ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Investigation Property of Propolis in Different Areas of Iran and Its Qualitative and Quantitative Chemical Composition

(**Case study: Collected Ilam and Kermanshah Province**)

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ABSTRACT

The aim of this study was to investigate the chemical composition propolis of west provinces of Iran. Propolis samples in different area from were collected and underwent the extract procedure was obtained. Propolis samples were investigated by GC/MS. The results showed that 70 compounds of propolis were identified chemical compositions were abundant in total Aliphatic, Aromatic (Benzofuran and etc), phenol and total flavonoid, and most WEP had strong antioxidant activity. Chemical compositions of Ilam and kermanshah Provincec were similar, but Iran propolis were different from those another country because The compounds identified indicated that the main plant sources of propolis that depends on its botanical and thus also on its geographical origin.

Keywords: Preopolise. Chemical composition, GC /mass.

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ISSN: 0975-8585

INTRODUCTION

Propolis is composed mainly by the plant resins and exudates that bees gather. Bees add wax, and also some secretions and pollen to it [1]. The compounds identified indicated that the main plant sources of propolis that depends on its botanical and thus also on its geographical origin. The typical components of poplar propolis are the phenolics: flavonoid aglycones, (flavones and flavanones), phenolic acids and their esters. The flavonoids are different from those in 'poplar type' propolis. The optimal conditions for propolis extraction have been studied in different publications [2,3,4]. In practice propolis is generally macerated (extraction in the solvent without stirring) with occasional shaking [5]. Other extraction methods as Soxlett, ultrasound or microwave or differential extraction are better, but need specific equipment and cannot be used under home conditions. 60-80 % aqueous ethanol solutions have a higher biological activity than tinctures, prepared with more or less water [6]. In practice many different propolis maceration procedures are given, the maceration time being sometimes as long as one year. The maceration time for best extraction of bioactive materials depends on different factors: e.g. on the extraction time, extraction method, on the solvent composition, on the propolis concentration and on the size of the propolis particles. Higher temperatures increase the extraction power, but due to the volatility of ethanol room temperature between 20 and 25 ^{o} C are optimal. Extraction has been studied for poplar propolis in detail by Bartosz Tylkowski and coworkers who developed a semi industrial and membran method of differential extraction for optimal and reproducible extraction of propolis[7,8].Optimum extraction of phenolics is when fine propolis particles (size 0.5-1 mm) are extracted using 95 % ethanol. These particles which don already after 3 days optimum extraction of phenolics is achieved under conditions of maceration,found out that when using 70 % ethanol the maceration of 20 g/ 100 ml of green propolis is optimal 30 days of extraction time, although there was no statistical difference between the extractions of 10 and 30 days [9]. This has been confirmed in another study on extraction of Baccharis propolis where a minimum of 5 days was suggested.

The health enhancing effect is found in the ethanol extractable part of propolis is called balsam. Balsam is substances as: phenols, phenolic acids, esters, flavons, flavonols, dihydroflavanons, phenolic glycerides, and Others component as: Aliphatics acids, alcohols, esters, aldehydes, ketones, benzoic acid and esters [10].The biological activity of ethanol extracts is generally tested with 70 to 100 % ethanol extracts. 60-80 % aqueous ethanol solutions have a higher biological activity than tinctures, prepared with more or less water. The antimicrobial activity of propolis is by far the most important biological property of propolis, which has deserved the highest scientific interest, considering the high number of performed studies [11,12]. It is recommended that collection is from spring to autumn. Propolis that has been in the bee hive during winter is much darker and is of lesser quality. Although the composition of propolis different considerably depending on its plants origin, all examined types of propolis revealed a strong antibacterial activity. The antibacterial activity of poplar propolis and other types of propolis of different geographical and botanical origin was similar [13].

MATERIAL AND METHODS

Material

It is astonishing, that while the composition of the different type of propolis differs greatly depending on its botanical origin, the biological effects of the different propolis types are very similar. Propolis samples in different area from Ilam and Kermanshah province were collected and underwent the extract procedure was obtained. While the solvent used for extraction were ethanol (70%), water, and acetone.. Fig.1 is show of picture propolis.

Figure 1: Popolis were collected from Ilam and Kermanshah province,Iran

ISSN: 0975-8585

Methods

The propolis extract was prepared according to a method presented by Guo,X.L and coworking, Hajime,A with modifications. Fine ground propolis was extracted with different solvent, namely water, ethanol, Acetone and tetra carbon chloride (same concentrations) at 40 ^{0}C in shaker Thus, 20 gram of propolis was extracted with100 ml solvents (water, ethanol 70%, Acetone and tetra carbon chloride,) at 40 ^{0}C in shaker for ten days in dark room. 60-80 % aqueous ethanol solutions have a higher biological activity than tinctures, prepared with more or less water 70 % Propolis is most widely used, Ethanol is one of the best solvent for extracting the bioactive substances (balsam). Store vessel in the dark for about two weeks, shaking occasionally, more than 2 weeks brings only a small improvement of yield. After that, the suspension was filtered (and the residue was extracted again. Then for seven days the suspensions was filtered every day. Filter through a paper filter (coffee filter will do) and store tincture closed in a clean dark vessel. If vessel is not brown or reddish, store in the dark, or pack vessel in aluminums foil. Ethanol-free propolis can be made by evaporating the ethanol in a water bath. The remaining pure balsam can be mixed to honey or other materials where ethanol-free material is required.. Figures 2a&b are shown extract and solution propolis in the aqueous ethanol 70% at 40^o C.

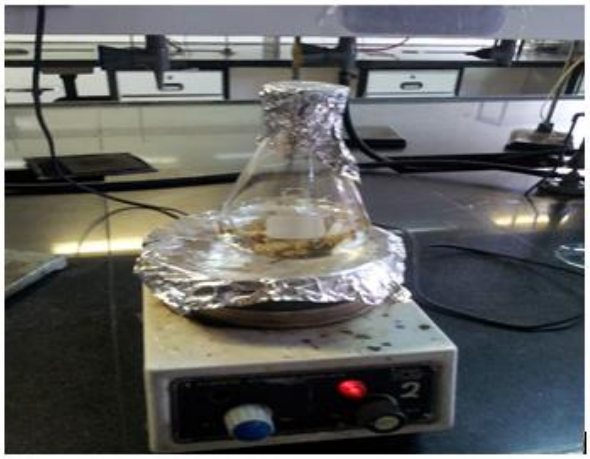

Figure 2a: Propolis with100 ml solvents at 40 ^{0}C in shaker

Figure 2b: Propolis component after filtration,

For Analysis chemical composition of propolise was used of GC-MS 6890-5973. Gas chromatography–mass spectrometry (GC–MS) analysis was carried out on a Agillent GC 6890 gas chromatograph coupled with a AgillentMSD5973 mass detector under electron impact ionization. The chromatographic column for the analysis was Zebron (ZB-1) methyl polysiloxane column (30m×0.25mm i.d.×0.25_m film thickness). The carriergas used was helium at a flow rate of 10 ml/min. Propolis samples were analyzed with the column held initially at 100 ◦C for 5 min and then increased to 150 ◦C and then kept at 150 ◦C for 2 min. Finally, temperature was increased to 280 ◦C with a 2 ◦C/min heating and the temperature was kept 280 ◦C for 60 min for samples. The injection was performed in split mode at 250 ◦C

RESULTS AND DISCUSSION

The chemical composition of propolis samples, which were collected from different area were investigated by GC–MS. More than 25 peaks obtain of chemical analyses that More than 75 individual compounds and quantities them were identified. The following compounds were identified in propolis samples: Phenol 2-methyl-5-(1-methylethyl),Phenol 5-methyl-2-(1-methylethyl), Phenol 5-methyl-2-(1-methylethyl), Decanoic acid (CAS) Capric acid, Decanoic acid (CAS)Capric acid, n-Decanoic acid, gamma. Dodecalactone,4-Octylbutan-4-olide, Tetrahydropyran 12-tetradecyn, Thiazole, 5-methyl, Dotriacontane, Triacontane (CAS) $$ n-Triacontane, 2H-Pyran-2-one, 6-heptyltetrahydroxed, 2H-Pyran-2-one, 6-heptyltetrahdoxid, 5-Hydroxyddodecanoic acid lactone,4-7-10-13-16-19-Docosahexaenoic,3,5-Disilaheptane; 3,3,5,5-tetra., 1t,3t-3-Divinyl-1c,2r-cyclohexan, 1,2-Benzenedicarboxylic acid,1,3-Cyclohexadiene, Benzofuran-2-one,3-methyl-3-aza,Tricyclo[4.1.0.02,4]heptane, Tricyclo [4.1.0.0(3,5)] Heptane, Tricyclo [4.1.0.0(2,4)] heptane,1H-Cyclopropa[a]naphthalene, 1H-Indene,1-ethylidene octahydroxid, Alpha.-Guaiene $$ Azulene, Ethyl citrate, 1,2,3-Propanetricarboxylic acid; triethyl 2-hydroxy-1,2,3-propane, 2H-Pyran-2-one, 6-heptyltetrahyd, 2,5-Hexanedione (CAS) $$ Diaceto, 2H-Pyran-2-one, 6-heptyltetrahydroxid, Octadecane, 1-

ISSN: 0975-8585

chloro-(CAS), Pentatri acontane,O ctadecane, 1-chloro- (CAS), Caryophyllene-(I1), Alloaromadendrene,1H-Cyclopropan,1H-Cycloprop[e]azulene, decahydroxid, Tetratetracontane, Pentatriacontane, 2-Propen-1-one, 1-(2,6-dihydroxyd);1-ethyl-4,5-dimethoxy-9-methyl-9; The components of propolis samples were identified and listed in Table 1.

Table 1: Wt% Propolise chemical compounds assessed by GC/MS of alcoholic extracts from propolis samples.

Component	Wt%	Component	Wt%
Phenol, 2-methyl-5-(1-methylethy... Phenol, 5-methyl-2-(1-methylethy... Phenol, 5-methyl-2-(1-methylethy...	14.13	Octadecane, 1-chloro- Pentatriacontane Octadecane,1-chloro-(CAS)	0.43
Decanoic acid (CAS) capric acid n-Decanoic acid	30.80	Caryophyllene-(I1) Alloaromadendrene$$1H-Cyclopro 1H-Cycloprop[e]azulene, decahydrid	1.18
Gamma Dodcalactone 4-Octylbutan-4-Olide Tetrahydropyran 12-tetradecyn	2.45	Tetratetracontane Tritetracontane Pentatetracontane	1.40
Thiazole, 5-methyl- (CAS) Dotriacontane Triacontane (CAS) $$ n-Triacontane	1.45	Octadecane, 1-chloro- (CAS) $$ Dodecane, 1,1'-oxybis- (CAS) $$ Octadecane, 1-chloro-	0.61
2H-Pyran-2-one, 6-heptyltetrahydro- 2H-Pyran-2-one, 6-heptyltetrahyd... 5-Hydroxyd -dodecanoic acid lactone	12.41	Cyclododecanemethanol (+-)-Tridec-2-en-12-ol $$ 12-Tri... (R)-(-)-14-Methyl-8-hexadecyn-1-ol	0.58
4,7,10,13,16,19-Docosahexaenoic ... 3,5-Disilaheptane, 3,3,5,5-tetra... 1t,3t-3-Divinyl-1c,2r-cyclohexan...	0.80	2,15-Hexadecanedione 3,3',5,5'-Tetramethoxy-2,2',4,4'... 2-Undecanone, 6,10-dimethyl- $$ ..	1.21
1,2-Benzenedicarboxylic acid, bu... 1,3-Cyclohexadiene, 1,3,5,5,6,6-... Benzofuran-2-one, 3-methyl-3-aza...	1.72	2-Propen-1-one, 1-(2,6-dihydroxy... 2-Propen-1-one, 1-(2,6-dihydroxy... 1-ethyl-4,5-dimethoxy-9-methyl-9...	14.44
Tricyclo[4.1.0.02,4]heptane, 3,3... Tricyclo[4.1.0.0(3,5)]Heptane Tricyclo[4.1.0.0(2,4)]heptane, 3...	1.03	Octadecanoic acid, 3-hydroxy-, m... Methyl 3-hydroxytetradecanoate Octadecanoic acid, 3-hydroxy-, m...	0.51
H-Cyclopropa[a] naphthalene, dec... 1H-Indene, 1-ethylideneoctahydro... Alpha.-Guaiene $$ Azulene, 1,2,...	6.41	1,2-Benzenedicarboxylic acid, bi... Phthalic acid, 2-hexyl ester 1,2-Benzenedicarboxylic acid, bi...	0.48
Ethyl citrate ,2,3-Propanetricarboxylic acid,... Triethyl 2-hydroxy-1,2,3-propane	4.90	Benzaldehyde, 2-hydroxy-5-nitro Ethyl(2-benzothiazolinethione-3 Allobarbital	0.89
2H-Pyran-2-one, 6-heptyltetrahyd... 2,5-Hexanedione (CAS) $$ Diaceto... 2H-Pyran-2-one, 6-heptyltetrahydro-	1.51	4H-1-Benzopyran-4-one, 5-hydroxy... 12-hydroxy-bbf $$ Benz[e]acephen... 1,2,3,6,7,8-Hexahydro-1,3,6,8-te...	0.75

Detailed Study of the qualitative chemical differences between samples and of mixed origin was performed by GC-MS analysis of alcohol extract after silyation. Fig. 3. Is show GC-MS analysis of alcohol extract of propolis from sample.

ISSN: 0975-8585

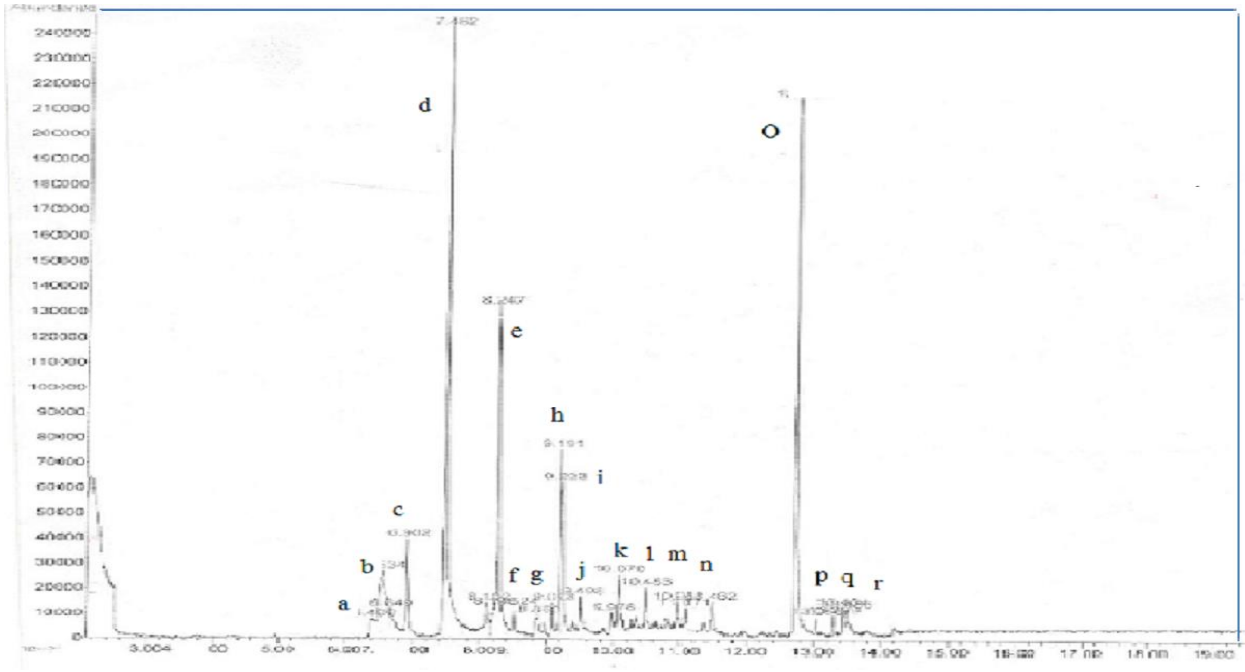

Figure 3: GC-MS of alchohol extract of propolist from samples.Main component: (a),(b),(c) Phenol, 2-methyl.. and Phenol, 5-methyl..;(d) Decanoic acid; (e) pyrans; (f),(g) Disilaheptanes,Benzofuran& Tricyclo heptanes; (h) naphthalene; (i) Ethyl citrate; (j) heptyltetrahyd;(k) Pentatriacontane;(l),(m),(n) Caryophyllene,tetracontane & decanes;(O) Different combinations of propane; (p),(q),(r) Benzenedicarboxylic acid, Benzaldehyde, &Benzopyran

In a recent study, the chemical composition of propolis sample from Ilam and Kermanshah province in West region Iran has been investigated. As Table 1 and Figure 3 are shows that Decanoic acid (CAS) capric acid (of Aliphatic acid group) with 30.8wt% to be high the content after this one Different combinations of propane with 14.44 wt% and Pyran component with 12.41wt% are high the content and Benzaldehyde component with less 2% to be low the content of propolis .

CONCLUSION

In the presence study most of the chemical com ofponents in samples were collected from propolis were the expected results, because propolis source in the Ilam and Kemanshah in the west area Iran was same. The chemical analyses of type's samples of propolis collected three different races were investigated. Propolis samples were investigated by GC/MS, and then more 70 compounds were identified. The compounds identified indicated was show that the propolis component dependent to main plant sources of propolis. In conclusion, our results confirm the importance of the amount of Aliphtic compounds (Decanoic acid (CAS) capric acid) of propolis and Benzaldehyde component with less 2% to be low the content of propolis. These findings confirm that the determination of the type of propolis, according to its plant source.

ACKNOWLEDGEMENT

Tis study was generously supported by Islamic Azad university Tehran branch.

REFERENCES

[1] Guo XL, Chen B, Luo LP, Zhang X, Dai XM, Gong SJ. J Agr Food Chem 2011;59(23):12610-12616
[2] Melliou E, Chinou L. Planta Medica 2004;70(6):515-519
[3] Fonseca YM, et al. ECam 2011: doi:10.1155/2011/863917
[4] Silici S, Kutluca S. J Ethnopharmacol 2005;99 (1): 69-73.
[5] Hajime A. Propolis extract with improve water-solubility. United States Patent 5922324, 1999.
[6] Sosnowski Z. Method for extracting propolis and water soluble dry propolis powder. United States Patent 432886, 1983.
[7] Bartosz T, Boryana T, Vassya B, Marta G, Georgi P, Agnessa N. J Membr Sci 2010;(348) 124-130

ISSN: 0975-8585

[8] Ramanuskiene K, Inkeniene, A M, Leskaukaite D. Farmacia 2012;60 (4): 535-543
[9] Özcan M. Grasas y Aceites (Sevilla) 2000;51 (4): 251-253
[10] Marcucci MC. Apidologie 1995;25:83-99
[11] Burdock GA. Food Chem Toxicol 1998;36(4):347-363
[12] Uzel A, Sorkun K, Oncag O, Cogulu D, Gencay M, Salih B. Microbiol Res 2005;160 (2):189-195.
[13] Crane E. History of other products from bees The world history of beekeeping and honey hunting, Gerald Duckworth & Co Ltd; London; 1999;pp 545-553.