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RESEARCH ARTICLE

The Effect of the Phytogeographical Area (Pine Trees) on the Effectiveness of Propolis against some Microorganisms

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ABSTRACT

Propolis samples were collected from the Mosul city, starting from the October until the end of the December. The effectiveness of propolis was tested against one type of yeast, *Candida albicans* ATCC 10231, and two types of bacteria, *Bacillus pumilus* ATCC 6633, and *staphylococcus aureus* ATCC 6538. Propolis has shown clear antimicrobial activity against three strains. *B. pumilus* were the most sensitive among their counterparts, as the inhibition was 24 % at a concentration 600 mg/ml, while the inhibition against *C. albicans*, and *staph. Aureus* was 19.40%, and 17.25% respectively at the same concentration. The GC-MS chromatogram of Mosul propolis extract appear 40 peaks, The most popular plant-components formative in Mosul propolis extract are 2-Propen-1-one, 1-(2,6-dihydroxy-4 -methoxyphenyl)-3-phenyl, (E) - (24.27%), Oleic Acid (10.48%), n-Hexadecanoic acid (8.66%), Phenylethyl Alcohol (6.35%), and 4H-1-Benzopyran-4-one, 2,3-dihydro (6.22%), 6-Methyl-2'-(1-naphthylmethylene) nicotinohydrazi (3.29%), Octadecanoic acid (2.47%), Pyrido [2,3-d] pyridazine, 5-(methyl) (2.1%), Guaiol (2.02%), beta.-Santalol (1.8%).

INTRODUCTION

The use of propolis by humans is not recent, but rather since ancient times. Some antiques were found dating back to ancient Egyptian times with bees drawn on them. It was also used by many ancient civilizations to preserve corpses from damage, corrosion, and the like. Rather, the matter went beyond. This by linking the name of the god they worship to the name of bees. It was also used in ancient times in the manufacture of perfumes by the Greek civilization (Rojczyk et al., 2020).

Aristotle researched the medicinal properties of propolis, and he was followed by many scientists, such as Galen, Pliny the Elder, and Dioscorides (Rojczyk et al., 2020).

The first person to use it in modern medicine to treat wounds and ulcers was Hippocrates. It has also been known to the Arabs since ancient times as black wax, and it was known to the Jews as Tzuri, propolis was used by the Arabs, Jews, and Persians as a treatment for many diseases and as a sterilizer for wounds (Hossain et al., 2022).

Unlike honey and pollen, propolis is not used by bees as food. Rather, it is used to build and restore the structure of the hive by filling cracks and openings, and installing wax frames inside the hive or attaching them to the trees on which it is located (Bankova et al., 2018).

At present, propolis is considered one of the natural compounds that can be used as nutritional supplements that are included in the compositions of many foods, which impart many vital properties as an anti-oxidant, anti-microbial, and anti-inflammatory agent (Kolayli, 2024).

Bioactive compounds in propolis

Resin materials make up about 50%, wax makes up 30%, while the rest is essential oils, minerals and vitamins (Dezmirean et al., 2021) In addition to many compounds of secondary and primary metabolism, such as amino acids, alkaloids, and terpenes (Zulhendri et al., 2021) **.**The exact chemical variation of propolis depends on the type of bees and the seasons of collecting propolis (Salatino & Salatino, 2021)**.** Most of the health benefits of propolis are attributed to the phenolic compounds it contains (Zulhendri et al., 2021).

The importance of phenolic compounds are one of the main components of secondary metabolic compounds which are produced through the process of photosynthesis and have a clear benefit for the plant, as they participate in the main and basic functions by being the defense mechanism against pathogens such as bacteria, fungi, and insects, in addition to the damage caused by ultraviolet rays and other radiations (Cauich-Kumul & Segura Campos, 2019).

Some certain active compounds found in propolis include phenethyl caffeate, galangin, caffeic acid phenethyl ester, Apigenin naringenin, galangin, O-comaric acid and, chrysin. All of these compounds contributed to propolis having many medicinal activities, such as anti-inflammatory, anti-cancer, and anti-viral, anti-fungal, and anti-bacterial (Šuran etal., 2021).

Antibacterial and Antifungal properties of propolis

Antibiotics manufactured have declined in recent years resistance by pathogenic microorganisms has led many scientists to investigate and search for natural compounds with vital biological properties to limit the spread and growth of resistant microbes. Propolis is one of the five bee products (honey, propolis, wax, royal jelly, and bee venom), each of which has its own use by the bees. (Oliveira etal. 2017).

Bees collect propolis from the resinous substances found on flowers and tree buds. This makes the nature of the phytogeographical region an important role in determining the active of propolis against many types of bacteria, such as Vibrio spp, Aeromonas spp and, E. coli, L. (Przybyłek etal., 2019).

In addition to its antibacterial medicinal properties, propolis works to activate the body's immune system. (Braakhuis, 2019)

The mechanism of the effect of propolis on bacteria is in its impact on the quality of a material of the cell membrane, and on bacteria movement by inhibiting the production of adenosine triphosphate. (Almuhayawi, 2020).

Some studies indicate that propolis has more antibacterial active toword gram-negative than gram-positive bacteria. This difference is attributed to its effect on the external layer of gram-negative bacteria, and the production of some hydrolytic proteins that work to break down the active components inside the cell wall. (Przybyłek and Karpiński, 2019).

Propolis's high content of vanillin, ferulic acid, quercetin, cinnamic acid, and chlorogenic acid has made it an effective natural substance against many molds and yeasts (Zulhendri et al., 2021). The mechanism of the effect of propolis on fungi, other than its effect on bacteria, does not affect their cell walls. It works by inhibiting the activity of the exophospholipase enzyme and thus prevents its adhesion to the surfaces of the host’s living cells (Ożarowski et al., 2022)

MATERIAL AND METHODS

Collection samples of propolis

Propolis collected from Mosul city, starting from the October until the end of the December, that is, for two consecutive months in the fall season. The samples were collected from apiaries that are located in areas with an abundance of pine trees.

Propolis extraction

The ethanolic propolis extract was prepared by adding 100 g of propolis to 400 ml of absolute ethanol (1:4 weight:volume), then the sample was crushed using a ceramic hob, and crushed again by using the blender mixer under the cooling, then filtered using a filter membrane (Whatman No 2) under vacuum and sediment using a centrifuge at a speed of 3000 rpm for 10 min, dried the sample using a Lyophilizer device and stored the samples in tightly sealed sterile plastic boxes until use (Gebara etal., 2003).

Sterilize propolis extract

The sample was resolve in 5 ml of DMSO sol to obtain a concentricity of 200 mg/ml, and sterilized using filter papers (0.02) µm, then other concentrations were prepared of it to test the effectiveness of propolis against microorganisms (Özkırım etal., 2019).

Determine the minimum inhibitory constrictions (MICs) of propolis

The MICs of Propolis were determined using the dilution method by preparing a series of increasing concentricity (1, 10, 20, 30, 40, and 50) mg/ml and then tubes were inoculated microorganisms under study, all tubes incubated at 37°C for 48 h.

To determine MICs of propolis: An amount of culture medium (0.1 ml) is taken from clear tubes into a dish containing solid culture medium (Muller Hinton agar), the dishes are incubated at 37°C for 48 hours, growing colonies are observed in each dish. (Andrews, 2002).

Microorganisms strain

Candida albicans ATCC 10231, Bacillus pumilus ATCC 6633, and staphylococcus aureus ATCC 6538.

Antimicrobial test of propolis

The wells method was used to test the effectiveness of propolis against the microorganisms under study with a diameter of 9 mm on Muler Hinton agar. Each hole was filled with 50 microliters at different concentrations (100, 200, 300, 400, 500, and 600) mg/ml. All dishes are incubated at 37 °C for 48 hours (Balouiri etal. 2016)

GC-MS ANALYSIS OF MOSUL PROPOLIS EXTRACT

GC-MS analysis was performed by using Gas Chromatograph: Agelint Technologies (7820A), GC Mass Spectrometer (5977E) USA. Analytical Column: Agelint HP-5ms Ultra lneit (30 m length x 250 µm inner diameter x 0.25 µm film thickness). Carrier Gas: He 99.99%, and injection volium 1µl, Pressure 11.933 psi. The oven conditions was primary held at 60 °C for 4 min before being increased to 180 °C at a rate of 10 °C/min for 15 min. The following data were utilized to optimize the mass spectra: the temperature is 180 °C, and the pass on temperature is 280 °C. The solvent retard time was 5 min, and the scan extent was 35–500 Da. The temperature was ultimately elevated to 280 °C. The GC's whole run schedule was 40.5 min. By estimating their mass band to data from the National institute of Standards and Technology (NIST) library, the substances were recognized (Mohiuddin etal., 2022).

RESULTS

The propolis sample collected in areas where pine trees prevail in Mosul city was distinguished by its dark green to right yellow color and a bitter taste that left numbness in the mouth (Fig. 1)**.**

Figure 1: Propolis sample from areas where pine trees prevail

Propolis has shown clear antimicrobial activity against Candida albicans ATCC 10231, Bacillus pumilus ATCC 6633, and staphylococcus aureus ATCC 6538, noted that the minimum inhibitory concentrations (MICs) of propolis against all microorganisms under study is 10 mg/ml., (Fig 2).

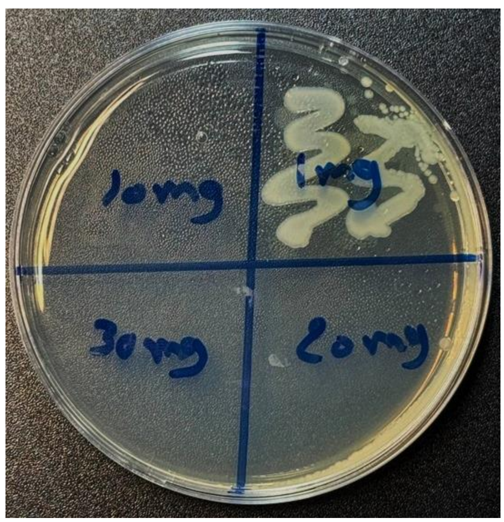

Figure 2: MICs of propolis against C.albicans 10 mg

The effectiveness of propolis against Bacillus pumilus ATCC 6633, Candida albicans ATCC 10231, and staphylococcus aureus ATCC 6538 was tested using the well diffusion method, where it was noted that the effectiveness of propolis increases with increasing concentrations used. B. pumilus were the most sensitive among their counterparts, as the inhibition was 24 % at a concentration 600 mg (Table 1), (Fig 3), while the inhibition against C. albicans, and staph. Aureus was 19.40%, and 17.25% respectively at the same concentration (Table 3, and 4).

Table 1: Propolis effect against B.pumilus

Concentration Mg/ml	**R1**	**R2**	**R3**	**M**	**Inhibition ratio%**
100	16.96	16.98	15.97	16.6367	16.64%
200	17.06	15.71	18.8	17.19	17.19%
300	17.9	16.66	17.28	17.28	17.28%
400	22.24	23.91	23.07	23.0733	23.07%
500	24.36	23.87	23.61	23.9467	23.95%
600	25.9	24.25	24.07	24.74	24.74%
Standard*	0.0	0.0	0.0	0.0	0.0

Standard*: DMSO without Propolis

Table 2: Propolis effect against C. albicans

Concentration Mg/ml	R1	R2	R3	M	Inhibition ratio%
100	14.42	17.96	16.19	16.19	16.19%
200	17.35	18	18.23	17.86	17.86%
300	17.38	18.51	17.94	17.94333	17.94%
400	17.55	18.28	18.44	18.09	18.09%
500	19.13	17.21	18.17	18.17	18.17%
600	19.53	19.27	19.4	19.4	19.40%
Standard*	0.0	0.0	0.0	0.0	0.0

Standard*: DMSO without Propolis

Table 3: Propolis effect against staph. Aureus

Concentration Mg/ml	R1	R2	R3	M	Inhibition ratio%
100	11.69	12.98	12.33	12.33	12.33%
200	11.94	11.44	13.69	12.35	12.36%
300	13.09	16.05	14.57	14.57	14.57%
400	13.28	13.28	17.28	14.61	14.61%
500	14.1	13.29	17.69	15.02	15.03%
600	17.87	16.97	16.92	17.25	17.25%
Standard*	0.0	0.0	0.0	0.0	0.0

Standard*:- DMSO without Propolis

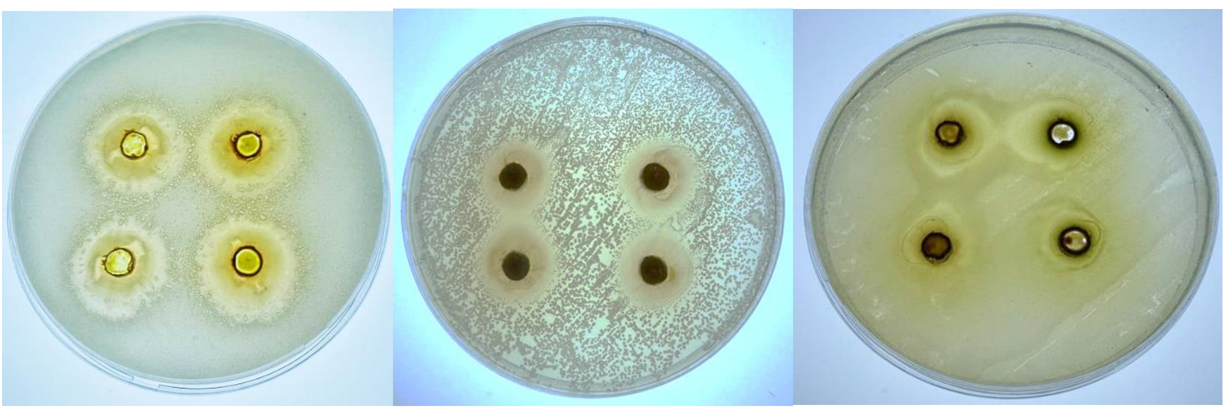

Figure 3: (A) The inhibition effect of propolis against *B. pumilus* at concentration 600 mg/ml.

(B) The inhibition effect of propolis against *C. albicans* at concentration 600 mg/ml.

(C) The inhibition effect of propolis against *staph. aureus* at concentration 600 mg/ml

The GC-MS chromatogram of Mosul propolis extractor appear in Fig. 4 displays a sum of 40 peaks equel to substances having a biological effect. recognized by paralleling their mass spectral crash samples to these of renowned substances recorded in the NIST list. The alchemical constituents recognized in Table 4 limited by store time, beak space (%). The most popular plant-components formative in Mosul propolis extract are 2-Propen-1-one,1-(2,6-dihydroxy-4 -methoxyphenyl)-3-phenyl, (E) - (24.27%), Oleic Acid (10.48%), n-Hexadecanoic acid (8.66%), Phenylethyl Alcohol (6.35%), and 4H-1-Benzopyran-4-one,2,3-dihydro (6.22%).

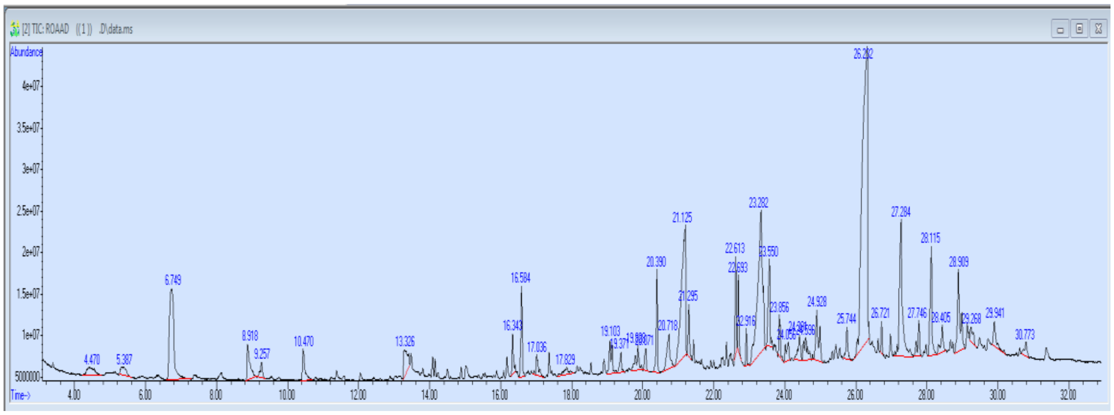

Figure 4: GC-MS chromatogram of important propolis combination.

Peak	Name	R.Time	Area%
1	Butyl (dimethyl) silyloxypropane	4.472	0.98
2	L-Histidine, N-[(phenylmethoxy)] car	5.389	0.73
3	Phenylethyl Alcohol	6.748	6.35
4	Benzofuran, 2,3-dihydro	8.921	1.62
5	Acetic acid, 2-phenylethyl ester	9.258	0.57
6	Benzene,[2-(methylthio) ethenyl]-(Z)	10.47	1.11
7	2,1,3-Benzothiadiazole	13.326	1.6
8	1H-Cyclopropa[a]naphthalene, 1,2,3,5,6,7,7a,7b -, [1aR-(1a.alpha.,7.alpha.,7.alpha.,7b.alpha.)]-etramethyl, [1aR-(1a.alpha.,7.alp ha.,7a.alpha.,7b.alpha.)]	16.338	0.97
9	Guaiol	16.581	2.02
10	alpha.-Bisabolol	17.039	0.74
11	5-Phenylpenta-2,4-diecoic acid	17.827	0.59
12	Silane, chlorodiethyl (dodec-9-ynyloxy)	19.099	1.49
13	2,6,9,11-Dodecatetraenal, 2,6,10-trimethyl	19.368	0.6
14	Glutaric acid, 2-methylpent-3-yl ester	19.826	1.16
15	Spiro [5.5]undecane, 1-methylene	20.069	0.61
16	Hexadecanoic acid, methyl ester	20.389	2
17	3,4-Dimethoxycinnamic acid	20.718	1.65
18	n-Hexadecanoic acid	21.125	8.66
19	Ethyl 14-methyl-hexadecanoate	21.298	1.07
20	beta.-Santalol	22.613	1.84
21	2-Nonadecanone	22.691	1.2

22	Methyl stearate	22.916	0.57
23	Oleic Acid	23.28	10.48
24	Octadecanoic acid	23.548	2.47
25	8-Nonen-2-one	23.86	1.05
26	Isophthaldiamidoxime	24.059	0.83
27	2-Propenoic acid, 3,(4-hydroxy-3-methoxyphenyl), (E)	24.388	0.96
28	17-Pentatriacontene	24.595	1.31
29	Pyrido[2,3-d] pyridazine, 5-(methyl)	24.924	2.1
30	9-Octadecenamide, (Z)	25.747	0.96
31	2-Propen-1-one, 1,(2,6-dihydroxy-4 -methoxyphenyl)-3,phenyl, (E)	26.231	24.27
32	Heptafluorobutyric acid, pentadecyl ester	26.725	0.56
33	4H-1-Benzopyran,4-one, 2,3-dihydro	27.287	6.22
34	1-[4]-[2]-(6-Amino- -8-ylsulfanyl) ethoxy phenyl ethanone	27.746	1.01
35	4H-1-Benzopyran,4-one, 5-hydroxy-7-methoxy-2-phenyl	28.118	3.2
36	4H-1-Benzopyran,4-one, 5,7-dihydroxy-2-(2-methoxyphenyl)	28.404	0.98
37	6-Methyl-2-(1-naphthylmethylene) nicotinohydrazide	28.906	3.29
38	Chrysin	29.269	0.66
39	4',5-Dihydroxy-7-methoxyflavanone	29.944	0.96
40	1-Heptacosanol	30.775	0.55

DISCUSSION

The effectiveness of Mosul propolis against Candida albicans ATCC 10231, Bacillus pumilus ATCC 6633, and staphylococcus aureus ATCC 6538 is due to it containing a group of active compounds that possess biological properties such as flavonoids, phenolic acids, alkaloids, terpenes, etc. It was found in a previous study on the propolis content of samples collected in three regions in Iraq (Akra, Sinjar and Mosul) the content of phenolic compounds was approximately (40%) (Allawi and, Al-Taie, 2020). In addition to the fact that the ethanolic solvent is considered a good solvent for many organic compounds which have antimicrobial activities, as phenolic compounds (Ding etal., 2021). The abundance of the aromatic compound 2-Propen-1-one, 1-(2,6-dihydroxy-4 -methoxyphenyl)-3-phenyl-, (E) in the propolis sample, at 24.27%, plays a major role in limiting the growth of microorganisms, as its effectiveness is estimated at moderate to strong, especially contra Candida albicans, with a minimum inhibitory impact of 23 µg (Fang etal., 2024). The second compound that was found in high abundance 10.48% in the Mosul propolis sample is oleic acid, which is considered a natural fatty compound that has antibacterial activity, especially gram-positive bacteria (Pushparaj etal., 2018). The compound n-Hexadecanoic acid has been shown in a previous study to have antimicrobial efficiency contra B. subtilis, E. coli, S. aureus, and K. pneumoniae, which is present in a sample of propolis with an area of 8.66% (Ganesan etal., 2022). There are other compounds, although the space they occupy is relatively small, but it is possible to give propolis medicinal activities such as: Chrysin, Octadecanoic acid, and 3, 4-Dimethoxycinnamic acid (Mani etal., 2018: Pu etal., 2010, and Hemaiswarya and Doble, 2010).

CONCLUSION

The propolis sample collected in areas where pine trees prevail in Mosul city has shown clear antimicrobial activity against Candida albicans ATCC 10231, Bacillus pumilus ATCC 6633, and staphylococcus aureus ATCC 6538. The GC-MS chromatogram of Mosul propolis extract appear 40 peaks of chemical compounds. The most popular plant-components formative in Mosul propolis extract are 2-Propen-1-one, 1-(2, 6-dihydroxy-4 -methoxyphenyl)-3-phenyl-, (E) - (24.27%), Oleic Acid (10.48%), n-Hexadecanoic acid (8.66%), Phenylethyl Alcohol (6.35%), and 4H-1-Benzopyran-4-one, 2,3-dihydro (6.22%).

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