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Influence of Egyptian Propolis as Antifungal Agent

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THE ANTIFUNGAL activity of Egyptian propolis against 9 genera of fungi was determined. These genera are belonging to *Cladosporium, Mucor, Scopulariopsis, Penicillium, Rhizopus, Fusarium, Aspergillus, Alternaria* and *Rhodoterola.* The results revealed that Egyptian propolis induced inhibition of fungal growth. This inhibition varied according to the type of fungi. The minimal inhibitory concentration of propolis ranged from 1.20 - 3.60 mg /ml. It was obvious that the minimum inhibitory concentration was 3.60 mg / ml for *Alternaria* and *Fusarium* whereas the concentration (1.20 mg / ml) was recorded for *Aspergillus* and *Penicillium.*

Propolis is the resinous substance collected by honeybee from various plant sources. It is known to exhibit antibacterial (Kedzia, 1987; Meresta and Meresta, 1988 and Hegazi *et al.*,1996-a); antiviral (Serkedjieva and Manolova, 1992, Hegazi *et al.*, 1993 and 1995, 1997); fungicidal (Pepeljniak *et al.*,1982; Dobrowolski *et al.*, 1991; Hegazi *et al.*, 1996-b).

Propolis is an extremely complicated mixture of natural substance (Walker and Crane, 1987). The available literature dealing with the Egyptian propolis is few (Hegazi, 1998). However, the honey and bee products were used in medicine from ancient times and in many cultures (Majno,1975) where The Holy Quran has a long Sorat with the name of bees (Al Nahl). The therapeutic characteristics of the propolis have been well known for a very long time. This is explained by its very pronounced anti-microbial characteristics. Thus the aim of this study was to throw more light on the influence of the Egyptian propolis as an antifungal agent.

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Material and Methods

Propolis

Egyptian propolis was collected from Mansoura City during the summer season. Ethanolic extract of propolis (EEP) was prepared according to Bankava *et al.* (1988) and Hegazi & Abd El Hady (1994). Fifty grams of propolis were cut into small pieces and extracted with ethanol overnight.

Extraction and sample preparation

Propolis was extracted with 70% ethanol. 2.5 mg of the balsam obtained after the evaporation of the alcohol was derivatized for 30 min at 100 °C with 50µl pyridine + 100 µl BSTFA and analyzed by GC/MS.

GC/MS analyses

A finnigan MAT SSQ 7000 mass spectrometer was coupled with a Varian 3400 gas chromatograph . DB-1 column, 30 m x 0.32 mm (internal diameter), was employed with helium as carrier gas (He pressure, 20 lbf in-2; injector temperature, 310°C; GC temperature program, 85-310°C at 3°C / min (10-min initial hold) . The mass spectra were recorded in electron ionization (EI) mode at 70 eV. The scan repetition rate was 0.5 s over a mass range of 39 amu to 650 amu.

Identification of compounds

The identification was accomplished using computer search user- generated reference libraries, incorporating mass spectra. Peaks were examined by single-ion chromatographic reconstruction to confirm their homogeneity. In some cases, when identical spectra have not been found, only the structural type of the corresponding component was proposed on the bases of its mass spectral fragmentation. Reference compounds were co-chromatographed where possible to confirm GC retention times.

Fungal strains

Nine genera of fungi prevailed in the examined samples. These fungi as follows: Cladosporium, *Mucor, Scopulariopsis, Penicillium, Rhizopus, Fusarium, Aspergillus, Alternaria* and *Rhodoterola.* These fungi were isolated and identified in the Microbiology section, Dep. of Parasitology, National Research Center, Dokki, Giza, Egypt.

Antifungal assay

The antifungal activity of tested propolis sample was carried out against nine genera of fungi as described in British Pharmacopoeia (1968). Sabouraud's dextrose agar medium (Moss and McQuown, 1969) supplemented with chloramphenicol and broth inoculated by the spore suspension (20ul/10 ml). Then added 20 % propolis. The tubes were incubated at 28°C for 48hr Spectrophotomeric measured of the growth as well as inhibition as turbidity at 420-nm wave length. The mean value of inhibition was calculated by triple reading in each test. Growth inhibition of different fungi were compared with the inhibition with Ketoconazole (50 µg/ml) antifungal drug. The minimal inhibitory concentration (MIC) of propolis was determined by ten-fold dilution method against 9 genera in in-vitro as well as antifungal activity (Hegazi *et al.*, 1996-b). Data were analyzed statistically using student "T" test according to Senedcor (1961).

Results

Ethanolic extract of propolis was revealed the presence of phenolic constituent that illustrated in Table 1. From Table 1, it was clear that the phenolic ester present as a major constituent (11.7 %), phenolic acids (1.6%); flavoinones; 3.5% aliphatic acids (11.4%) .

The antifungal activity of Egyptian propolis was reported in Table 2 . The results revealed that Egyptian propolis induced inhibition of fungal growth. This inhibition varied according to the type of fungi (Table 2). The minimal inhibitory concentration of propolis ranged from 1.20 - 3.60 mg /ml (Table 2). It was obvious that the highest minimum inhibitory concentration was 3.60 mg / ml for *Alternaria* and *Fusarium* whereas the lowest concentration (1.20 mg / ml) was recorded for *Aspergillus* and *Penicillium* .

Discussion

The results of the antifungal activity of the Egyptian propolis are in agreement with the findings of Mertzner *et al.* (1979) found that the antimicrobial activity of propolis can be attributed to its component: flavonoids, pinocembrin, galangin, pinobanksin, pinobanksin-3-acetate p-coumaric acid benzyl ester and caffeic acid ester. Relatively good antimycotic activity was shown by 5,7-dihydroxy flavanone (pinocembrin) this compound also present in the Egyptian propolis (1.1 %). Also esters as Isopentenyl caffeate (0.9 %), Dimethylallyl caffeate (1.3 %), Dodecyl caffeat (1.1 %), Tetradecyl caffeate (3.1 %) and Hexadecyl caffeate (4.4%) were present and gave its action on different fungi .

TABLE 1. Chemical composition (%TIC) of 70% ethanolic extract of Egyptian propells.

Compound	Egyptian propolis (% TIC)[a]
Acids (aliphatic)	
Palmitie acid[c]	3.0
Stearic acid[c]	0.9
Oleic acide[c]	4.0
Tetracosanoic acid[c]	1.6
Succinic acid[c]	0.3
Lactic acid[c]	1.3
Piruvic acid[c,e]	0.3
Acids (aromatic)	
Benzoic acid[c]	0.2
trans-p-coumaric acids[c]	0.5
Caffeic acid[b]	0.3
Ferulic acid[b]	0.2
Dimethoxycinnamic acid[c]	0.4
Esters	
Ethyl palmitate[c]	0.5
Ethyl oleate[ce]	1.2
Isopentenyl caffeate[b]	0.9
Dimethylallyl caffeate[b]	1.3
Dodecyl caffeat [de]	1.1
Tetradecyl caffeate[de]	3.1
Tetradecenyl caffeate[de]	0.3
Hexadecyl caffeate[de]	4.4
Benzyl caffeate[c]	0.6
Phenylethyl caffeate[c]	-
Sugars	
D-glucose[c]	6.1
Sorbose[c]	3.1
Fructose[c]	3.1
Sucrose[c]	1.6
Mannitol[c]	0.2
Flavonoids	
Pinocembrin[b]	1.1
Galangin[b]	0.7
Chrysin[b]	0.8
Pinostrobin[c]	0.6

TABLE 1. Cont .

Pinobanksin[c]	0.3
3-O-acetylpinobanksin[c]	1.1
Triterpenic alcohols	
Lanosterol[c]	1.2
Cycloartenol[ce]	7.1
Triterpenic alcohol of amyrine type[d]	4.8
ß-amyrine[ce]	4.7
Others	
Phosphoric acid[c]	2.7
Tricosane[c]	0.5
Glycerol octadecyl ether[ce]	1.8

[a]The ion current generated depends on the characteristics of the compound concerned and it is not a true quantitation.
[b] Identified by comparison with authentic samples (RT,mass spectrum).
[c] Identified by mass spectra (computer searches on commercial libraries).
[d]Tentatively identified by analysis of mass spectrum.
[e] For the first time in propolis.

TABLE 2. Determination of antifungal activity and minimal inhibitory concentration of Egyptian propolis .

Fungi	Normal fungal growth	Growth inhibition Ketoconazole (50 ug) *	Growth inhibition Propolis*	MIC (µg/ml)	
				K	P
Cladosporium	1.14 ± 0.001	0.895 ± 0.001	0.12 0 ± 0.005	4800	2200
Mucor	1.19 ± 0.002	0.902± .0062	0.150 ± 0.005	5600	1800
Scopulariopsis	1.31± 0.007	0.638 ± 0.003	0.210 ± 0.006	2400	1600
Penicillium	1.20 ± 0.045	0.770 ± 0.025	0.350 ± 0.012	2400	1200
Rhizopus	1.24 ± 0.005	0.610 ± 0.002	0.550 ± 0.003	5600	3200
Fusarium	1.21 ± 0.042	1.270 ± 0.001	0.300 ± 0.001	6400	3600
Asprgillus	1.72 ± 0.012	1.700 ± 0.002	0.280 ± 0.002	3400	1200
Alternaria	1.33 ± 0.006	0.460 ± 0.003	0.250 ± 0.0005	8400	3600
Rhodotyrlla.	1.05 ± 0.025	1.233 ± 0.004	0.240 ± 0.0012	3200	2600

K = Ketoconazole (50 ug) P = Propolis
* = Growth inhibition measured spectrophotometrically at 420-nm weave length

The minimum inhibitory concentration of Egyptian propolis against different fungi ranged from 1.20 - 3.60 mg/ ml according to the type of tested fungi. Some authors *e.g.* Olivieri *et al.* (1981) found that 4 - 40 mg total flavones (extracted from propolis) had *in vitro* inhibitory activity against some fungi and yeasts. Also, Pepeljnjak *et al.* (1982) found that concentration of 15 - 30 mg/ml pure propolis extract inhibited the growth of : *Candidia albicans, Aspergillus flavus, Aspergillus ochraceus, Penicillium virdicatum* and *Penicillium natatum.* Also

Kovacs (1984) found pure propolis extract in a concentration of 15-30 mg/ml was needed to inhibit the growth of *Candidia albicans, Aspergillus flavus, A. ochraceus, Penicillium viridicatum* and *P. notatum. Milena et al.* (1989) used 10 % propolis extracts against 17 fungal pathogens. They found propolis extract inhibited Candidia and all tested dermatophytes. Lori (1990) found that propolis concentration of 5% or 10 % prevented growth of fungus and the lower concentration did not completely suppress growth. Also, Hegazi *et al.* (1996-b) found that the minimal inhibitory concentration of Egyptian propolis ranged between 10 and 30 mg/ml. It was obvious that the highest minimum inhibitory concentration was against *A. parasiticus* (30 mg / ml) while the lowest concentration was against *A. flavus* (10 mg / ml). The inhibition zone of different EEP using the agar diffusion method against *Mucor, Penicillium, Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus* and *Aspergillus parasiticus* was 18, 26, 32, 26, 21 and 24 mm respectively.

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تأثير صمغ النحل المصرى كمضاد للفطريات

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تم تحديد الكفاءة القاتلة لصمغ النحل المصرى ضد تسعة عائلات من الفطريات ؛ وهذه الفطريات تشمل : الكلادوسبوريم - الميوكر - السكرولوبيسيس - البنيسيليم - الرايزوبس - الفيوزاريوم - الأسبريجليس - الألترناريا - والروديتيلا . حيث أسفرت النتائج أن صمغ النحل المصرى يحدث تثبيط لنمو الفطريات ؛ وهذا التثبيط أختلف تبعا لنوع الفطر . كما أن القدرة المثبطة القليلة تراوحت بين ١,٢٠ - ٣,٦٠ مليجرام / ملليميلتر. كما ظهر واضحا أن القدرة المثبطة القليلة ٣,٦٠ مليجرام / مليميلتر كانت ضد الألترناريا والفيوزاريوم بينما التركيز ١,٢٠ مليجرام / مليميلتر كانت ضد الأسبريجليس والبنيسيليم .