Egyptian Propolis: 2. Chemical Composition, Antiviral and Antimicrobial Activities of East Nile Delta Propolis

Faten K. Abd El Hady and Ahmed G. Hegazi*

Departments of Chemistry of Natural products and Parasitology*, National Research Center, Dokki, Giza, Egypt. P. code: 12622.
E-mail: ahmedgaffer@mailer.suc.eun.eg and samira@mena.org.eg

* Author for correspondence and reprint requests

Z. Naturforsch. **57c,** 386–394 (2002); received December 18, 2000/October 9, 2001

Propolis, Polyphenols, Antiviral and Antimicrobial Activities

Three propolis samples from East Nile Delta, Egypt were collected. Propolis samples were investigated by GC/MS,103 compounds were identified, 20 being new for propolis. Dakahlia propolis was a typical poplar propolis but it contained two new caffeate esters and two new triterpenoids. Ismailia propolis was characterized by the presence of new triterpenic acid methyl esters and it did not contain any aromatic acids, esters and flavonoids. Sharkia propolis was characterized by the presence of caffeate esters only, some di- and triterpenoids.

The antiviral (Infectious Bursal Disease Virus and Reo-Virus) and antimicrobial (*Staphylococcus aureus; Escherichia coli* and *Candida albicans*) activities of propolis samples were investigated. Dakahlia propolis showed the highest antiviral activity against Infectious Bursal Disease Virus (IBDV) and the highest antibacterial activity against *Escherichia coli* and the highest antifungal activity against *Candida albicans.* While Ismailia propolis had the highest antiviral activity against Reo-virus. Sharkia propolis showed the highest antibacterial activity against *Staphylococcus aureus* and moderate antiviral activity against infectious bursal disease virus and reovirus.

Introduction

Propolis (bee glue) is a resinous hive product. It consists of exudate from plants mixed with beeswax and used by bees as glue in general-purpose as sealer and draught-excluder for beehives. Propolis has been long used in folk medicine of different nations as early in Egypt as 3000 BC (Hegazi, 1998). Egyptian propolis has recently become a subject of increasing attention for biologists and chemists (Hegazi, *et al*., 1993, 1995,1996a, b, 1997, 2000 b; Hegazi and Abd El Hady, 1994, 2000; Abd El-Hady, 1994; Abd El-Hady and Hegazi, 1994; Bankova *et al*., 1997; Christov *et al*., 1998, and Kujumgiev *et al*., 1999). Hegazi and Abd El Hady (2001) found significant differences in antimicrobial activity and chemical composition of Upper Egypt propolis. Thus, the aimed of this study was to determine the chemical composition, antiviral, and antimicrobial activities of propolis collected from 3 different provinces in the East area of Nile Delta, Egypt.

Materials and Methods

Propolis

Propolis samples were collected from Dakahlia, Ismailia and Sharkia provinces east area of Nile Delta, Egypt. These samples were collected during March 2000.

Extraction and sample preparation

One gram of each sample was cut into small pieces and extracted at room temperature with 50 ml of 70% ethanol (twice after 24 hours). The alcoholic extract was evaporated under vacuum at 50° C until dryness. The percentage of extracted matter was as follows: Dakahlia propolis 0.8 g/dry weight, Ismailia propolis 0.33 g/dry weight and Sharkia propolis 0.40 g/dry weight. 2.5 mg of the dried matter was prepared for chromatography by derivatization for 30 min at 100 °C with 50 µl pyridine + 100 µl bis-(trimethylsilyl)trifluoroacetamide (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 × 0.32 mm (internal diameter), was employed with helium as carrier gas (He pressure, 20 Mpa/cm^2; injector temperature, 310 °C; GC temperature program, 85–310 °C at 3 °C/min (10 min. intial 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 39–650 atomic mass units (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.

Viral strains

Infectious Bursal Disease Virus was locally isolated from Animal Health Research Institute, Dokki, Giza, while the Reo virus vaccinal strain S 1133 was kindly supplied by Animal Health Research Institute, Dokki, Giza, Egypt.

Evaluation of embryo infected dose fifty/ml (EID_{50}/ml)

This method of titration of viruses was done according to the procedure of Anon (1971). Ten fold serial dilutions of virus were made in phosphate buffer saline (PBS, pH 7.2) and inoculated into the allantoic cavity of 9 days old embryonated chicken eggs (obtained from Faculty of Agriculture, Cairo University). Five eggs were inoculated per dilution, each egg received 0.2 ml of virus inoculum. Inoculated eggs were incubated at 37–38 °C for 6 days, The inoculated eggs were observed daily for mortality of embryos. Determination of the 50% of embryo infected dose fifty titer *(EID_{50}/ml)* was calculated after the method of Reed and Muench (1938).

Antiviral assay

The antiviral activity of Reo and IBDV viruses was determined to evaluate the infectivity titer in embryonated chicken fibroblast. Primary monolayer cultures of chicken embryo fibroblast (CEF) cells were prepared in plastic plates (Falcon 3002, Becton Oxnard, CA) from 9 to 11 day old chicken embryo. Infected monolayers of CEF in microtiter plates (cloned five times) were inoculated with the Infectious Bursal Disease Virus or Roe virus. 0.2 ml of ten fold dilution of each virus in phosphate buffer saline (PBS, pH 7.2) were mixed with equal volume of 1/100 of the original propolis (100 mg/ml) propolis samples (from different provinces) and incubated for 30 min at room temperature. Then inoculated into infected monolayers of CEF in microtiter plates (cloned five times) with the Infectious Bursal Disease (Komine *et al*., 1989) and Roe virus (Taylor *et al*., 1966) in a dose of 50 µl/well to evaluate the infectivity of the virus as well as the antiviral effect of propolis. After 120 h., the cells were observed microscopically for their cytopathic effects. Monolayer cells were stained with crystal violet. The dilution which gave the lowest lethality was undertaken to evaluate the antiviral effect of propolis to IBDV or Reo virus The calculation was done according to the method adopted by Reed and Muench (1938) as the mean tissue culture infective dose fifty ($TCID_{50}$).

Antibacterial assay

Two bacterial strains were used: *Staphylococcus aureus* (209) and *Escherichia coli* (H-480). These bacteria were kindly supplied from Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria. The bacterial suspension was prepared and adjusted by comparison against 0.5 Mc-Farland turbidity standard (5×10^7 cells/ml) tubes. It was further diluted to obtain a final of 5×10^6 cells/ml. *Staphylococcus aureus* was enriched on polymyxin agar (Finegold and Sweeny, 1961) as a selective media while *E. coli* was enriched on MacConkey broth. Both bacteria were subcultured on nutrient broth for further bacterial propagation (Cruickshank *et al.*, 1979). The broth was inoculated by the 0.20 µl/10 ml broth either with *Staphylococcus aureus* and *E. coli*, then added 40 µl of 20% propolis. The tubes were incubated at 37 °C

for 24 h. The growth of control bacterial strains as well as inhibitions of the bacterial growth due to propolis were measured by turbidity at 420 nm wavelength. The mean values of inhibition were calculated from triple reading in each test. The minimum inhibitory concentration (MIC) of propolis was determined by the ten-fold dilution method against bacterial strains in *in-vitro* (Hegazi *et al*., 1996a). Data were analyzed statistically using student "T" test according to Senedcor (1961).

Antifungal assay

The antifungal activity of propolis was carried out as described in British Pharmacopoeia (1968) against *Candida albicans* (562). *Candida albicans* was kindly supplied from Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria. . Sabouraud's glucose agar and broth inoculated by the spore suspension (0.20 µl/10 ml). Then added 40 µl of 20% propolis. The tubes were incubated at 28 °C for 48h. The growth as well as inhibition were measured as turbidity at 420 nm. The mean values of inhibition were calculated from triple reading in each test. The minimum inhibitory concentration (MIC) of propolis was determined by the ten-fold dilution method against *Candida albicans* in *in-vitro* (Hegazi *et al*., 1996b). Data were analyzed statistically using student "T" test according to Senedcor (1961).

Results and Discussion

Propolis samples were collected from three provinces in East area of Nile Delta region, Dakahlia, Ismailia and Sharkia, each of them characterized by some types of predominant trees or shrubs. These samples were extracted at room temperature with 70% ethanol, the extracts were silylated and subjected to GC/MS analysis. The results obtained are summarized in Table I. The three propolis samples showed qualitative similarities in 3 compounds: palmitic acid, oleic acid and glycerol, the concentration of the first two acids was significantly higher in Dakahlia sample, while glycerol was significantly higher in Ismailia sample.

The Dakahlia sample showed the presence of the characteristic groups of poplar propolis. These groups are aliphatic acids, aromatic acids, aromatic acid esters and flavonoids and it was also characterized by the presence of some triterpenoids. From the aliphatic acids, it contained a significant concentration of palmitic, oleic, stearic, tetracosanoic and hexacosanoic acids. The Dakahlia sample showed high concentrations of the following aromatic acids: benzoic, cinnamic, trans-p-coumaric, 3,4-dimethoxycinnamic, ferulic and caffeic acids. Between the 19 esters identified , the Dakahlia sample was characterized also by the presence of 11 caffeate esters, from which two are new to propolis: tetradecenyl caffeate(isomer) and tetradecanyl caffeate. It also contained some triterpenoids, from which two are new to propolis : lupeol and α-amyrin. In contrary to Dakahlia sample, the Ismailia sample did not contain any aromatic acids and aromatic esters (except phthalate ester) and flavonoids (except hexamethoxyflavone). It contained 6 aliphatic acids from which three are new to propolis: pentonic acid-2-deoxy-3,5-dihydroxy-γ-lactone and its isomer and 2,3,4,5-tetrahydroxypentanoic acid-1,4-lactone (isomer). Also it was characterized by the presence of 5 new triterpenoids to propolis: four 3-oxo-triterpenic acid methyl esters belonging to oleanane and ursane types and one triterpene from the β-amyrin type. Four new sugar and sugar derivatives have been identified in Ismailia sample: galactitol, gluconic acid, galacturonic acid and 2-O-glycerylgalactose. Also 1,2,3-trihydroxy butanal (isomer), 1-methoxy-1,3-dihydroxypropane and dihydroxyacetone dimer were identified for the first time to propolis. Sharkia propolis is extremely different from the Ismailia sample. It shared with Dakahlia sample 6 aliphatic acids, 4 aromatic acids and one flavanone (pinobankasin). It was characterized by the presence of caffeate esters only (8 esters, from which 2 are new to propolis) as well as 3 diterpenes, 5 triterpenes and 2 flavonoids. It was also characterized by the presence of three isomers of myristicin.

From the above mentioned data it is clear that Dakahlia sample is a typical poplar propolis (Christove *et al*., 1998, Hegazi *et al*., 2001). In this investigation 65 compounds were identified in the Dakahlia sample while in Christove's study there were 39 compounds. The study of Christove *et al*., 1998 was done on poplar sample collected from Banisiewief province (Upper Egypt) while Dakahlia is located in the East area of Nile Delta region.

Table I. Chemical composition assessed by GC/MS of alcoholic extracts of East Nile Delta propolis samples.

Compound	Dakahlia	Ismailia	Sharkia
	% *TIC*[a]		
Aliphatic Acids			
Lactic acid	0.25	–	0.55
Hydroxyacetic acid	0.06	–	0.02
5-Hydroxy-n-valeric acid	0.28	–	–
2,3-Dihydroxypropanoic acid	–	–	0.06
Pentonic acid- 2-deoxy-3,5-dihydroxy-γ-lactone [b]	–	0.02	–
Pentonic acid- 2-deoxy-3,5-dihydroxy-γ-lactone (isomer) [b]	–	0.02	–
Malic acid	0.70	–	0.13
Succinic acid	–	–	0.14
2,3,4,5-Tetrahydroxypentanoic acid-1,4-lactone	–	0.11	–
2,3,4,5-Tetrahydroxypentanoic acid-1,4-lactone (isomer) [b]	–	0.01	–
Nonanoic acid	0.20	–	–
Decanoic acid	0.20	–	–
Dodecanoic acid	0.50	–	–
Tetradecanoic acid	0.50	–	–
Palmitic acid	13.30	0.06	1.40
Heptadecanoic acid	–	–	0.05
Linoleic acid	1.50	–	–
Oleic acid	12.30	0.02	3.20
Stearic acid	6.40	–	1.90
Octadecenoic acid	–	–	–
Eicosanoic acid	– –	– –	0.30
Tetracosanoic acid	8.00	–	–
Hexacosanoic acid	2.00	–	–
2- Hydroxy hexacosanoic acid [b]	– –	–	0.40
Aromatic acids			
Benzoic acid	2.70	–	0.01
2-Phenyl- 2-hydroxy acrylic acid	0.40	–	–
4-Hydroxy benzoic acid	0.60	–	0.02
Dihydrocinnamic acid	0.30	–	–
Cinnamic acid	2.80	–	–
4-Methoxy-cinnamic acid	0.80	–	–
cis-p-Coumaric acid	0.40	–	–
trans-p-Coumaric acid	2.30	–	–
3,4-Dimethoxy-cinnamic acid	2.90	–	0.05
Isoferulic acid	1.10	–	–
Ferulic acid	2.40	–	–
Caffeic acid	4.40	–	0.30
Esters			
Methyl palmitate	0.27	–	–
Ethyl palmitate	0.13	–	–
Stearic acid methyl ester	0.20	–	–
Phthalate ester	–	0.30	3.80
Benzyl benzoate	0.40	–	–
Benzyl-trans-4- coumarate	0.03	–	–
Cinnamyl-trans-4- coumarate	0.09	–	–
3-Methyl-3-butenyl isoferulate	0.07	–	–
3-Methyl-2-butenyl isoferulate	0.14	–	–
3-Methyl-3-butenyl caffeate	0.64	–	0.50
2-Methyl-2-butenyl caffeate	0.18	–	0.12
3-Methyl-2-butenyl caffeate	0.90	–	0.80
Benzyl caffeate	0.32	–	–
Phenylethyl caffeate	0.30	–	–
Cinnamyl caffeate	0.10	–	–

Table I (continued).

Compound	Dakahlia	Ismailia	Sharkia
		% *TIC*[a]	
Esters			
Tetradecyl caffeate	0.18	–	0.84
Tetradecenyl caffeate	0.05	–	0.14
Tetradecenyl caffeate (isomer) [b]	0.13	–	1.26
Tetradecanyl caffeate [b]	0.05	–	0.65
Hexadecyl caffeate	0.15	–	0.40
Di and Triterpenes			
Pimaric acid	–	–	0.50
Dehydroabietic acid	0.14	–	1.09
Abietic acid	–	–	1.00
Lupeol [b]	0.42	–	–
Cycloartinol	0.58	–	0.80
Lanosterol	–	–	0.30
Lanosterol with another double bond	–	–	0.16
α-Amyrin [b]	0.30	–	0.20
β-Amyrin	0.21	–	0.80
Triterpene of β-amyrin type [M^+] m/z = 498	0.44	0.73	–
3-Oxo-triterpenic acid methyl ester (oleanane type) [b] [M^+] m/z = 468	–	0.14	–
3- Oxo-triterpenic acid methyl ester (oleanane type) [b] [M^+] m/z = 468	–	0.21	–
3- Oxo-triterpenic acid methyl ester (ursane type) [b] [M^+] m/z = 468	–	0.46	–
3- Oxo-triterpenic acid methyl ester (ursane type) [b] [M^+] m/z = 468	–	1.16	–
Triterpene of β-amyrin type [b] [M^+] m/z = 498	–	0.13	–
Flavonoids			
2′,6′-Dihydroxy-4′-methoxychalcone (Pinostrobin chalcone)	–	–	0.45
Hexamethoxyflavone	–	0.05	–
Pinostrobin	0.04	–	–
Pinocembrin	6.06	–	–
Pinobankasin	0.30	–	0.80
Pinobankasin-3-acetate	1.16	–	–
Chrysin	0.35	–	–
Galangin	0.40	–	–
5,7- Dihydroxy-3-butanoyloxyflavanone	0.30	–	–
Sugars			
Methylglucose	–	0.03	0.08
Xylitol	–	1.00	0.03
Inositol	–	0.03	–
Galactitol [b]	–	0.11	–
Glycerol octadecyl ether (unidentified)	0.07	–	–
Gluconic acid [b]	–	0.13	0.13
Galacturonic acid [b]	–	0.03	–
2-O-Glycerylgalactose [b]	–	0.04	0.03
Others			
Glycerol	1.41	7.50	2.03
Phosphoric acid	0.04	–	0.07
1,4-Dihydroxy benzene	0.06	–	–
4 -Hydroxy-benzaldehyde	0.04	–	–

Table I (continued).

Compound	Dakahlia	Ismailia	Sharkia
		% *TIC*[a]	
Others			
4 -Hydroxy-acetophenone	0.10	–	–
Vanillin	0.50	–	–
1,2,4-trihydroxy butane	–	–	0.01
1,2,3-trihydroxy butanal	–	0.60	0.07
1,2,3-trihydroxy butanal (Isomer) [b]	–	0.01	–
2,4-bis(dimethyl benzyl)-6-t- butyl phenol	–	0.04	–
1,8-dihydroxy-3- methyl anthraquinone	–	–	1.43
Myristicin	–	–	0.27
Myristicin (isomer)	–	–	0.09
Myristicin (isomer)	–	–	0.04
1-Methoxy-1, 3-dihydroxypropane [b]	–	1.63	–
Dihydroxyacetone dimer [b]	–	0.17	–

[a] TIC = The ion current generated depends on the characteristics of the compound concerned and it is not a true quantitation.
[b] For the first time in propolis.

There were some differences in the chemical composition of these two poplar propolis samples, these variations are species specific.

Four triterpenic acid methyl esters belonging to ursane and oleanane types were identified for the first time in propolis. Ismailia province is rich with *Eucalyptus* trees, so these triterpenoids could originate from this plant. Younes *et al*., (1986) isolated some ursonic acid derivatives from the leaves of Egyptian *Eucalyptus rostrata*. Contrary to the propolis sample collected from an *Eucalyptus* forest in Sao Paulo , Brazil investigated by Marcucci *et al*. (1998) which contained aromatic acids and esters and did not contain any triterpenoids. In this investigation, the Ismailia sample contained new triterpenoids to propolis and did not contain any aromatic acids and esters.

Embryo infected dose fifty/ml (EID_{50}/ml) of Infectious Bursal Disease Virus was 2.8×10^7 (viral particles), while embryo infected dose fifty ml (EID_{50}/ml) of the Reo virus vaccinal strain S 1133 was 1.03×10^8/ml.

Propolis samples from Dakahlia, Ismailia and Sharkia provinces in the East area of Nile Delta, have been investigated to determine the minimum lethal dose of the propolis on embryonated chicken fibroblasts which revealed that its dilution of 1/100 of the original propolis (100 mg/ml) gave less mortality, no cytopathic effect on chicken embryos fibroblast.

Table II. Antiviral activity of Egyptian propolis.

Virus	IBDV	Reo
Virus only	2.8×10^7 *	1.03×10^8
Virus + Dakahlia propolis**	1.1×10^3	1.8×10^5
Virus + Ismailia propolis	5.2×10^4	3.3×10^4
Virus + Sharkia propolis	4.0×10^6	1.1×10^5

IBDV = Infectious Bursal Disease Virus
Reo = Reo virus
* Calculation of the mean 50% infective dose for tissue culture ($TCID_{50}$) / ml (Reed and Muench, 1938).
** Propolis concentration: 50 µl/well yielding 1/100 of the original propolis, concentration of 100 mg/ml.

The effect of propolis on the infectivity titers as measured by the mean tissue culture infective dose ($TCID_{50}$) of IBDV and Reo viruses is illustrated in Table II. It was clear that all propolis samples from different provinces revealed reduction in the infectivity mean titers of the IBDV and Reo viruses. It was obvious that the reduction varied from propolis sample to another. Dakahlia propolis gave the highest reduction (calculated as the mean tissue culture infective dose fifty ($TCID_{50}$) as 1.1×10^3/ml against IBDV while Ismailia propolis showed the highest reduction of 3.3×10^4/ml against Reo virus (calculated as the mean 50% infective dose for tissue culture $TCID_{50}$). But Sharkia propolis showed a moderate activity against both viruses.

Regarding to the inhibitory effect of propolis on replication of IBVD and Reo viruses, it was clear that propolis induced different variations in the inhibitory effect of both viral strains. Propolis induced inhibitory effects on small pox vaccine virus (Ktivotuchko *et al*., 1975); influenza virus (Maolova *et al*., 1985); Newcastle disease virus (Hegazi *et al*., 1993); herpes simplex virus (Amoros *et al*., 1994), rift valley fever virus (Hegazi *et al*., 1997), HIV (Faff and Hiszem 1998), avian influenza virus (Kujumgiev *et al*., 1999); and infectious Bursal disease virus and reo Virus (Hegazi *et al*., 2000b). The infectivity of both viruses was reduced, but this reduction was varied according to the propolis origin. The reduction of the infectivity depends on the chemical composition of different propolis sample collected from the three provinces and confirmed by the chemical analysis in this investigation. These findings of the difference in the chemical composition were previously reported as considerable difference in the biological activities (Kujumgiev *et al*., 1999).

The antimicrobial activity of propolis collected from three provinces of East Nile Delta, Egypt against *Staphylococcus aureus; Escherichia coli,* and *Candida albicans* are recorded in Table III. All propolis samples showed an inhibition in the growth of all examined bacteria but the inhibition varied according to the propolis origin. It was obvious that propolis collected from Dakahlia had the highest antimicrobial activity against *Escherichia coli* and *Candida albicans*. But Sharkia propolis had the highest antimicrobial activity against *Staphylococcus aureus*. The variation in the antimicrobial activity seems to be due to the differences in the chemical composition of different propolis samples. The highest antimicrobial activity of Dakahlia propolis to *Escherichia coli* and *Candida albicans* probably attributed to the presence of some aliphatic and aromatic acids, eleven caffeate esters; triterpenes as (lupeol, cycloartinol) and flavonoids as (pinostrobin, pinocembrin, pinobankasin, pinobankasin-3-acetate, chrysin, galangin and 5,7-dihydroxy-3-butanoyloxy flavanone). While Sharkia propolis had the highest antimicrobial activity against *Staphylococcus aureus* due to the presence of caffeate and phthalate esters, di and triterpenes as (pimaric acid, abietic acid, lanosterol); flavonoids as (pinostrobin chalcone and pinobankasin) and three isomers of myristicin. The results of the antimicrobial activity of such propolis samples are in agreement with the findings of Mertzner *et al*., (1979) who found that the antimicrobial activity of propolis can be attributed to its components as pinocembrin, galangin, pinobanksin, pinobanksin-3-acetate, p-coumaric acid

Table III. Antimicrobial activity of Egyptian propolis.

Treatment	*Staphylococcus aureus*		*Escherichia coli*		*Candida albicans*	
	Growth inhibition	MIC [μg/ml]	Growth inhibition	MIC [μg/ml]	Growth inhibition	MIC [μg/ml]
Pathogen normal growth	1.275 ± 0.0064*	–	1.256 ± 0.0017	– –	1.758 ± 0.0023	– –
Dakahlia propolis	0.576± 0.004	3200	0.146 ± 0.0081	1200	0214 ± 0.0013	1320
Ismailia propolis	0.509± 0.0045	2800**	0.467± 0.0005	2800	0.383± 0.0075	1400
Sharkia Propolis	0.305± 0.005	2400**	0.383± 0.002	2200	0.675± 0.002	3380
Tetracycline (50 μg)	0.095± 0.0001	1000	0.469 ± 0.0003	1400	1.700 ± 0.002	6400
Ketoconazole (50 μg)	1.233± 0.004	8400	1.270 ± 0.0011	5600	0.638 ± 0.003	3200

* Growth inhibition = Inhibition of the growth measured by turbidity at 420 nm.
** MIC: Minimum inhibition concentration.

benzyl ester and caffeic acid esters. Relatively good antimycotic activity was previously identified in the Egyptian propolis by Hegazi and Abd El Hady (2000, 2001). Also Kujumgiev *et al*., (1999) found that all investigated propolis samples were active against fungal and Gram-positive bacterial strains.

The comparison between the activity of different therapeutic agents (against bacteria and fungi) as tetracycline and ketoconazole in relation to different propolis samples revealed that the propolis samples effectively inhibited growth of the pathogens. The minimum inhibitory concentration (MIC) of propolis samples was determined by ten-fold dilution *in-vitro* against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. The results of MIC are illustrated in Table III. There were differences in their minimum inhibitory concentration as indicated by the difference in chemical composition determined in this study. The MIC ranged from 2400 to 3200 μg/ml for *Staphylococcus aureus* while it was 1200 to 2800 μg/ml for *Escherichia coli*. But it ranged from 1320 to 3380 μg/ml in case of *Candida albicans*. The variation in the antibacterial activity of propolis referred to the differentiation in the chemical composition of propolis from area to area. This variation produced variable synergistic effects of the phenolic compounds (Kujumgiev *et al*., 1999; Hegazi *et al*., 2000a; Hegazi and Abd El Hady, 2000b, 2001).

The present results confirm the striking variability of the chemical composition of Egyptian propolis and demonstrate the need for further investigation of Egyptian plants possessing resinous exudates as probable source of propolis.

Acknowledgements

The authors are grateful for the financial support by the National Research Center of Egypt (Contracts 1/1/2/3/1 and 1/3/1/2/14).

Abd El-Hady F. K.(1994), Gas chromatography – mass spectrometry (GC/MS) study of the Egyptian propolis-2 – flavonoid constituents. Egypt. J. Appl. Sci. 9, 91–109.

Abd El-Hady F. K. and Hegazi A. G. (1994), Gas chromatography – mass spectrometry (GC/MS) study of the Egyptian propolis 1 – aliphatic, phenolic acids and their esters Egypt. J. Appl. Sci. **9**, 749–760.

Anon (1971),Methods for examining poultry biologics and for identifying and quantifying avian pathogen. National Academy of Sciences, Washington D. C.

Amoros M., Lurton E., Boustie J., Girre L., Sauvager F. and Cormier M. (1994), Comparison of anti herpes simplex virus activities of propolis and 3-methyl-but-2-enyl caffeate. J. Nat. Prod. **57**, 644–647.

Bankova V. , Christov R. Hegazi A. G., Abd El Hady F. K. and Popov S. (1997), Chemical composition of propolis from poplar buds. Proceedings of International Symposium on Apitherapy Cairo, 8–9th March 1997, p. 26–37.

British Pharmacopoeia, (1968), Biological Assay of Antibiotics. The Pharmaceutical Press, London, pp. 1313–1317.

Christov R., Bankova V, Hegazi A.G, Abd El-Hady F. K. and Popov S. (1998), Chemical composition of Egyptian propolis. Z. Naturforsch. **53c**, 197–200.

Cruickshank R., Duguid J. P., Masion B. P. and Swain R. H.(1979), Medical Microbiology. 12th Edition: Churchill Livingstone, Edinburgh, London, New York.

Faff O. and Hiszem A. G. (1998), Antiviral effect of propolis by inhibiting viral nucleic acid polymerases. PCT Int. Appl. WO 99 49, 830 (Cl. A 61 K), 7 Oct. 1999, DE Appl. 19, 813, 802, 27 Mar. 1998; 13 pp. (Ger).

Finegold S. M. and Sweeny E. E. (1961), New selective and differential medium for coagulase positive staphylococci allowing rapid growth and strain differentiation. J. Bacteriol. **81**, 636.

Hegazi A. G. (1998), Propolis an overview. J. Bee Informed **5**, 22–23 and **6**, 23–28.

Hegazi A. G. and Abd El Hady F. K. (1994), Influence of propolis on chicken immune response. Egypt. J. Immunol. **1**, 92–97.

Hegazi A. G. and Abd El Hady F. K. (2000), Influence of Egyptian propolis as antifungal agent. In: International Conference of Propolis. Argentina, September 2000, p. 114.

Hegazi A. G. and Abd El Hady F. K. (2001), Egyptian propolis: 1- Antimicrobial activity and chemical composition of Upper Egypt propolis. Z. Naturforsch. **56c**, 82–88.

Hegazi A. G., Abd El Hady F. K. and Abd Allah F. A. M. (2000a), Chemical composition and antimicrobial activity of European propolis. Z. Naturforsch. **55c**, 71–75.

Hegazi A. G., Awadalla K. Y. and Mansour S. M. (1997), Influence of honey and propolis on Rift Valley Fever Virus. Proceeding of International Symposium On Apitherapy, Cairo 8–9th, March, 1997, p. 70.

Hegazi A. G., El Berdiny F., El Assily S., Khashabah E., Hassan N. and Popov S. (1993), Studies on some aspects of antiviral activity. 1-Influence of propolis on NDV. Vet. Med. J. Giza **41**, 53–56.

Hegazi A. G., El Miniawy H. F. and El Miniawy F. A. (1995), Effect of some honey bee products on immune response of chicken infected with virulent Newcastle disease virus (NDV). Egypt. J. Immunol. **2**, 79–86.

Hegazi A. G. Farghali A. A. and Abd El Hady F. K. (2000b), Antiviral activity and chemical composition of European and Egyptian propolis. In: International Conference of Propolis. Argentina, September 2000, p. 99.

Hegazi A. G., Hazzaa M. and Tosson E. A. (1996-a), Influence of propolis on normal and infected rats with correlation to serum glucose and liver glycogen. J. Union Arab. Biol. **3** (B), 77–86.

Hegazi A. G., Hazzaa M. and Abd El Aziz A. (1996b), Antifungal activity of Egyptian propolis. J. Union. Arab. Biol. 3 (B), 67–75.

Komine K., Ohta H., Kamata S., Uchida K., Yoshikawa Y., Yamanouchi Y. and Okada H. (1989), Complement activation by Bursal Disease Virus growth in lymphoid cells. Jap. J. Vet. Sci. **51**, 1259–1262.

Ktivotuchko V. F., Degtyarenko V. I., Zaitseva T. L. and Ganchenko M. G. (1975); Antiviral effects of propolis. Vitusy I Virusnye Zabolevaniya **3**, 61–64 (in Russian).

Kujumgiev A., Tsvetkova I., Serkedjieva Y., Bankova V., Christov R. and Popov S. (1999), Antibacterial, antifungal and antiviral activity of propolis of different geographic origin. J. Ethnopharmacol **64**, 235–240

Maolova N. H., Maximova. A., Gegova. G. A., Serlcedjieva Y. P., Uzunov. S. T., Matelcov N. Y. and Bankova V. S. (1985), On the antinfluenza action on fractions from propolis. C. R. Acad. Bulg. Sci. **38** (6), 735–738.

Marcucci M. C., Rodriguez J., Ferreres F. Bankova V., Groto R. and Popov S. (1998), Chemical composition of Brazilian propolis from Sao Paulo state. Z. Naturforsch. **53c**, 117–119.

Mertzner J., Bekemeier H., Paintz M. and Schneidewind E. (1979), Antimicrobial activity of propolis and its constituents. Pharmazie **34**, 97–102.

Senedcor G. W. (1961), Statistical Methods, 5th Ed. Iowa State University Press, Iowa USA.

Reed L. V. and Muench H. (1938), A simple method of estimating of fifty percent end points. Am. J. Hyg. **27**, 493.

Taylor D. L., Olson N. O. and Burrell R. G. (1966), Adaptation of an avian arthritis viral agent to tissue culture. Avian Dis. **10**, 462–470.

Youies M. G., Amer M. S. and Sheta A. E. (1986), Chemical examination of local plants. Part X VI. Isolation of 9 α- hydroxyursonic acid from the leaves of Egyptian *Eucalyptus rostrata*. Arab Gulf J. Scient. Res. **4**, 79–86.