Study The Effect of Propolis. Extract Against Some C. Albicans, C. Glabrata

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Abstract
The advent of fungi capable of infecting humans is becoming a major public health problem. Fungi with clinical importance can be classified as either primary or opportunistic fungal infections kill about 1,350,000 people per year, and more than 300 million people are infected the present study was conducted to investigate the effect of phytochemical compounds extract from (propolis) by using solvents which is Ethanol on fungal isolated from collected from various and hospitals and the medical province of Babil 2020 in Iraq. Antifungal activity was achieved in vitro by using the food poisoning method against Fungi species by preparing three concentrations for each solvent (5,10 and15) mg/ml and compared with positive control represented by anti-fungal ketoconazole 5mg/ml and negative control represented by 10% DEMSO. The aim of this investigation was to control fungi species isolated from various clinical cases by using propolis. The data collected from the study revealed that the Ethanolic extracts of propolis showed a significant reduction at $p < 0.05$ in the growth fungi species especially at 15 mg/ml compared with negative control. We conclude that the alcoholic extract of the propolis showed high efficiency in inhibiting the growth of the fungi under study.
Keywords: Anti-fungal activity, propolis extract, C.albcanis ,C.glabrata

1. Introduction

Propolis can be used to treat recurrent valvo vaginal candidiasis (RVVC) and can be an alternative to antibiotics for patients who are unable to take antibiotics due to another medical condition. The efficacy of propolis against antifungal nystatin has been shown to be satisfactory. In human cells, propolis extract solution (PES) has low toxicity and may be used as an alternative treatment for chronic vaginitis. PES also has antifungal properties and can be used as an antibiofilm material for RVVC to fight C. albicans biofilm growth and antifungal drug resistance (Capoci et al., 2015).[1]
Honeybees contain propolis, a natural resinous mixture made up of substances collected from plant parts, buds, and exudates. The word propolis comes from the Greek pro, which means "at the entrance to," and polis, which means "community" or "city," meaning that this natural product is used in hive protection. Propolis is also used as bee glue. Bees use propolis in the construction and repair of their hives for sealing holes and cracks and smoothing out the internal walls (Burdock, 1998; Bankova et al., 2000) [2][3] and as a defensive
shield against external invaders such as snakes, lizards, and so on, as well as wind and rain. Propolis is obtained bybees from a variety of plants in temperate climate zones (Wagh, 2013).[4]
Propolis is a complex mixture of plant-derived and bee-released compounds. In general, raw propolis is made up of 50% resins, 30% waxes, 10% essential oils, 5% pollen, and 5% organic compounds (Burdock, 1998. Park et al., 2002; Pietta et al., 2002).[5][6][7] More than 300 constituents have been found in various samples (Marcucci, 1995; Park et al., 2002; Pietta et al., 2002.Castro, 2001) [8][9][10][11], with new ones being discovered during chemical characterization of new forms of propolis (Bankova et al., 2000; Banskota et al., 1998; Alencar et al., 2007) [12][13][14]

2. Materials and Methods

Plant material:(*propolis)* had been purchased from the local market, identified based on the taxonomic features by a botanist. Materials of these plants were cleaned, dried, and kept according to Von Rudloff, (1975) [15]

materials extraction

Phytochemicals compounds were extracted by using digestion methods. By using 50 gm of propolis materials powder instant in 250 ml of Ethanol solvents separately for oreganum then shake it well for 1 hour, after that leave it for 72 hours in water path at 45C° to complete the process of extraction [16], and then dried. A stock solution of 100 mg/ml was prepared in 10% Ethanol then sterilized by a Millipore filter (0.22µm) and stored at (-20C°) until use [17].

Isolation and diagnosis of fungi species

The fungal species used in the present study were, *candida Albicans*, *candida glabrata* were collected from patients with different clinical cases and different age and gender (wounds, skin UTI, vanginal). A sample collected by using swab media from the infected area and cultured it in the culture media [18]. Fungal isolated were then diagnose based on the taxonomic key and diagnosed depend on the shape, size, color and growth, SDA culture planning by the loop method incubated for 5-7 hr. at 28 C [19].

Antifungal activity assay of extract

SDA medium was prepared and autoclaved after that a known volume (2ml) of each plant extracts is placed in the center of the Petri dishes and complete the volume to 20ml with SDA medium to obtain the required final concentrations (5, and 10,15mg/ml) of

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the medicinal plants after complete solidification of the medium, 5 mm disc of seven days old culture of the test fungus were placed aseptically in the center of the Petri plates and incubated at 28 ± 2C° 24-48h simultaneously 0.02ml of the antibiotic solution was added to each assay plate to check the bacterial contamination as suggested by [20]. Antifungal ketoconazole2mg/ml [21] was used as positive control DIMSOas negative control observations were recorded on the 24-48h. The colony diameter was recorded in terms of millimeters. For each treatment, three replicates were maintained. The fungi toxicity of extracts was calculated in terms of percent inhibition of mycelia growth by using the formula [22]. Percent Inhibition = (dc- dt/ dc) * 100 Where:
dc = Average increase in mycelia growth in control. dt = Average increase in mycelia growth in treatmen

3. Statistical Analysis

All statistical calculations were performed by using SPSS software (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp. USA) and Microsoft Excel (2010, Microsoft Corp. USA). All the results were expressed as mean. A p. value < 0.05 was considered statistically significant. Analysis of variance was employed to evaluate the presence of significant differences. LSD was carried out to find the significant difference using [23].

4. Results

Fungal isolated Fungal isolated from cutaneous infections for spp of fungi were isolated which is, *candidaAlbicans, candida glabrata*

Gas Chromatography – Mass Spectrum Analysis

The results of GC-MS analysis of the Bioactives of plant extract that have pharmacological actions are presented in (figure 1). Bioactives are the chemical compounds often referred to it as secondary metabolites, eight bioactive compounds (Table 2) were produced from *propolis* were identified in the Ethanolic extract.

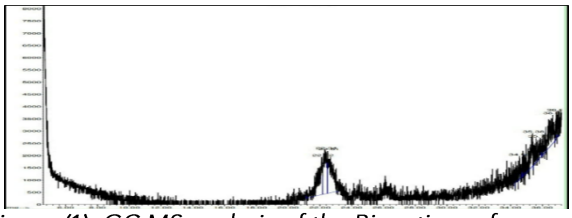

Figure (1): GC-MS analysis of the Bioactives of propolis extract.

Table (2) The major phytochemical compounds of propoles extract detected by GC-MS analysis that have pharmacological action.

No.	Compounds	RT (min)	Area %	Molecular Weight (gm/ml)	Formula	Nature of the compound	Bilogacal active	Chemical structure
1	Carbutamide	22.34	18.12	271.34	C11H17N3O3S	Phenols	Anti microbal, Anti fungle, Insecticide,	
2	Citric acid	22.51	16.77	192	C_{6H8O7}	Fatty acid	Anti microbal/	
3	Octadecanoic acid	22.11	21.38	284	$C_{18}H_{36}O_2$	Fatty acid	Antibacterial, Anti fungal,	
4	Octadecanal	34.50	5.17	252	$C_{18}H_{38}O$	Fatty alchol	Anti fungal, Antibactral, Anti larva,	
5	Phenol,4-bromo-2- (1,2-dimthyl)-	35.70	11.19	295	C_{11}H10BrONO2	Phenols	Anti fungal, Anti tumor, Anti oxdant,	
6	pyridate	35.38	7.36	378	$C_{19}H_{23}ClN_2O_2S$	Phenols	Anti microbile	
7	Iron,Tricarbonyl	36.89	16.36	220	C_9H8feO_3	Phenoles	Anti flammtary Anti fungle, Anti microbile	
8	Carbonic acid	36.89	3.18	62.025	CH_2O_3	Organic citrus	Anti micerbal, Anti flammatry	

The results of antifungal activity of ethanolic extract extracted from against Fungi species isolated in the study are presented in (table 2) activity of (propolis) was screened by food poisoning methods
The result of ethanolic extracts of propolis showed significant reduction at P≤ 0.05 in the growth of

candida spp Antifungal activity was applied at (5,10, and15) mg/ml. Mycelial inhibitionC. albicans and c. glabrata anti-fungal activity were applied (5,10, and15mg/ml) in table (1) mycelial inhibition ranging of ethanol extract showed (56.6%, in 5mg/ml and 65.5%, in 10mg/ml and 92.07%, in 15 mg/ml) (figur2, A) compared with positive control(B) and negative (C). and the rate of inhibition was in the fungus C.glabrata (22.1% in 5mg/ml,28.8%,in 10mg/ml and 64.4% in 15mg/ml).(figur2,C) compared with negative control (A) and positive (B) were inhibition percentage was (0.00% for negative control and 100% for positive control).

Table (1): Antifungal activity of phytochemical compounds alcoholic extracted from (propolis) against c.albcans and c.glabrata

Concentrations Mg/ml	C.albcans	C.glabrata
Control (-)	0±0.00	0±0.00
5mg/ml	56.6±0.63	22.1±0.63
10mg/ml	65.5±0.00	28.8±0.63
15mg/ml	92.07±0.127	64.4±0.56
Ketoconozol(+) 5mg/ml	100±0.00	100±0.00
L.S. D	1.571	
*Mean± standard deviation		

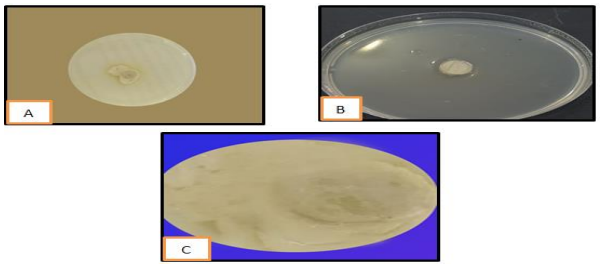

Figure (2): Antifungal activity of phytochemical compounds alcoholic extracted from (propolis) against c.albcans A: extract of propolis 15 mg/ml B: positive control (ketoconazole 5mg/ml), c: negative control 10% DMSO treatment

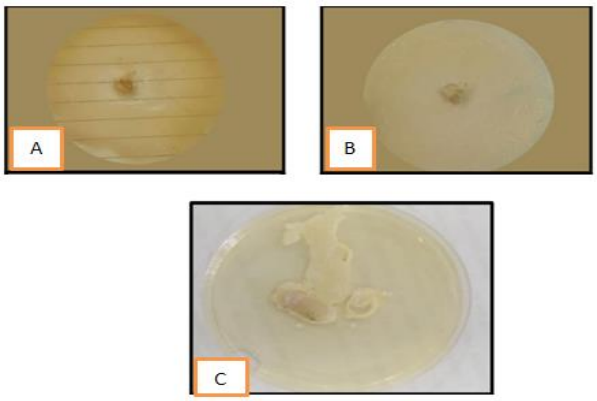

Figure (13): Antifungal activity of phytochemical compounds alcoholic extracted from (propolis) against C.glabrata A: negative control 10% DMSO treatment B: positive control (ketoconazole 5mg/ml), c: extract of propolis 15 mg/ml

5. Discussion

The results showed that the inhibitory effect of propolis extract the tested fungi depended on the type of extract (alcoholic) and its concentration in addition to the type of fungal isolate, as the alcoholic extract had a high inhibitory activity and it was evident that the rates of fungal colony diameters increased with the increase in the concentration of the extract used at the time in which the inhibition percentages were directly proportional to the increase in the concentration of the extract.Thispresent study is consistent with a numerous studies, Akgula and Kivanc [24] found that, among ten tested spices,Propolis has received the attention of clinicians and researchers because of its diverse pharmacological activities and low toxicity (da Silva Frozza et al., 2013; Agarwal et al., 2012 ;Bankova, 2009). Another study by Kacaniova et al., (2009) [25][26][27][28] which revealed the antifungal activity of Propolis against Candida species.

The study Kabanova et al., (2009) [29] was purely based on discs diffusion and reported the fungistatic activity of propolis against C. albicans. Also, the current study consistent with the study findings done by Dalben-Dota et al., (2010) [30], which revealed that excellent performance in an in vitro test against vaginal yeasts (C. albicans, C. glabrata) by inhibiting their growth at a maximal concentration.

Other studies Campos et al., 2015; Capoci et al., 2015; Shehu et al., 2016; Maureira et al., 2017and Dezmirean et al., 2017[31][32][33][34][35] which revealed that in the ethanolic extracts of the propolis demonstrated activity against C. albicans. Furthermore, the antibacterial and antifungal activity of bee propolis has been attributed, at least partially, to its phenolic content (flavonoids, phenolic acids, and their esters), such as the flavonoid galangin (Freires et al., 2016; Ota et al., 2001) [36][37], although the composition of the propolis varies depending on the plant and bee species of each location (Ramón-Sierra et al., 2019) [38].

6. Conclusions

1- The alcoholic extract of the propolis showed high efficiency in inhibiting the growth of the fungi under study.

2- It was found through the detection with the GC-MS technology that propolis contains a range of active compounds Important as phenols, flavonoids, steroids, and fatty acids.

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