouraud broth containing different concentrations of each ethanol extract of propolis ranging from 6.12 µg/mL to 50 µg/ml. The positive control consisted of Sabouraud broth inoculated only with microbial suspension. The uninoculated tube containing extract only served as a negative control. The tubes were incubated for 48 hours at 25 °C. Microbial growth is indicated by turbidity at the bottom of the tube. The MIC is defined as the lowest concentration of the given ethanol extract of propolis capable of inhibiting visible yeast growth in a liquid medium. The first tube, in ascending order, which shows no turbidity at the bottom of the tube, corresponds to the MIC.

Determination of Minimal Fungicidal Concentration

The Minimal Fungicidal Concentration (MFC) is the minor concentration of extract that leaves only 0.01% or less of survivors of the initial inoculum after 48 hours of incubation at 25 °C. To determine the MFC, 10 x 10^{-6} mL of each broth from MIC and above was inoculated onto Petri dishes containing Sabouraud-dextrose agar. After incubation, the number of microorganisms was determined. The MFC is the concentration at which 99.9% or more of the initial inoculum was destroyed.

Total phenolic content

The method used to determine the total polyphenol contents in the extract involved the Folin–Ciocalteu technique, which was based on the method developed by (Gülçin et al., 2005). However, some minor modifications were made. To carry out the procedure, 25 µL of hydro-alcoholic extracts were mixed with 125 x 10^{-6} mL of Folin-Ciocalteu reagent (0.2 N) and 100 x 10^{-6} mL of 7.5% Na_2CO_3. The resulting mixture was then incubated at room temperature for 2 hours, after which the absorbance was measured at 760 nm. The total polyphenol content was determined using a standard curve prepared with gallic acid and was expressed as milligrams of gallic acid equivalent (GAE) per gram of sample (Kumazawa et al., 2002).

Flavonoid content

The method used to determine the levels of flavones in the extracts was based on the technique developed by (Miguel et al., 2014), with slight modifications. Specifically, 100 x 10^{-6} mL of Al_2Cl_3 (20%) was added to

100 x 10^{-6} mL of the extract, and the resulting mixture was allowed to stand at room temperature for 1 hour. The absorbance was then measured at 420 nm. The total flavonoid content was calculated using a calibration curve as quercetin equivalents (mg QE/g).

Statistical Analysis

Statistical analyses were performed using JMP SAS 11.0.0 (SAS Institute Inc., Cory, NC, USA) software. To investigate the phenolic and flavonoid contents and antimicrobial activity, a one-factor ANOVA factorial design (extract) was used to analyze phenolic and flavonoid contents and bacterial and fungal inhibition diameters in each propolis extract. The statistical model included the fixed effect of the propolis extract. When statistically significant differences were detected, Tukey's post hoc test was used to compare means and standard error, considering the significance level of $P < 0.05$. The data are expressed as mean ± standard error (SE). Correlations were calculated to establish the relationship between polyphenols, flavonoids, and the antimicrobial activity of propolis extract. Correlations were compared using Pearson's bivariate at $P < 0.05$.

RESULTS

Antibacterial activity

Bacterial growth inhibition zone diameters (mm)

According to the agar diffusion method used to evaluate the antibacterial activities of the four propolis samples, significant antibacterial activities were observed against Gram-positive bacteria (*Staphylococcus aureus*), while their activities against Gram-negative bacteria (*Escherichia coli*, and *Klebsiella pneumoniae*) were less pronounced (as shown in Table 2). EEP1 and EEP3 extracts displayed the highest inhibition diameter against *S. aureus*, *E. coli*, and *K. pneumoniae* ($P < 0.05$), while EEP2 and EEP4 had the lowest inhibition diameter ($P < 0.05$). None of the four extracts showed any activity against *Pseudomonas aeruginosa* (Table 2). Gentamicin was used as a positive control. According to CLSI charts, the interpretation used for this antibiotic is susceptible (S) for diameter zone ≥ 15 mm, intermediate (I) for 13-14 mm, and Resistant (R) for ≤ 12 mm.

Evaluation of Minimum Inhibitory Concentrations (MIC), Minimum Bactericidal Concentrations (MBC), and MBC/MIC Ratio of Propolis.

Examining propolis extracts' average MIC values confirmed that *Staphylococcus aureus*, a Gram-positive strain, is more susceptible than the Gram-negative strains, *Escherichia coli* and *Klebsiella pneumoniae* (as shown in Table 3). The ethanol extracts from Moroccan propolis collected from four different regions (EEP1: Agadir, EEP2: Marrakech, EEP3: Rabat, EEP4: Settat) demonstrated the most excellent antibacterial effect on *Staphylococcus aureus*, with MIC values ranging between 100 µg/mL and 200 µg/mL, and for *Escherichia coli*, with values ranging between 100 µg/mL and 250 µg/mL.

However, *Klebsiella pneumoniae* had the highest MIC of all the tested extracts, with concentration values ranging from 250 µg/mL to 300 µg/mL. EEP1 (Agadir) and EEP3 (Rabat) were more effective in inhibiting bacterial proliferation at lower concentrations than EEP2 (Marrakech) and EEP4 (Settat). Similarly, EEP1 and EEP3 showed higher bactericidal activity at lower concentrations than EEP2 and EEP4. It is important to note that no antibacterial activity was observed against *Pseudomonas aeruginosa* (as indicated in Table 3).

The activity ratio of MBC/MIC was studied for the various bacterial strains, and the results are presented in Table 4. The activity ratio ranged between 1 and 2 for all studied strains. The highest MBC/MIC ratios were observed in EEP2 and EEP3, followed by EEP4 and EEP1. The MBC/MIC ratio measures the effectiveness of an antimicrobial agent against a particular bacterial strain. A higher ratio indicates that the agent is more effective at killing the bacteria, while a lower ratio indicates that the agent is less effective. The results suggest that EEP2 and EEP3 may be more susceptible to the antimicrobial agent than the other strains studied, as evidenced by their higher MBC/MIC ratios (Table 4).

Table 2. Diameters of the inhibition zones (mm) of bacterial growth according to different concentrations of ethanolic extract of Moroccan propolis collected from 4 regions (mean ± standard error)

EEP	Microorganisms				Product
	S. aureus	*E. coli*	*K. pneumoniae*	*P. aeruginosa*	Gentamicin
EEP1	20,4 ± 0,73[a]	18,63 ± 0,34[a]	15,53 ± 0,53[a]	< 6	≥ 15mm
EEP2	13,73 ± 0,18[b]	12,83 ± 0,09[b]	13,1 ± 0,06[b]	< 6	≥ 15mm
EEP3	19,93 ± 0,17[a]	17,67 ± 0,23[a]	15,37 ± 0,09[a]	< 6	≥ 15mm
EEP4	13,43 ± 0,26[b]	12,77 ± 0,38[b]	12,7 ± 0,06[b]	< 6	≥ 15mm

EEP: ethanolic extract of propolis; EEP1: from Agadir, EEP2: from Marrakech, EEP3: from Rabat, and EEP4: from Settat; *S. aureus: Staphylococcus aureus; E. coli: Escherichia coli; K. pneumoniae: Klebsiella pneumoniae; P. aeruginosa: Pseudomonas aeruginosa*.

The values expressed are the means of three repetitions.

The letters (a, b, c) following the values in each column indicate a significant difference between each extract for each bacterium at $P < 0.05$.

Table 3. Minimum inhibitory (MIC) and bactericidal (MBC) of the ethanolic extract of Moroccan propolis against different bacterial species

Strains	MIC µg/mL				MBC µg/mL			
	EEP1	EEP2	EEP3	EEP4	EEP1	EEP2	EEP3	EEP4
S. aureus	100	200	100	200	100	400	100	200
E. coli	100	200	100	250	100	400	150	250
K. pneumoniae	250	300	175	300	250	400	175	400
P. aeruginosa	R	R	R	R	R	R	R	R

R: Resistant.

EEP: ethanolic extract of propolis, EEP1: from Agadir, EEP2: from Marrakech, EEP3: from Rabat, and EEP4: from Settat; *S. aureus: Staphylococcus aureus; E. coli: Escherichia coli; K. pneumoniae: Klebsiella pneumoniae*.

Antifungal activity

The findings indicated that the four propolis ethanol extracts had notable antifungal effects against *Candida albicans*. EEP1 originated from Agadir, and EEP3, which originated from Rabat, demonstrated the most potent antifungal activity against *Candida albicans*, resulting in inhibition diameters s of 28.7 ± 0.11 mm and 27.33 ± 0.07 mm, respectively. Conversely, the results for the extracts obtained from Settat and Marrakech were the least effective, with a value of 24.4 ± 0.11 ($P < 0.05$) (Table 4).

Regarding the MIC and MFC, all extracts showed antifungal activity with values ranging from 6.12 µg/mL to 25 µg/mL for MIC and 12.5 µg/mL to 50 µg/mL for MFC (Table 5).

Total polyphenols and flavonoid contents

The study analyzed propolis extract samples from four regions of Morocco (as presented in Table 6) to determine their total polyphenol and flavonoid contents. The results indicated a significant difference ($P < 0.05$) between the total phenolics and flavonoids found in the extracts, depending on where the samples were collected. The propolis extract from Rabat had the highest polyphenol concentration, followed by Agadir, Settat, and Marrakesh. Similarly, the samples from Rabat had the highest concentration of flavonoids, while the lowest concentration was found in those from Marrakesh (Table 6).

Table 4. Bacteriostatic or bactericidal power of ethanolic extract of Moroccan propolis collected from 4 regions

EEP	Microorganisms	MBC/MIC	Power
EEP1	*S. aureus*	1	Bactericidal
	E. coli	1	Bactericidal
	K. pneumoniae	1	Bactericidal
	P. aeruginosa	R	
EEP2	*S. aureus*	2	Bactericidal
	E. coli	2	Bactericidal
	K. pneumoniae	1.3	Bactericidal
	P. aeruginosa	R	
EEP3	*S. aureus*	2	Bactericidal
	E. coli	2	Bactericidal
	K. pneumoniae	1.3	Bactericidal
	P. aeruginosa	R	
EEP4	*S. aureus*	1	Bactericidal
	E. coli	1	Bactericidal
	K. pneumoniae	1.3	Bactericidal
	P. aeruginosa	R	

R: Resistant.

EEP: ethanolic extract of propolis, EEP1: from Agadir, EEP2: from Marrakech, EEP3: from Rabat, and EEP4: from Settat; *S. aureus: Staphylococcus aureus; E. coli: Escherichia coli; K. pneumoniae: Klebsiella pneumoniae; P. aeruginosa: Pseudomonas aeruginosa.*

Correlation test

Based on the data presented in Table 7, a significant positive correlation was observed between the Total Phenolic and Flavonoid Content in Propolis and the diameters of the inhibition zones of *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Candida albicans*. Additionally, the diameters of the inhibition zones of *Escherichia coli* showed a significant positive correlation with the inhibition diameters s of *Klebsiella pneumoniae* and *Staphylococcus aureus*.

Table 6. Total phenolic and flavonoid contents of Ethanolic extract of propolis (EEP) samples collected from different Moroccan regions (EEP1 from Agadir, EEP2 from Marrakech, EEP3 from Rabat, and EEP4 from Settat; mean ± standard error)

EEP	Total phenolic (mg GAE/g)	Total flavonoid (mg (QE/g))
EEP1	190.08±0.81^{b}	71.83±1.16^{b}
EEP2	76.79±1.81^{d}	13.03±0.39^{d}
EEP3	240.56±1.74^{a}	90.38±1.37^{a}
EEP4	126.12±1.51^{c}	47.42±0.53^{c}

Means with different superscript letters within a column are significantly different at $P < 0.05$. GAE: gallic acid equivalent (GAE) per gram of sample; QE: quercetin equivalents.

Table 5. Diameters of the inhibition zones (mm), and the Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of *Candida albicans* fungal growth in response to different concentrations of ethanolic extract of Moroccan propolis collected from four distinct regions (mean ± standard error)

EEP	Inhibition zone (mm)	MIC µg/Ml	MFC µg/mL
EEP1	28.7±0.11^{A}	6.12	12.5
EEP2	24.4±0.11^{D}	25	50
EEP3	27.33±0.07^{B}	6.12	12.5
EEP4	26.4±0.12^{C}	12.5	25

Values within columns followed by letters (A. B. C) are statistically different at a significance level of $P < 0.05$.

EEP: ethanolic extract of propolis, EEP1: from Agadir, EEP2: from Marrakech, EEP3: from Rabat, and EEP4: from Settat.

Table 7. Correlation Coefficients Between Total Phenolic and Flavonoid Content in Propolis and the diameters of the inhibition zones of *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Candida albicans*

		Total phenolic (mg GAE/g)	Total flavonoid (mg (QE/g))	Diameters of the Inhibition zones (mm)			
				S. aureus	*E. coli*	*K. pneumoniae*	*C. albicans*
Total phenolic (mg GAE/g)		1,00	0.98*	0.89*	0.87*	0.86*	0.80*
Total flavonoid (mg (QE/g))			1,00	0.85*	0.83*	0.81*	0.85*
Diameters of the inhibition zones (mm)	*S. aureus*			1,00	0.89*	0.96*	0.83
	E. coli				1,00	0.99*	0.86
	K. pneumoniae					1,00	0.79
	C. albicans						1,00

* Significant at $P < 0.05$; *S. aureus*: *Staphylococcus aureus* ; *E. coli* : Escherichia coli; *K. pneumoniae* : *Klebsiella pneumoniae*; C. albicans : *Candida albicans*.

GAE: gallic acid equivalent (GAE) per gram of sample; QE: quercetin equivalents.

DISCUSSION

The aim of this investigation was to assess the antimicrobial effectiveness of ethanolic extracts of propolis (EEP) sourced from four distinct regions in Morocco (Rabat, Settat, Marrakech, and Agadir) against a range of microorganisms, including *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Candida albicans*. The findings demonstrated that the ethanolic extract of Moroccan propolis exhibited significant antibacterial and antifungal properties, with a heightened efficacy against Gram-positive bacteria. Moreover, the results indicated that propolis collected from Rabat and Agadir showcased more pronounced antimicrobial potential compared to that obtained from Settat or Marrakech.

This study finds support in a body of existing research. Indeed, propolis has been extensively studied for its antimicrobial properties against a wide range of pathogens, including bacteria, fungi, viruses, and parasites (Pobiega et al., 2019). Propolis exerts its effects either through direct interaction with microbial cells or by bolstering the immune response of host cells (Bouchelaghem, 2022). Additionally, some studies have proposed that propolis may induce structural damage to microorganisms, suggesting a potential mechanism for its antimicrobial activity (Przybyłek and Karpiński, 2019; Daraghmeh and Imtara, 2020). In this study, ethanolic propolis extracts showed an antibacterial effect on *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae*, with the greater sensitivity of the extract towards Gram-positive bacteria (*Staphylococcus aureus*). This latest finding is consistent with previous research (Castaldo and Capasso, 2002; De Vecchi and Drago, 2007; Sa-eed et al., 2023) that has shown the antimicrobial activity of propolis is mainly due to its ability to disrupt the cell wall of bacteria. However, the absence of any antibacterial activity against *Pseudomonas aeruginosa* can be explained by the fact that it is a bacterium from a nosocomial infection, and it is known for its multidrug resistance.

In addition, our findings demonstrate that all four ethanolic propolis extracts exhibited significant antifungal activity against *Candida albicans*. These results are consistent with previous studies (Ramón-Sierra et al., 2019; Dudoit et al., 2020; Cerqueira et al., 2022). The antifungal activity of propolis can be attributed to its constituents, such as 3-acetylpinobanksine, pinobanksine-3-acetate, pinocembrin, p-coumaric acid, and caffeic acid, as reported by Oliveira et al. (2006). These compounds have been found to exhibit antifungal properties, providing a possible explanation for the observed activity of propolis extracts against *Candida albicans*.

Interestingly, the results of this study also suggest that the antimicrobial potential of propolis collected from different regions of Morocco may vary. Specifically, propolis collected from Rabat and Agadir exhibited more substantial antimicrobial potential than that collected from Settat or Marrakech. This finding is consistent with previous research (Hegazi and Hady, 2001) suggesting that the composition and potency of propolis can be influenced by factors such as geographic location, plant source, and bee species (Poklukar, 2001; Ożarowski et al., 2022). In fact, the variation in antimicrobial potential among propolis samples collected from Rabat, Agadir, Settat, and Marrakech can be attributed to a combination of factors. These include the unique botanical composition specific to each region, influenced by varying climates, soil type, and altitude (Toreti et al., 2013). Additionally, geographical location impacts the types of plants available for bee foraging, leading to differences in the bioactive compounds present in the propolis (Dezmirean et al., 2017). Seasonal variations, climatic conditions, and soil characteristics further contribute to these disparities (Mountford-McAuley et al., 2023). Bee foraging behavior, constrained by their hive's proximity, ensures that the local plant species significantly influence the propolis composition (Dezmirean et al., 2017). Collectively, these environmental and biological factors culminate in the observed differences in antimicrobial efficacy.

In addition to evaluating the antimicrobial activity of propolis, the study also assessed the concentration of flavonoids and polyphenols to investigate potential correlations with the observed antimicrobial effects. The results revealed notable variations among the propolis samples. Specifically, the propolis extract from Rabat and Agadir exhibited the highest flavonoids and polyphenols concentrations, followed by Settat, and Marrakesh. Importantly, these findings demonstrated a significant correlation between the concentrations of flavonoids and polyphenols and the antimicrobial activity of the ethanolic propolis extract. In fact, these outcomes are consistent with prior research findings, which have consistently reported a relationship between the antimicrobial activities of propolis extracts and the concentration of flavonoids and polyphenols present in them (Choi et al., 2006; Tosi et al., 2007). These bioactive compounds, known for their wide range of biological activities, have been identified as crucial constituents responsible for the antimicrobial efficacy of propolis (Fernández-Calderón et al., 2020). Flavonoids and polyphenols, naturally occurring in various plant species, exhibit potent antioxidant, anti-inflammatory, and antimicrobial properties (Gutiérrez-Venegas et al., 2019; Othman et al., 2019). Their antimicrobial activity is attributed to their ability to interact with microbial cells, resulting in cellular damage and growth inhibition (Górniak et al., 2019). Additionally, these compounds have been demonstrated to interfere with the activity of bacterial enzymes and disrupt cell membrane function (Abdu et al., 2020; Donadio et al., 2021). Moreover, it has been proposed that the synergistic action of different polyphenolic compounds in propolis extracts may contribute to their antimicrobial activity (Donadio et al., 2021). The diverse array of polyphenols and flavonoids in propolis extracts may account for the broad spectrum of antimicrobial activity observed against various microorganisms.

The findings of this study have important implications for developing new antimicrobial agents. Propolis represents a promising source of natural compounds that could create new antimicrobial agents to combat the growing problem of antibiotic resistance (Almuhayawi, 2020).

CONCLUSION

The results of this study demonstrate that the ethanolic extract of Moroccan propolis possesses potent antimicrobial activity against a range of microorganisms and that this activity is correlated with the concentration of flavonoids and polyphenols present in the extract. The finding that propolis collected from different regions of Morocco may exhibit different levels of antimicrobial activity suggests that further research is needed to understand this natural substance's properties fully. Nonetheless, the results of this study highlight the potential of propolis as a source of natural compounds that could be used to develop new antimicrobial agents.

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