Chemical Composition and Antimicrobial Activity of European Propolis

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Three propolis samples from Austria, Germany and France were investigated by GC/MS, where eleven compounds were being new for propolis. The samples showed some similarities in their qualitative composition. Phenylethyl-*trans*-caffeate, benzyl ferulate and galangin were predominant in German propolis. Benzyl caffeate was predominant in French sample. Pinocembrin was predominant in French and Austrian propolis and *trans*-p-coumaric acid was predominant in all samples.

The antimicrobial activity against *Staphylococcus aureus; Escherichia coli,* and *Candida albicans* was evaluated. German propolis showed the highest antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli.* While Austrian propolis has the highest activity against *Candida albicans.* French propolis was effective against all pathogens but less than German and Austrian propolis.

Introduction

Propolis (bee glue) is the material used by bees as a glue, general-purpose sealer and draught-excluder for beehives. It is a resinous hive product. It consists of exudate from plants mixed with beeswax. Propolis has been long used in folk medicine of different nations as early as 3000 BC (Hegazi, 1998). Now propolis possesses variable biological activities: antibacterial (Hegazi *et al.,* 1996a), antiviral (Hegazi *et al.,* 1993 and 1997), fungicidal (Hegazi *et al.,* 1996b), antiulcer, anti-tumour etc. (Marcucci , 1995; Cheng and Wong, 1996).

The chemical composition of propolis appeared to be extremely complex and more than 180 compounds have been identified so far (Marcucci, 1995), the most important ones being polyphenols. Now it is known that bees collect propolis from different plant buds (Crane, 1988). In temperate climatic zones (Europe, North America, Mongolia, Uruguay, New Zealand) the main source of propolis is poplar buds, mainly these of Populus nigra (Greenaway *et al.,* 1987; Wollenweber *et al.,* 1987; Bankova *et al.,* 1992; Bonvehi *et al.,* 1994; Markham *et al.,* 1996), but in some cases other poplar species can be used as an additional supply of propolis (Greenaway *et al.,* 1989; 1990a; 1990b). In such cases chemical composition of propolis and connected with it biological activity will be changed. So, the aim of this work is to investigate the chemical composition of propolis from different countries as well as the variation in their antimicrobial activities.

Materials and Methods

Propolis

Propolis samples were collected in Austria (Vienna), Germany (Hannover) and in France (Monbliahue).

Bacterial and fungal strains

Staphylococcus aureus, Escherichia coli and *Candida albicans* were used. These strains were isolated and identified in the section of Microbiology and Immunology, Dept. of Parasitology and Animal Diseases, National Research Center, Egypt.

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 till dryness. The percentage of extracted matter was as follows: Austrian propolis 0.352 gm/

dry weight, French propolis 0.20 gm/dry weight and German propolis 0.187 gm/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 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 kg/cm^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 39–650 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.

Antibacterial assay

Two bacterial strains were used: *Staphylococcus aureus* and *Escherichia coli.* The bacterial suspension was prepared and adjusted by comparison against 0.5 Mc-Farland turbidity standard (5x10^7 organisms / ml) tubes. It was further diluted to obtain a final of 5x10^6 organisms / ml. *Staphylococcus aureus* was enriched on polymyxin agar (Finegold and Sweeney, 1961) as a selective media While *Escherichia coli.* was enriched on MacConkey broth. Both bacteria were subculture 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* or *Escherichia coli.* Then added 40 µl of 20% propolis extract. The tubes were incubated at 37 °C for 24 hr. The growth of control bacterial strains as well as inhibitions of the bacterial growth due to propolis were measured by spectrophotometeric assay as a turbidity at 420 nm wavelength. The mean values of inhibition were calculated from triple reading in each test. The minimal inhibitory concentrations (MIC) of propolis samples were determined by ten-fold dilution method against bacterial strains in in-vitro (Hegazi *et al.,* 1996a).

Antifungal assay

The antifungal activity of tested propolis samples was carried out against Candida albicans as described in British Pharmacopoeia (1968). 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 48 hr. The growth as well as inhibition were measured by spectrophotometeric assay as turbidity at 420 nm wave length. The mean value of inhibition were calculated from triple reading in each test. Data were analyzed statistically using student "T" test according to Senedcor (1961).

Results and Discussion

Propolis samples have been collected from three different European countries with different climates (Austria, Germany and France). The main plant source of propolis being always poplar buds. These samples have been extracted with 50 ml of 70% ethanol (twice after 24 hours). The alcoholic extracts were subjected to preliminary investigation by thin layer chromatography (TLC). The spots of flavonoids and phenolic esters showed similarity in the three samples, but the amount of some flavonoids and phenolic esters in the German and French samples were much larger than Austrian propolis. The samples were silylated and subjected to GC/MS analysis. The results obtained are summarized in Table I. It is evident that the Austrian, German and French propolis showed significant qualitative similarities, which is an indication for a common plant source. The quantitative differences obtained could be due to the participation of different poplar species.

There are few groups of polar compounds in the investigated samples, which are characteristic to poplar buds also. To the first group of such com-

Table I. Chemical composition assessed by GC/MS of alcoholic extracts of Austrian, German and French Propolis.

Compound**	Austria	Germany % TIC a	France
Acids (aliphatics)			
Hydroxyacetic acid [b]	0.20	0.05	0.40
5-Hydroxy-*n*-valeric acid [b]	0.10	0.04	0.10
2,3-Dihydroxypropanoic acid	–	0.03	–
Nonanoic acid	–	0.03	–
Malic acid	–	0.02	0.20
Acids (aromatics)			
Benzoic acid	3.10	1.30	4.00
trans-cinnamic acid	4.80	0.40	2.00
2-Phenyl-2-hydroxyacrylic acid [b]	0.30	0.05	–
4-Methylmandelic acid [b]	0.30	0.05	0.30
4-Methoxyhydrocinnamic acid	0.30	–	0.20
cis-p-coumaric acid	0.30	–	0.20
trans-p-coumaric acid	7.00	6.70	6.10
3,4-Dimethoxy-cinnamic acid	–	0.23	2.20
Ferulic acid	2.60	0.05	2.10
Caffeic acid	2.60	2.60	5.20
Esters			
Benzenepropanoic acid ethyl ester [b]	–	–	0.10
Pentenyl coumarate	0.10	0.30	–
Benzyl-*trans*-4-coumarate	3.50	2.70	4.00
Cinnamyl-*trans*-coumarate	1.10	3.40	2.10
3-Methyl-2-butenyl-isoferulate	1.00	0.70	–
Cinnamyl-isoferulate	–	2.80	1.30
Benzyl-ferulate	3.00	8.00	7.30
Cinnamyl-ferulate	–	0.20	–
Ethyl caffeate	–	0.30	–
Butanyl-caffeate [b,c]	–	0.08	–
Benzyl – caffeate	3.10	1.50	14.50
Phenyl-ethyl – caffeate	2.40	5.80	5.10
Phenyl-ethyl – caffeate (isomer) [b]	–	17.00	–
Cinnamyl – caffeate	0.60	5.60	3.10
Flavonoids			
Pinocembrin	15.30	6.90	17.20
Pinobankasin	2.70	4.80	4.80
Pinobankasin-3-acetate	6.10	9.30	9.00
Chrysin	2.50	3.50	5.30
Galangin	6.40	21.60	10.00
5,4′-Dihydroxy-7-methoxyflavone	–	0.44	–
5,7-Dihydroxy-3-butanoyloxy flavanone	1.00	3.00	1.30
5,7-Dihydroxy-3-pentenoyloxy flavanone [b]	–	0.50	–
5,7-Dihydroxy-3-pentanoyloxy flavanone	–	0.20	–
Other aromatic compounds			
2,3-Butanediol [b]	–	0.40	0.50
Guiacol [b]	0.30	0.23	1.40
2,3,5,6,Tetrahydroxy-methylglucofuranoside [b]	0.50	1.60	0.60

[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.
** Only the compounds which showed significant differences between the three propolis samples were tabulated.

pounds belong aromatic acids, found in all investigated samples. *Trans-p*-coumaric acid has the largest quantity in all samples. The following acids were identified for the first time in propolis: 2-phenyl-2-hydroxyacrylic acid identified in Austrian and German samples, 4-methymandelic

acid was identified in all samples. Beside aromatic acids poplar propolis is characterized by the presence of significant amounts of esters of these acids. Phenylethyl caffeate, benzyl ferulate and benzyl-*trans*-4-coumarate appeared in all samples with high concentration. French sample has the highest concentration of benzyl caffeate. Propolis contains three new esters: benzenepropanoic acid ethyl ester in French propolis, butanyl-caffeate and phenylethyl-caffeate isomer (17%) in German propolis.

Flavonoid aglycones, especially flavanones are typical components of poplar propolis. These results are in agreement with the statement. All investigated samples contain significant amount of flavanones, but there are difference in the concentration of the individual compounds. Pinocembrin and pinobankasin acetate are the main flavanones of the European samples. 5,7-dihydroxy-3-pentenoyloxy flavanone was identified in German sample for the first time in propolis. The flavone galangin appeared in high unexpected significant amount in German propolis (21.6%).

Between the main components of the samples investigated appeared to be hydroxyacetic acid, 5-hydroxy-n-valeric acid, 2,3-butanediol, guiacol and methylglucose which were identified for the first time in propolis.

The primary source of the plant exudate incorporated into propolis in the North Hemisphere is bud exudate of poplar trees (Greenaway *et al.,* 1987, 1988, 1990; Papay *et al.,* 1985, 1987; Bankova *et al.*1989, 1994; Wollenweber *et al.,* 1987) and the composition of propolis is therefore directly related to the composition of the poplar bud exudate collected by the bees. Each species or clone of poplar has its own characteristic mixture of compounds in its bud exudate (Wollenweber, 1975; Greenaway *et al.,* 1989) and there can be considerable difference in bud exudate composition between different poplar species (Greenaway *et al.,* 1989, 1990a, 1990b, 1990c). Propolis is potentially, therefore, a very variable product.

This communication here reported the comparative GC/MS investigation of three propolis samples from three European countries (Austria, Germany and France). 41 compounds have been identified, which included 11 identified for the first time in propolis. The relative concentrations of these compounds vary greatly and these variations are species-specific. Wollenweber (1975) reported that some *Populus* spp., such as aspens, are characteristically high in cinnamic acid derivatives and low in flavonoids. Here, Austrian propolis has the highest significant concentration in *trans-p*-coumaric acid. German propolis is characteristically high in benzyl ferulate, phenylethyl caffeate and its isomer, pinobankasin acetate and galangin. French sample has the highest significant concentration in benzoic acid, caffeic acid, benzyl coumarate, benzyl caffeate and pinocembrin.

Generally in this work, German propolis appeared with significant high concentrations of flavonoids followed by French propolis. This is probably due to the participation of different poplar species, which is probably *Populus deltoides,* where it contains galangin, pinocebrin, pinobanksin together with its related compounds as principal compounds (Greenaway *et al.,* 1990a).

The antimicrobial activity of propolis collected from some European countries against *Staphylococcus aureus; Escherichia coli,* and *Candida albicans* were recorded in Table II. All propolis samples showed an inhibition in the growth of all examined pathogens but the inhibition varied according to the propolis origin. It was obvious that German propolis showed the highest antimicrobial

Table II. Effect of different European propolis on antimicrobial activity.

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 ± 0.006*	–	1.256 ± 0.0017	–	1.758 ± 0.022	–
Austrian propolis	0.112 ± 0.008	2400**	0.629 ± 0.0005	1600	0.175 ± 0.001	1200
French propolis	0.164 ± 0.009	4600	0.740 ± 0.0019	3400	0.187 ± 0.005	1512
German propolis	0.101 ± 0.020	1400	0.364 ± 0.0039	1200	0.193 ± 0.011	4048
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	2400

* Growth inhibition = Inhibition of the growth measured by turbidity on 420 nm analyzed by spectrophotometer.
** MIC: Minimal inhibition concentration.

activity against *Staphylococcus aureus* and *Escherichia coli*, but Austrian propolis has the highest activity against *Candida albicans*. French propolis was effective against all pathogens but less than German and Austrian propolis. The minimal inhibitory concentration (MIC) of propolis samples were determined by ten fold dilution in-vitro against *Staphylococcus aureus, Escherichia coli* and *Candida albicans*. The MIC results are illustrated in Table II.

The antimicrobial activity of Propolis reflected to its constituent which differs from area to area depending on its chemical composition as observed by Shub *et al.* (1978) in USSR, Meresta and Meresta (1983) in Poland; Pepeljnjak *et al.* (1985) in Croatia, Yugoslavia; Petri *et al.* (1988) in Hungary and Serra and Escola (1995) from Brazil, Uruguay and China. Abd El Fattah *et al.* (1993) and Hegazi *et al.* (1996) from Egypt. The variation of the antibacterial activity of propolis from area to area referred to the chemical composition of propolis, which had a synergistic effect of various phenolic compounds. Also geographic areas differ due to plant flora which reflected in the propolis constituents.

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Abd El Fattah M. A., Hemeida, H. H. and Nour, M. E. (1993), The antimicrobial and antioxidant activity of propolis as a natural honey bee product. 1- The antimicrobial activity of propolis. Bull. Of Fac. Agri. Uni. Cairo **44**, 637–648.

Bankova V., Popov S. and Marekov N. (1989), Isopentenyl cinnamates from poplar buds and propolis. Phytochemistry **28**, 871–873.

Bankova V., Dyulgerov A., Popov S., Evstatieva L., Kuleva L., Pureb O.,and Zamjanzan Z. (1992), Propolis in Bulgaria and Mongolia: phenolic compounds and plant origin. Apidologie **23**, 79–85.

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

Bonvehi J. S., Ventura Coll F. and Escola Jorda R. (1994), The composition, active components and bacteriostatic activity of propolis in dietetics. J. Aoccs. **71**, 529–532.

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

Cheng P. C. and Wong G. (1996), Honey bee propolis: prospects in medicine. Bee World **77**, 8–15.

Crane E. (1988), Beekeeping: Science, Practice and World Resources. Heinemann, London, pp. 367–372.

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

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

Garg A. P. and Muller J. (1993), Fungitoxicity of fatty acids against dermatophytes. Mycoses **36**, 51–63.

Greenaway W., Scaysbrook T. and Whatley F. R. S. (1987), The analysis of bud exudate of *Populus x euramericana*, and propolis, by GC/MS. Proc. R. Soc. Lond. B **232**, 249–272.

Greenaway W., Scaysbrook T. and Whatley F. R. S. (1988), Compostion of propolis in Oxfordshire, U. K. and its relation to poplar bud exudate. Z. Naturforsch. **43c**, 301–305.

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. Chromatogr. **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**, 587–593.

Greenaway W., Davidson C. G., Scaysbrook T., May J. and Whatley F. R. S. (1990b),Hybrid origin of *Populus x jackil* confirmed by gas chromatography-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., 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., Kawther Y. Awadalla and S. M. Mansour (1997), Influence of honey and propolis on Rift Valley Fever Virus. Proceeding of International Symposium On Apitherapy, Egypt. 70.

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.

Hegazi A. G. (1998), Propolis, an overview. J. Bee Informed 5:5, 22–23, 6:723–28.

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

Markham K. R., Mitchel K. A., Wilkins A. L., Daldy J. and Lu Y. (1996), HPLC and GC/MS of the major organic constituents in New Zealand propolis. Phytochemistry **42**, 205–211.

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

Papay V., Toth L., Soltesz M., Nagy E. and Litkei G. (1985), Flavonoids and Bioflavonoids Symposium Proc. 7th. Hungarian Bioflavonoids Symposium, Szeged, Hungary. Studies in organic chemistry 23 (L. Farkas *et al.*, ed.), 233–240, Elsevier, Amsterdam 1986.

Papay V., Soltesz M., Csizmadia B. and Toth L. (1987), Chemical and pharmacological study of propolis sample of various locations Acta Pharm. Hung. **57**, 143–151.

Pepeljujak S., Jalsen jak and Maysinger D. (1985), Flavonoid content in propolis extracts and growth inhibition of *Bacillus subtilis*. Pharmazie **40**, 122–123.

Petri G., Lemberkovics E. and Foldvari M. (1988), Examination of Differences between Propolis (Bee Glave) Produced from Different Floral Environments. Elsevier Science Publ., 439–446.

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

Serra J. and Escola R. (1995), Studies of the bacteriostatic activity of propolis. Deutsche Lebensmittel RundSch. **91**, 242–246.

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

Wollenweber E. (1975), Flavonoidmuster als systematisches Merkmal in der Gattung *Populus*. *Biochem.* Syst. Ecol. **3**, 35–45.

Wollenweber E., Asakawa Y., Schillo D., Lehmann U. and Weigel H. (1987), A novel caffeic acid derivative and other constituents of *Populus* bud excretion and propolis. Z. Naturforsch. **42c**, 1030–1034.