Egypt. J. Vet. Sci. Vol. 42 pp. 39 - 51 (2008)

Comparative Study on Chemical Composition and Antimicrobial Activity of Egyptian and Canadian Propolis

A.G. Hegazi and Faten K. Abd El-Hady
National Research Centre, Cairo, Egypt.

TWO PROPOLIS samples from Egypt and Canada were investigated by GC/MS, 103 compounds were identified, mainly terpenoids and phenolics, 17 being new for propolis. The samples showed some similarities in their qualitative composition of the phenolics. In Canadian propolis benzoic acid, trans-p-coumaric acid, benzyl-trans-4-coumarate and pinocembrin were predominant. The Egyptian propolis showed the presence of 7 di- & triterpenoids, from which three are new.

The antimicrobial activity of propolis collected from Egyptian propolis showed the highest antimicrobial activity against *Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi, Asprgillus flavus; Asprgillus fumigatus; Mucor and Candida albicans*, while Canadian propolis has the highest activity against *Staphylococcus aureus; Bacillus subtilis; Erwinia carotovora; Asprgillus ochraceus and Asprgillus niger*.

Keyword : Propolis, GC/MS, Phenolics, Terpenes, Antimicrobial activity.

Egyptian propolis (bee glue) is a resinous hive product. It used by bees as glue for general-purpose, sealer and draught-excluder for beehives. It was used for a long time as early as 3000 BC (Hegazi, 1998). It has recently become a subject of increasing interest for chemists and biologists It had various biological and therapeutic activities. Propolis possesses variable biological activities: antiviral activity (Abd El-Hady *et al.*, 2007), antibacterial (Hegazi and Abd El Hady, 2002); fungicidal (Hegazi *et al.*, 2000); antiulcer and anti-tumor (Marcucci, 1995 and Hegazi *et al.*, 1998), antiprotozoa (Higashi and de Castro, 1995), inhibitory effect on the vitality and hatchability of immature *F. gigantica eggs* (Hegazi *et al.*, 2007-a) and antiparasitic (Hegazi *et al.*, 2007-b). Little is known about Canadian propolis. Only two investigations has been published on its phenolic constituents (Garcia-Viiguera *et al.*, 1993 and Christov *et al.*, 2006). Thus this publication aimed to compare between the chemical composition as well as the antimicrobial activity of Egyptian and Canadian propolis.

Correspondence anthor: E - Mail: ahmedhegazi128@gmail. com

Materials and Methods

Propolis

Propolis sample was collected in Canada, Burnaby, Vancouver, British Colombia and provided kindly by Dr. Simon, H. Wallis, Psipharm Consulting, Burnaby, Vancouver, Canada. While Egyptian propolis sample was collected from Al Mansoura, Dakahlia Governorate.

Thin layer chromatography (TLC)

Propolis samples have been collected from two countries with different climates as Egypt and Canada, the main plant source of propolis not being always poplar buds. These samples have been extracted with 70% ethanol. The alcoholic extracts were subjected to preliminary investigation by thin layer chromatography (TLC).

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 balsam obtained after the evaporation of the alcohol was prepared for chromatography by derivatization for 30 min at 100 °C with 50µl pyridine + 100µl 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, 0.32 mm x 25 m (diameter), was employed with helium as carrier gas and the temperature programmed from 40 to 260°C at 5°C / min. (3-min. initial hold, 10-min. final hold). The mass spectra were recorded in electron ionization (EI) mode at 70 eV, ion source temperature 150°C. The scan repetition rate was 0.5 s over a mass range of 39 amu to 400 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.

Antibacterial assay

Six bacterial strains were used: *Staphylococcus aureus, Bacillus subtilis. Pseudomonas aeruginosa; Salmonella typhi, Escherichia coli and Erwinia carotovora.* The bacterial suspension was prepared and adjusted obtain a final of $5X10^6$ organisms/ml (Mc-Farland turbidity standard). These bacterial strains were enriched on nutrient broth as well as on selective broth for bacterial

propagation (Cruickshank *et al.*, 1979). The growth rate as well as inhibition concentration were measured by Spectrophotomeric as turbidity at 420 nm wave length. The mean value of inhibition were calculated triple reading in each test. The minimal inhibitory concentration (MIC) of propolis samples were determined by tenfold dilution method against bacterial strains in in-vitro as well as antimicrobial activity (Hegazi *et al.*, 1996).

Antifungal assay

The antifungal activity of the tested propolis samples was carried out against *Asprgillus ochraceus, Asprgillus niger, Asprgillus flavus, Asprgillus fumigatus, Mucor and Candida albicans* as described by Hegazi *et al.*, (2000). Nutrient broth inoculated by the spore suspension (0.20 ul/10 ml) fungi or yeast, then 20 % propolis was added. The tubes containing yeast were incubated at 28°C for 48hr.The growth as well as inhibition concentration were measured by Spectrophotomeric as a turbidity at 420 nm wave length .The mean value of inhibition were calculated triple reading in each test. Data were statistically analyzed using student "T" test according to Senedcor (1961).

Results and Discussion

Propolis samples have been extracted with 70% ethanol. The alcoholic extracts were subjected to preliminary investigation by thin layer chromatography (TLC). The spots of phenolic acids and phenolic esters showed similarity in the two samples, but the amount of some phenolic acids and phenolic esters in the Canadian sample were much larger. The percentage of extracted matter was as follow: Egyptian propolis 0.80 gm/dry weight, while Canadian propolis 0.64 gm/dry weight.

The samples were silylated and subjected to GC/MS analysis. The results obtained are summarized in Table I . It is evident that the two propolis samples showed some similarities in their qualitative composition. Both of them contained lactic, 2,3-hydroxypropanoic, malic,palmitic, linoleic, oleic and stearic acid. Hydroxyacitic and 5-hydroxy-n-valeric acid were newly. 2-Hydroxystearic acid was identified in Canadian sample for the first time in propolis.

The following aromatic acids were identified in both samples: dihydrocinnamic, trans-cinnamic, 4-hydroxybenzoic, cis-p-coumaric, 4-methoxycinnamic, isoferulic, ferulic and caffeic acid. Benzoic and trans-p-coumaric acid were very significantly higher in Canadian propolis than in Egyptian one. cis-Cinnamic and vanillic acid were only identified in Canadian propolis, while 3,4-dimethoxycinnamic acid in Egyptian one. 2-phenyl-2-hydroxyacrylic acid was newly identified. α-oxo-benzeneacetic acid was identified in Canadian sample for the first time in propolis.

Eleven caffeate esters were identified in Egyptian propolis, from which, tetradecenyl caffeate (isomer) and tetradecanyl caffeate were identified for the first time in propolis. Three caffeate esters were only identified in Canadian. Beside the above mentioned esters, methyl palmitate and methyl stearate identified in Egyptian sample for the first time in propolis and 4-methoxydihydrocinnamic acid ethyl ester and 1-(4-hydroxy-3-methoxyphenyl)1-

ethoxyacetic acid ethyl ester in Canadian. Benzenepropanoic acid ethyl ester was newly identified. trans-Benzyl-4-coumarate and phenylethyl caffeate were significantly higher in Canadian propolis. Ethyl palmitate, 3-methyl-3-butenyl-isoferulate and 3-methyl-2-butenyl-isoferulate were only found in Egyptian propolis, while cinnamyl cinnamate, benzyl-cis-4-coumarate, phenylethyl-trans-coumarate and benzyl ferulate in Canadian. Finally, 9-octadecenamide was identified in Canadian sample for the first time in propolis. Canadian propolis contained six mono- and sesquiterpenes, five of them were completely identified, while one sesquiterpene alcohol was only found in Egyptian propolis.

The diterpene, dehydroabietic acid was found in both samples, while pimaric acid which was identified for the first time in propolis, was found only in Egyptian sample.

Contrary to Canadian propolis the Egyptian sample contained some triterpenic alcohols, including lupeol, α-amyrin, β-amyrin, cycloartinol and pentacyclic triterpenoid from the β-amyrin type. To the best of our knowledge, triterpenic compounds with ursan/ lupan skeleton have not been found in propolis till now. Lupeol and α-amyrin were identified for the first time in propolis. Surprisingly in Canadian propolis we did not find the above mentioned triterpenes.

About nine flavonoids as: dihydrochalcones, flavanones and flavones were identified. pinocembrine was the highest one in both samples.

Beside phosphoric acid, which was common in the two samples, phosphoric acid monomethyl ester and phosphoric acid dioctadecyl ester were identified for the first time in propolis in both samples and Egyptian propolis respectively.

In Canadian propolis we found high concentration of carbohydrates than in Egyptian one. Beside the main compounds from this group: glucose and fructose, which are characteristic for hives, we identified sorbose and another pentose, temporary identified as arabinose. Galactose and methylglucose were also identified.

Egyptian propolis showed some significant similarities in many compounds (Hegazi and Abd El-Hady, 2002), have been identified nine new compounds for propolis. These results confirm the variability of the chemical composition of tropical propolis (Tomas-Barberan *et al.*, 1993; Aga *et al.*, 1994; Bankova *et al.*, 1996). The explanation could be analogous: the Egyptian propolis which could be gathered from more than one plant source, one of them has to be a poplar species, probably *P. nigra*. This indicated by the high concentration of esters of phenolic acids, the presence of pentenyl caffeates and the typical flavanones. The presence of long chain caffeate esters still remain unclear, however, investigation of poplar bud exudates from Egypt is necessary to find if they are poplar metabolites. Our work is in agreement with that of (Hegazi and Abd El-Hady, 2002), where we found that Egyptian propolis is characterized by the presence of large number of caffeic acid esters, beside the large number of the other phenolic acid esters.

The presence of substances unusual for poplar buds, such as sterols and triterpenes, are an indication that these could be another plant source for propolis in Egypt. Triterpene alcohols of amyrine type were found in Egyptian and Brazilian propolis (Hegazi & Abd El-Hady, 2002 and Bankova, 2005). Egyptian and Canadian propolis contained the usual flavonoids of poplar propolis (Greenaway *et al.*, 1990 and Bankova *et al.*, 1994).

The comparison of the chemical composition of the present investigated sample with that of earlier studied Canadian propolis (Garcia-Viiguera *et al.*, 1993) showed some little significant similarities in many compounds. Besides we identified five new compounds for Egyptian propolis. These results confirm the variability of the chemical composition of the temperate propolis (Greenaway *et al.*, 1989, 1990a, 1990b, 1990c, Bankova *et al.*, 1994 and (Hegazi & Abd El-Hady, 2002). The significant difference is that: we identified a large number of phenolic acid esters for benzoic, coumaric, ferulic and caffeic acids. In addition to that some monoterpenes, sesquterpenes, diterpene, aromatic ketones, aldehydes and alcohols were identified in the present work.

Some similarities were found when comparing the aliphatic and phenolic acids, but here, we identified some much more and different acids. Benzoic and trans-p-coumaric acids appeared with highly significant amounts in the present investigation. In contrary, the flavonoids in the earlier study (Garcia-Viiguera *et al.*, 1993) appeared with highly significant amounts of chalcones and flavones, specially 2`,4`,6`-trihydroxychalcone, chrysin and galangin, while in our investigation all the flavonoids appeared in low concentration, except the flavanone pinocembrin.

The antibacterial activity of propolis collected from Egypt and Canada against *Staphylococcus aureus; Bacillus subtilis;; Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi and Erwinia carotovora* was recorded in Table 2. All propolis treatments showed an inhibition in the growth of all examined bacteria but the inhibition varied according to the propolis origin.. The propolis collected from Egypt showed the highest antibacterial activity against *Pseudomonas aeruginosa* (0.275 ± 0.0024)), *Escherichia coli* (0.146± 0.0081) and *Salmonella typhi* (0.136± 0.0041). The Canadian propolis showed the highest antibacterial activity against *Staphylococcus aureus* (0.178 ± 0.0028), *Bacillus subtilis* (0.285 ± 0.0028), and Erwinia carotovora (0.272 ± 0.0046).

The results of antifungal activity against moulds and yeast using extracted propolis samples from Egyptian and Canadian propolis were recorded in Table 3. All the examined propolis samples showed antifungal activity with different degree of inhibition. It was clear that Egyptian propolis showed the highest antifungal activity against *Asprgillus flavus* (0.137± 0.006); *Asprgillus fumigatus* (0.335± 0.0011); *Mucor* Sp.(0.732± 0.004) and *Candida albicans* (0.214± 0.013), where the Canadian propolis showed effective results against *Asprgillus ochraceus* (0.275± 0.0058) and *Asprgillus niger* (0.281± 0.0012).

The antimicrobial activity of propolis collected from Egypt and Canada against *Staphylococcus aureus; Bacillus subtilis;; Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi; Erwinia carotovora; Asprgillus niger ; Asprgillus flavus; Asprgillus fumigatus; Asprgillus ochraceus ; Mucor Sp.* and *Candida albicans* was evaluated in this investigation. The result of antimicrobial activity of propolis collected from Egypt showed the highest activity against *Pseudomonas aeruginosa, Escherichia coli* and *Salmonella typhi, Asprgillus flavus; Asprgillus fumigatus Mucor Sp.* and *Candida albicans*. These findings may be due to the presence of 3,4-dimethoxycinnamic acid, eleven caffeate esters, methyl palmitate, methyl stearate, benzenepropanoic acid ethyl ester, ethyl palmitate, 3-methyl-3-butenyl-isoferulate, 3-methyl-2-butenyl-isoferulate, pimaric acid, triterpenic alcohols, including lupeol, α-amyrin, β-amyrin, cycloartinol and pentacyclic triterpenoid from the β-amyrin type.

The Canadian propolis showed the highest antibacterial activity against *Staphylococcus aureus, Bacillus subtilis ,Erwinia carotovora, Asprgillus ochraceus* and *Asprgillus niger*. This activity was probably due to presence of benzoic and trans-p-coumaric acid Which significantly higher in Canadian propolis than in Egyptian propolis. α-oxo-benzeneacetic acid, 4-methoxydihydrocinnamic acid ethyl ester, 1-(4-hydroxy -3- methoxyphenyl, 1- ethoxyacetic acid ethyl ester , trans - Benzyl-4-coumarate, phenylethyl caffeate ethyl palmitate, 3-methyl-3-butenyl-isoferulate, 3-methyl-2-butenyl-isoferulate, cinnamyl cinnamate, benzyl-cis-4-coumarate, phenylethyl - trans-coumarate and benzyl ferulate, 9-octadecenamide , Lupeol and α-amyrin. the absence of some fatty acids as nonanoic acid, tetradecanonic acid eicosenoic acid, eicosanoic acid, docosanoic acid and hexacosanoic acid. Such finding was previously reported by Gizmarik and Trupl (1976) who found that the antifungal activity decreased with increasing carbon chain length in the even-numbered carbon chain series, while odd-numbered carbon fatty acids showed irregularities. Undecanoic acid (C11:0) was the most toxic in the C7:0 –C18:0 series. Polyunsaturated fatty acids were more toxic than their corresponding saturated acids.

The antimicrobial activity of propolis reflected to its constituent which differs from area to area depending on its chemical composition Propolis differ from region to region to its antimicrobial activity as in USSR (Shub *et al.*, 1978); in Poland (Meresta and Meresta, 1983). Pepeljnjak *et al.* (1982) in Croatia and Yugoslavia; in Hungary Petri *et al.* (1988) and Hegazi *et al.* (1996) from Egypt. The variation of the antibacterial activity of propolis from area to area refereed to the chemical composition of propolis, which had a synergistic effect of various phenolic compounds as well as flavonoids. Also geographic areas differ due to plant flora which reflected in the propolis constituents.

In the present investigation Gram positive bacteria are more affected than Gram negative bacteria. The most sensitive bacteria are *Streptococcus faecalis* where the most resistant organism is *Escherichia coli.* These findings come in

full conformity with many authors as Pepeljnjak *et al.* (1982); Petri *et al.* (1988); Abd El Fattah *et al.*, (1993) and Hegazi *et al.* (1996 & 1997).

It is clear that, there is a significant difference between Egyptian and Canadian propolis sample and between the investigated samples and the earlier investigations of Egyptian and Canadian propolis. These differences could be a poplar species-specific and or the probable participation of another plant source as well as the climate variations.

Acknowledgement: The authors are grateful for the financial support by the National Research Center of Egypt (Contract 1/1/2/3/1).

TABLE 1. Chemical composition assessed by GC/MS of alcohol extracts of Egyptian and Canadian Propolis.

Compound	Egypt TIC%[a]	Canada TIC%[a]
Acids (aliphatics)		
Lactic acid	0.25	0.40
Hydroxyacetic acid	0.06	0.20
Oxalic acid	-----	0.02
5-Hydroxy-n-valeric acid	0.28	0.50
2,3-Dihydroxypropanoic acid	0.01	0.05
Nonanoic acid	0.02	------
Decanoic acid	0.02	------
Malic acid	0.07	1.90
Dodecanoic acid	0.05	------
Tetradecanoic acid	0.05	------
Palmitic acid	1.33	1.20
Linoleic acid	0.15	0.20
Oleic acid	1.23	0.70
Stearic acid	0.64	0.40
2-Hydroxy stearic acid[b]	-----	0.02
Tetracosanoic acid	0.80	------
Hexacosanoic acid	0.20	------
Acids (aromatics)		
Benzoic acid	0.27	16.30
Dihydrocinnamic acid	0.03	0.20
cis-Cinnamic acid	------	0.01
trans- Cinnamic acid	0.28	2.40
α-oxo-Benzene acetic acid[b]	------	0.10
2-Phenyl- 2-hydroxy acrylic acid	0.04	0.07
4-Hydroxy benzoic acid	0.06	0.30
Vanillic acid	------	0.10
cis-P-Coumaric acid	0.04	0.80
4- Methoxy-cinnamic acid	0.08	0.04
trans- P-Coumaric acid	0.23	17.20

TABLE 1. Continue .

Compound	Egypt TIC%	Canada TIC%
3,4-di-Methoxy-cinnamic acid	0.29	------
Isoferulic acid	0.11	0.20
Ferulic acid	0.24	2.70
Caffeic acid	0.44	1.80
Aromatic aldehydes and ketones		
4 –Hydroxy-benzaldehyde	0.04	0.50
4 –Hydroxy-acetophenone	0.10	0.05
Vanillin	0.05	2.00
Aliphatic and aromatic alcohols		
Guiacol	------	1.50
Glycerol	1.41	0.90
1,4-Dihydroxy benzene	0.01	0.40
Esters		
Benzenepropanoic acid ethyl ester	------	0.10
Benzylybenzoate	0.04	0.80
4-Methoxy dihydrocinnamic acid ethyl ester [b]	------	0.04
Cinnamyl cinnamate	------	0.06
1-(4-Hydroxy-3-methoxyphenyl)1-ethoxyacetic acid ethyl ester [b]	------	0.10
Methyl palmitate [b]	0.27	-----
Ethyl palmitate	0.13	-----
Stearic acid methyl ester [b]	0.20	-----
Benzyl- cis-4- coumarate	------	0.80
Benzyl- trans-4- coumarate	0.03	7.30
Phenyl-ethyl-trans- coumarate	------	0.60
Cinnamyl- trans- coumarate	0.09	0.50
3-Methyl-3-butenyl-isoferulate	0.07	-----
3-Methyl-2-butenyl-isoferulate	0.14	-----
Benzyl –ferulate	------	1.40
3-Methyl-3-butenyl- caffeate	0.64	-----
2-Methyl-2-butenyl- caffeate	0.18	-----
3-Methyl-2-butenyl- caffeate	0.90	-----
Benzyl – caffeate	0.32	1.00
Phenyl-ethyl – caffeate	0.30	2.80
Cinnamyl – caffeate	0.10	0.06
Tetradecyl – caffeate	0.18	-----
Tetradecenyl – caffeate	0.05	-----
Tetradecenyl – caffeate (isomer) [c]	0.13	-----
Tetradecanyl – caffeate [c]	0.05	-----
Hexadecyl – caffeate	0.15	-----
9- Octadecenamide [b]	------	0.10
Monoterpenes and Sesquiterpenes		
3-Cyclohexene-1-methanol--α -α-4-trimethyl	------	0.03
Guaiol	------	0.10

TABLE 1. Continue.

Compound	Egypt TIC%	Canada TIC%
2-Naphthalenemethanol decahydro-α -α-4a-trimethyl-8-methylene	------	0.40
Sesquiterpene alcohol	------	0.40
Sesquiterpene alcohol	0.05	-----
α -Bisabolol	------	0.20
2-Naphthalenemethanol,2.3,4.4a,5,6,7,8.octahy-dro -α -α-8-tetramethyl	------	0.70
Diterpenes and Triterpenes		
Dehydroabietic acid	0.14	0.14
Pimaric acid [b]	0.58	-----
Lupeol [b]	0.42	-----
α -Amyrin [b]	0.30	-----
β-Amyrin	0.21	-----
Cycloartinol	0.58	-----
Triterpene of β-amyrin type	0.44	-----
Flavonoids		
2`,6`-Dihydroxy-4`-methoxy dihydrochalcone	-----	0.30
2`,4`,6`-Trihydroxy dihydrochalcone	-----	0.02
Pinostrobin	0.04	0.60
Pinocembrin	6.06	6.60
Pinobankasin	0.30	0.50
Unknown flavonoid (M+ = 430)	0.55	------
Pinobankasin-3-acetate	1.16	0.40
Chrysin	0.35	0.90
Galangin	0.40	1.00
5,7- Dihydroxy-3-butanoyloxy flavanone	0.30	0.10
Sugars		
2,3,5,6,Tetrahydroxy-methylglucofuranoside	------	1.90
Pentose (temporary identified as arabinose)	0.15	0.20
Fructose	1.20	5.90
Sorbose	0.45	4.80
Galactose	-----	2.30
Glucose	0.36	2.30
Sugar (unidentified)	------	0.60
Glucose dimer	------	0.40
Others		
Phosphoric acid monomethyl ester [b]	0.01	0.01
Phosphoric acid	0.04	0.10
Phosphoric acid dioctadecyl ester [b]	0.49	------
Alkane C21	0.24	------
Alkane C23	0.46	------
1- Glycerol ether (unidentified)	0.07	0.05

[a] 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.
[c] Tentatively identified by analysis of mass spectrum.

TABLE 2. Effect of Egyptian and Canadian propolis as antibacterial activity.

	Staphylococcus aureus	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Salmonella typhi*	*Erwinia carotovora*
Egyptian	0.567± 0.006	0.393± 0.0064	0.146± 0.0081	0.275± 0.0024	0.136± 0.0041	0.515± 0.0076
Canadian	0.178± 0.0028	0.285± 0.0004	0.625± 0.0099	0.315± 0.035	0.24± 0.005	0.272± 0.0046
Bacteria	1.275± 0.0064	1.373± 0.0696	1.2560± 0.00170	1.1222± 0.0876	1.2795± 0.0161	1.5947± 0.013

TABLE 3. Effect of Egyptian and Canadian propolis as antifungal activity.

	Asprgillus ochraceus	*Asprgillus niger*	*Asprgillus flavus*	*Asprgillus fumigatus*	*Mucor*	*Candida albicans*
Egyptian	0.515± 0.0017	0.394± 0.0033	0.137± 0.006	0.355± 0.0011	0.732± 0.004	0.214± 0.0013
Canadian	0.275± 0.0058	0.281± 0.0012	0.557± 0.0952	0.431± 0.005	0.824± 0.0025	0.325± 0.0014
Fungi	1.788± 0.0288	1.864± 0.029	1.7407± 0.319	1.9357± 0.291	1.863± 0.305	1.7585± 0.229

References

Abd El-Hady, F.K., Hegazi, A.G. and Wollenweber, E. (2007) Effect of Egyptian propolis on the susceptibility of LDL to oxidative modification and its antiviral Activity with Special Emphasis on Chemical Composition. *Z. Naturforsch*, **62c**, 645-655.

Aga, H., Shibuya, T., Kurimoto, M. and Nakajimaaa, Sh. (1994) Isolation and identification of antimicrobial compounds in Brazilian propolis. *Biosci. Biotech. Biochem.*, **58**. 945-946.

Bankova, V., Popov, S., Bocari, G. and Haxhialushi, E. (1994) Phenolics in Albanian poplar buds and their relationship to propolis. *Fitoterapia*, **65**, 326-330.

Bankova V., Marcucci, M.C., Simova, S., Nikolova, N., Kujumgiev, A. and Popov, S. (1996) Antibacterial diterpenc acids from Brazilian propolis. *Z. Naturforsch*, **51c**, 277-280.

Bankova, V. (2005) Recent trends and important developments in propolis research. *eCAM;* **2**, 29–32.

Christov, R., Trusheva, B., Popova, M., Bankova, V. and Bertrand, M. (2006) Chemical composition of propolis from Canada, its antiradical activity and plant origin. *Nat Prod Res*. **20(6)**. 531-536.

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

Garcia-Viiguera, C.; Ferreres, F. and Tomás-Barberán, F.A. (1993) Study of Canadian propolis by GC/MS and HPLC. *Z. Naturforsch.* **48c**. 731-735.

Gizmarik, J. and Trupl, J. (1976) Effects of propolis on skin fungi *Pharmazie*, **31**(1). 55-57.

Greenaway, W., May, J. and Whatley, F.R.S. (1989) Flavonoid aglycone identification by gas chromatography-mass spectrometry in bud exudate of Populus balsamifera. *J. Chromatography*, **472**. 393-400.

Greenaway, W., Scaysbrook, T. and Whatley, F.R.S. (1990) The composition and plant origin of propolis : A report of work at Oxford. *Bee World*. **71**. 107-118.

Greenaway, W., English, S. and Whatley, F.R.S. (1990a) Phenolic Composition of bud exudates of Populus deltoides. *Z. Naturforsch.* **45c**. 587-595.

Greenaway, W., Davidson, C.G., Scaysbrook, T., May, J. and Whatlay, F.R.S. (1990b) Hybrid origin of Populus x jackil confirmed by gas chrometography-mass spectrometry analysis and its bud exudate. *Z. Naturforsch.* **45c**. 594-598.

Greenaway, W., English, S. and Whatley, F.R.S. (1990c) Variation in bud Exudate composition Populus nigra assessed by chromatography-mass spectrometry. *Z. Naturforsch*, **45c**, 931-936.

Hegazi, A.G. and F.K. Abd El Hady. (2002) Egyptian propolis: 3- Antioxidant, antimicrobial activity and chemical composition of propolis from reclaimed land. *Z. Naturforsch*, **57c**, 395-402.

Hegazi, A.G., Hazzaa, M. and Abd El Aziz, A. (1996) Antifangal activity of Egyptian propolis. J. Uniion. *Arab. Biol.* 3(B), 67-75.

Hegazi, A.G., Faten, K. Abd El Hady and Fyrouz Abd Allah (2000) Chemical composition and antimicrobial activity of European propolis. *Z. Naturforsch*, 55c. 70-75.

Hegazi, A.G., Nagia Moharm, Nour, M.S. and Khair, A.M. (1997) Influence of different geographic zones on antimicrobial activity of Egyptian propolis. *Proceeding International Symposium On Apitherapy, Cairo 8-9th, March, 1997*, pp. 253-262.

Hegazi, A.G., Faten, K. Abd El Hady and Shalaby, H.A.(2007 a) Inhibitory Effect of Egyptian Propolis on Fasciola gigantica Eggs with Reference to its Effect on Clostridium oedematiens and Correlation to Chemical Composition. *Pakistan Journal of Biological Sciences*, **10**(19), 3295-3305.

Hegazi, A.G., Faten K. Abd El Hady and Shalaby, H.A. (2007 b) An in vitro effect of propolis on adult worms of Fasciola gigantica. *Vet. Parasit.*, **144**, 279–286.

Hegazi, A.G. Nagia Z. Moharm, Fayrouz Abd Allah, Ehsan A.B. El Sheikh, Nabila, A. Ellithy and Khair, A.M. (1998) Influence of ethanolic extract of Egyptian propolis on on immune status in mice bearing Ehrlich, *J. Union of Arab Biologists*, **10**(A), 387-396.

Hegazi, A. G. (1998) Propolis an over view Bee Informed 5(2 3),5,22-23, 6,723-28.

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.

Higashi, I. K.O. and De Castro. S.L. (1995) Effect of different formulations of propolis on mice infected with Trypanosoma cruzi. *J. Ethnopharmacol.* 46(1),55-58.

Marcucci, M.C.(1995) Propolis: Chemical composition, biological properties and therapeutic activity. *Apidologie*, **26**, 83-99.

Meresta, L. and Meresta, T. (1983) Research on in vitro antibacterial activity of propolis extracts. *Bulletin of the Veterinary Institute in Pulawy*, **25** (1-4), 12-14.

Pepeljnjak, S., Jalsenjak, and Maysinger, D. (1982) Inhibition of growth and biosynthesis of ochratoxin A in Asprgillus sulphureus by propolis extract. *Pharmazie*, **37** (6), 439-440.

Petri, G, Lemberkovics, E. and Foldvari, M. (1988) Examination of differences between propolis (bee glave) produced from different floral environments. Elsevier Science publishers, 439-446.

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

Shub, T. A., Kagramonova, K. A., Kivman, G.YA., Tikhonov, A.I. and Gritsenko, V.I. (1978) Antimicrobial activity of propolis extracts. *Pharmacentical Chemistry Journal* **11** (9), 1242 – 1244.

Tomás-Barberán F.A., Garcia-Viiguera C, Vit-Olivier P., Ferreres F., and Tomas-Lorente, F. (1993) Phytochemical evidence for the botanical origin of tropical propolis from Venezuela. *Phytochemistry*, **34**, 191-196.

(*Received* 19/5/2009;
accepted 1/6/2009)

دراسة مقارنة عن التركيب الكيميائى والكفاءة المضادة للميكروبات لصمغ النحل المصرى والكندى

أحمد جعفر حجازى و فاتن كمال عبد الهادى
المركز القومى للبحوث – القاهرة - مصر .

درست عينتان لصمغ النحل المصرى والكندى بواسطة كروماتوجرافيا الغاز وطيف الكتلة حيث تم التعرف على ١٠٣ مركب غالبيتها تربينات وفنولات وجد منها ١٧ مركبا جديدا لصمغ النحل كما أن العينتان أظهرتا تشابها نوعيا فى التركيب الكيميائى. فصمغ النحل الكندى وجد حمض البنزويك وحمض الترانس ب كوكماريك وبنزايل ترانس ٤ كوماريك والبنوسمبرين ؛ بينما صمغ النحل المصرى عن تواجد ٧ داى و اربينويدات ثلاث منها جديدة تم التعرف عليها لاول مرة.

الكفاءة المضادة للميكروبات لصمغ النحل المجمع من مصر أظهر أعلى كفاءة ضد ميكروب السيدوموناس أرجينوزا والميكروب القولونى والسلمونيلا تيفاى وقطر الأسبريجلس فلافيس والأسبريجيلس فيوميجيتيس والميوكر وخميرة الكنديديا البيكانز بينما صمغ النحل الكندى أعطى أعلى كفاءة مضادة ضد الميكروب المكور العنقودى الذهبى والبسيلس ساتليس والأورونيا كاروتوفيرا والأسبريجليس اوكريشيس والأسبريجليس نيجر.