



Microwave Ultrasound Assisted Extraction: Determination of Quercetin for Antibacterial and Antioxidant Activities of Iranian Propolis

G. Heidari^a, G. D. Najafpour^{*a}, M. Mohammadi^a, A. A. Moghadamnia^b

^a Department of Chemical Engineering, Babol Noshirvani University of Technology, Babol, Iran

^b Department of Pharmacology and Physiology, School of Medicine, Babol University of Medical Science, Babol, Iran

PAPER INFO

Paper history:

Received 13 May 2019

Received in revised form 21 June 2019

Accepted 05 July 2019

Keywords:

Propolis

Extraction

Total Flavonoids

Microwave-ultrasound-assisted Extraction

Quercetin

ABSTRACT

Propolis was used as medicine, cosmetic and beverage additives since past decades. The most important active compounds in the propolis are phenolic and flavonoids, which are in the resin category. The purpose of this study was to measure the total amount of flavonoids and quercetin in propolis of northern Iran and compare obtain data with different extraction methods. The soxhlet, maceration and microwave-ultrasound-assisted extraction (MUAE) were conducted for comparison of the extraction efficiency. Total amount of flavonoids measured by Spectrophotometry and the amount of quercetin were determined by using high performance liquid chromatography (HPLC). Here in this work, the best extraction yield for the total flavonoids was obtained 70.88 % and for quercetin was 44.53% at 300 W, 1.5 min for microwave and ultrasound 10 min and 40 °C. Also extraction yield of quercetin for soxhlet (as reference method) was determined 67.85% and for maceration was 13.9%. UV-vis, HPLC and FTIR analyses were used to determine the extraction yield and chemical structure of the active compounds. Then antioxidant and antibacterial effects of propolis extract were evaluated and the obtained results were compared with the related literature.

doi: 10.5829/ije.2019.32.08b.01

1. INTRODUCTION

Propolis is one of the most valuable bee product. A substance found in many different colors such as green, red, and dark browns [1]. According to a botanical category, propolis can be divided into two sub-categories called blue (B) and orange (O); which is based on color of the separated compounds by the high performance thin layer chromatography (HPTLC) plate under the UV light [2]. In fact, the bee collects resinous and sticky materials from plants and flowers; then, combines it with salivary secretions to protect the hive against contamination and microorganisms [3]. Propolis has many biological properties, including anti-cancer, antibacterial, anti-virus, antioxidant and anti-parasite [4]. Propolis can be obtained from different species of plants. So far, about 300 to 400 molecules have been identified in chemical composition of propolis, including flavonoids, steroids, terpenoids, lignans, benzoguran, aliphatic acids and many other compounds [3]. Flavonoids are subgroups of

a larger group called polyphenolic compounds, in which a variety of flavonoids are classified according to their structure. They are naturally found in sources such as plants, vegetables, wine and flowers. Flavonoids are low molecular weight compounds with a central core called flavan. The general chemical structure of flavonoids is shown in Figure 1; which consists of three chains [5]. The flavonoids are hydroxylated in one of 3, 5, 7, 3', 4' or 5' positions according to the type of flavonoids [6]. Robinin and quercetin are the most famous flavonoids found in the diet [5]. Quercetin is found on onions, propolis, broccoli and etc [7]. Propolis composition depends on a variety of factors, including geographical conditions, harvest season and plant species in the region [8]. However, a general composition of 50% resin, 30% wax, 10% essential oil and 10% other materials were defined for propolis [9]. Today, people have become more motivated to supplements. Propolis has attracted a lot of attention, because it contains plenty of flavonoids [10]. Propolis has recently been used in medicine in various

*Corresponding Author's Email: najafpour@nit.ac.ir (G. D. Najafpour)

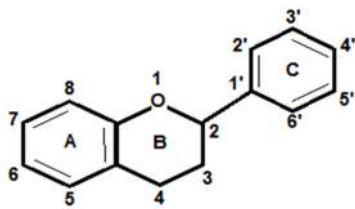


Figure 1. The general chemical structure of flavonoids

forms, including mouthwashes and creams. Also in the cosmetic industry has found a special application [11]. The propolis has compact and hard wall structure that makes digestion very difficult for the release of bioactive compounds [12]. For this reason, the extraction process is performed on the propolis to release the active compounds. As a result, these active compounds can be used with high quality in medicine, pharmaceutical compounds and other applications. Methanol, acetone, ethyl acetate and ethanol are the most suitable organic solvents for the phenolic compounds extraction. The maceration and soxhlet methods are mentioned as the traditional methods. Soxhlet was first used since 1879 for extraction; which could extract most bioactive compounds with high yields. In this way, it is generally used as a basis for comparing a variety of extraction methods. Maceration is considered one of the easiest and cheapest extraction methods [13]. In traditional routes, there are disadvantages such as high solvent consumption, low efficiency, health hazards, low mass transfer and time-consuming processes [14, 15]. Nowadays, new methods such as ultrasound assisted extraction, microwave assisted extraction are used to overcome these extraction problems. New extraction methods have objectives such as low cost process, low pollution and high performances [16]. In ultrasound extraction method, sound waves play a major role in the extraction. The frequency of this sound waves are more than 20 kHz, which is above human hearing. Audible frequency of sound wave for humans is 16 Hz to 16 KHz. In this method, ultrasound waves induced cell wall destruction. As a result, the solvent is easily penetrate into the cell, which increase the mass transfer and extraction efficiency [17]. In recent years, the microwave extraction method has become more prominent and complementary compounds have been extracted by this method. The organic solvent in contact with the sample is heated by microwave energy. This heating is caused by the collision of waves with polar compounds and thus leads to extraction. Microwave heating has features such as special uniform heat distribution, selective heating, increased reaction rate and consequently reduced extraction time. The ultrasonic and microwave have

many advantages over traditional methods, including low solvent consumption, operator safety, short testing time, high efficiency, low energy requirement [18]. According to the above statements, various extraction methods are used to release active substances present in propolis. An important property of propolis is its anti-bacterial property, which has been used in pharmaceutical applications from ancient times. Studies have demonstrated that propolis has affected on Gram-positive bacteria. It also has a lower impact on Gram negative bacteria [19]. Although, the mechanism of propolis effect on bacteria is not clear. Also comprehensive information on its effect on microbial physiology is not available [20]. Since propolis has different properties depending on the geographic region as well; since many studies have not been carried out on Iranian propolis. In this study, active compounds of propolis was extracted by various methods and important properties such as antibacterial and antioxidant were identified.

2. MATERIALS AND METHODS

2. 1. Materials The raw propolis used in this study was obtained from Savadkooch, Iran. It was stored at -20 °C until analysis. The propolis was dried and kept in fine powder before use. Standard quercetin (>98%) used for spectrophotometric and HPLC analysis was supplied by Merck (Darmstadt, Germany). Methanol HPLC grade were purchased from Chem-Lab (Zedelgem, Belgium). Potassium acetate, aluminum chloride, were acquired from Merck and distilled water was obtained from a Milli-Q water system (Darmstadt, Germany).

2. 2. Methods

2. 2. 1. Extraction Methods In this study, a variety of methods was used to extract flavonoids from propolis, including maceration, soxhlet, as well as a sequential microwave-ultrasound assisted extraction (MUAE) method. Use of the appropriate method for extraction is important factors that affecting the yield and quality of the product. The solvent is another influential factor. In this study, methanol was used to investigate their effect on extraction process. In all methods, 10 ml of each solvent is used. Trusheva et al. [21] demonstrated that the use of solvent: propolis ratio more than (10:1) is excessive. After extraction, the samples were centrifuged for 10 minutes at 6000 rpm and room temperature. Insoluble particles were separated, then supernatant was filtered using 0.45 µm Whatman filter paper. Afterwards, samples were placed in an oven at 50°C and given sufficient time (overnight) to evaporate the trace amount of any remaining solvent until the sample weights were unchanged. In this way, the extracts from each method were prepared. These steps were repeated three times for each set of experiment and also for high accuracy.

2. 2. 2. Extraction by Maceration Maceration was adapted, the method was described by Anđelković, et al. [22]. About 1 g of propolis powdered was cooled at -20°C , then materials were placed in a Erlenmeyer flask, 10 ml solvent (methanol: water, 80:20) was added. Samples were stirred for 24 hours at room temperature, then centrifugation and filtration were performed.

2. 2. 3. Extraction by Soxhlet This method was used as a basis for comparing the efficiency of other extraction methods. 12 g of dried powder of propolis was placed in cartridge and extracted with 300 ml of solvent containing 240 ml of methanol and 60 ml of distilled water for 6 hours at 60°C . Then the solvent was evaporated using a rotary evaporator and the sample was dried in an oven at 50°C . 5 milligrams of dried powder was dissolved in 5 ml of methanol. After dilution, total flavonoids were measured by colorimetry and quercetin content was determined by using HPLC analysis.

2. 2. 4. Extraction by Microwave-Ultrasound-Assisted Extraction (MUAE) In this method microwave and ultrasound were performed sequentially. First, 10 ml solvent (methanol: water, 80:20) was added to 1 g of propolis in the Erlenmeyer. Then, the container of propolis and solvent was placed inside the microwave with maximum power of 1150W and frequency of 2.45GHz. Finally transferred to the ultrasonic bath (Elmasonic S10H). The bath power was 280 W with 50/60 Hz frequency. In this process, parameters such as microwave power (100-450W), microwave irradiation time (30-150s), ultrasound temperature (30-70 $^{\circ}\text{C}$) and ultrasound time (5-45 min) were optimized. A series of hints during the extraction process should be followed, including: covering the container with the parafilm and cooling the sample container during the microwave process to prevent solvent evaporation. When the sample is exposed to microwave radiation, the temperature rises and causes the solvent to evaporate. To avoid this, the sample cools down in the ice bath in every 10 seconds (radiant heating- cooling with an ice bath, heating caused by radiation). During the experiment, temperature was measured by placing the thermocouple (Autonics, TCN4L-24R) in the solvent. Then extracts were prepared for subsequent analysis.

2. 2. 5. Total Flavonoids Content Total flavonoids Measurement was performed using aluminum chloride colorimetric method [23]. Quercetin was chosen as a reference for the calibration curve. 0.5 ml of standard at various concentrations was added to 1.5 ml methanol 99%. Then, 0.1 ml of aluminum chloride (10%) and 0.1 ml of 1M potassium acetate dissolved in 2.8 ml distilled water. After 30 min remaining in dark, the absorbances of samples were measured at 415 nm (Analytik Jena AG, Germany). Samples were analyzed at room temperature.

2. 2. 6. HPLC Analysis Quantitative analyzes of extracted quercetin were performed using high performance liquid chromatography (HPLC, Knauer, Germany). The device was equipped with a C18 column with dimensions of $6.4 * 250$ mm, with pre-column, pumping system and ultraviolet detector 2500 series (UV). The mobile phase was consisting of acetonitrile and formic acid-water at flow rate of 0.8 ml/min. The measurement was carried out at wavelength of 270 nm. First, the calibration curve of standard quercetin was drawn up in concentrations of 2.5, 5, 10, 15, 30 ppm. Then, the extracted samples were injected into the system.

2. 2. 7. Yield of Extracts The flavonoid extraction yield was calculated for maceration, soxhlet and the sequential microwave-ultrasound assisted extraction method as follows: The extract weight (g) (based on dry weight) was divided into raw propolis weight (g) then multiplied by 100.

$$\text{Yield (\%)} = x = \frac{\text{extract weight (g)}}{\text{raw propolis (g)}} \times 100 \quad (1)$$

2. 2. 8. Evaluation of Antibacterial Activity by Disc Diffusion Method In this study, bacterial strains including *Escherichia coli* (ATCC 25922) obtained from biotechnology research laboratory (Noshirvani University of Technology) and *Staphylococcus aureus* (ATCC 25923) was obtained from Babol University of Medical Sciences. To activate the strains, first contents of the vials were transferred to the test tubes in accordance to the instructions under sterile conditions. Then, tubes were covered with cotton and placed in incubator for 24 hours at 37°C , so that the bacteria grow and create opacity. Then the liquid medium was taken by sterile loop, and cultured on the nutrient agar environment. The cultured discs were incubated at 37°C for 24 hours. Then a colony of microorganisms was transferred into nutrient broth and incubated for 24 hours at 37°C and 120 rpm. Then, cultures were used as a microbial solution. During the experiment, 200 microliter of the bacterial suspension was transferred to the nutrient plates then distributed on the media uniformly. 150 microliter of different concentrations of extract (2000, 4000, 6000 and 8000 mg/l) were prepared in methanol and the discs impregnated to each concentration of the extract. A disk was impregnated to solvent as a control. After drying under sterile conditions, the discs were placed using sterile forceps at the surface of agar, and fixed on the culture medium. The plates were kept in incubator at 37°C and after 24 hours the inhibitory zone was measured by coulisse in millimeters.

2. 2. 9. The Fourier Transform Infrared (FTIR) The identification of functional groups of propolis extract

was performed by FTIR spectrometer (WQF-510A, China) in the range of 400 to 4400 (cm^{-1}) and compared with standard quercetin.

2. 2. 10. Antioxidant Assay by DPPH Method

Radical scavenging activity (RSA) of propolis extract and standard BHA was assessed on the basis of the free radical scavenging effect of the methanolic solution of 1,1 diphenyl-2-picrylhydrazyl (DPPH). Different concentrations of propolis extract and standard antioxidant were prepared in different tubes. Then solutions containing 1 ml of different concentrations of propolis extract and 5 ml of methanolic solution of DPPH (0.1 mM) were prepared. For Blank, 1 ml of methanol was replaced with propolis extract. The absorbance was calculated at 517 nm based on methanol. RSA% was measured as follows:

$$\text{RSA}\% = \left(1 - \frac{\text{sample absorbance}}{\text{Blank absorbance}}\right) \times 100 \quad (2)$$

3. RESULTS AND DISCUSSION

Extraction of flavonoids from propolis was investigated by different methods. Calibration curve based on quercetin was prepared at concentration of 0.5 to 30 mg/l ($R^2 = 0.99$). The colorimetric method of aluminum chloride is based on the aluminum chloride forms a stable complex with the hydroxyl group of flavonoids. It also forms an unstable complex with the orthodihydroxyl group of the chain A or B of flavonoids (See Figure 1) [23]. HPLC calibration curve plotted as above described method then chromatogram of standard quercetin and extracted quercetin of propolis were compared. According to Figure 2b major peak for the extracted sample was at 3.31 minutes. Since quercetin standard peak was also appeared at 3.36 minutes (Figure 2a). Therefore, it can be concluded that extracted sample contains quercetin. Then amount of quercetin in the extract was calculated by different extraction methods.

3. 1. Comparison of Traditional Methods for Flavonoid Extraction

Yield of extraction for maceration and soxhlet were 13.9 and 67.85%, respectively. As expected, extraction efficiency for soxhlet was considerably higher than maceration method. That is due to the fact of this method, solvent in the presence of heat, was constantly refluxed. In this way, solvent well penetrated into the structure of substance, and quercetin was easily extracted with generous amount of solvent. But, these traditional methods are required excessive amount of solvent for long duration of extract having full reflux. For this reason, in this study a newer method for extraction of quercetin from propolis was investigated and process parameters were optimized, which is described below.

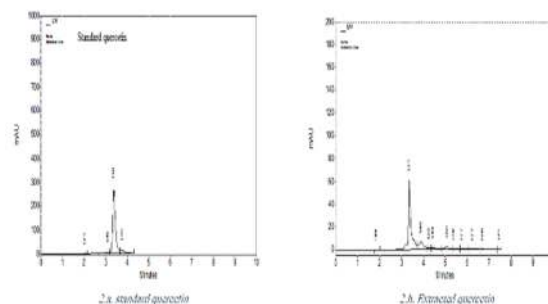


Figure 2. HPLC chromatogram of standard (2.a) and extracted quercetin (2.b)

3. 2. Effect of Microwave Power on Extraction Using MUAE

To determine the effect of microwave power on the yield of extraction by microwave-ultrasound assisted extraction, other parameters such as irradiation time were designated constant at 1.5 minute, sonication time at 10 minute and sonication temperature at 40 °C. Microwave power settings were optimized from 100 to 450 W. Figure 3 shows the effect of microwave power on the total flavonoids and quercetin extracted in this method. The maximum extraction yield was obtained at 300 W, which was 70.88% for total flavonoids and 44.53% for quercetin. According to this figure, increasing microwave power has a significant affected on extraction. Increasing power from 100 to 300 W resulted in an increase in the solvent temperature from 27 to 48 °C. At this temperature, the cell wall is degraded and solvents well penetrated into the cell; that has boosted the yield.

However, with an increase in power more than 300W, the yield was decreased. It seems that degradation of quercetin structure and evaporation of solvent at high temperature and power were the reasons for reduction on yield of extraction. This figure also shows increasing and decreasing extraction yield for total flavonoid is proportional to quercetin extraction yield. Which indicates that the highest percentage of flavonoids in the propolis of this region is quercetin.

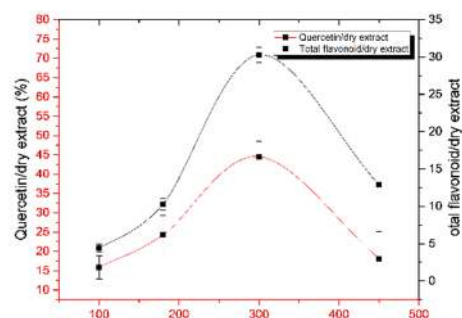


Figure 3. Effect of microwave power on extraction using MUAE

3. 3. Effect of Microwave Time on Extraction Using MUAE

Extraction time is an important factor in optimizing the extraction process. Figure 4 shows the effect of microwave irradiation time on extraction yield using MUAE method. Radiation times were 30, 60, 90, 120 and 150 seconds. Other parameters were constant at 300 W for microwave power, 40°C for ultrasound temperature and 10 min for ultrasound time. From 30 to 90 seconds, yield of extraction increased from 15.41 to 44.53%. Then with increasing time from 90 to 150 seconds, the extraction efficiency decreases from 44.53 to 5.71%. Therefore, the highest extraction efficiency occurred at 90 seconds. It seems deterioration of quercetin structure and also reduction of solid to solvent ratio in over longer period of time were the main reasons for reduction of extraction yield. As in previous step, increasing and decreasing for total flavonoid extraction yield and quercetin extraction yield is fit.

3. 4. Effect of Ultrasound Time on Extraction Using MUAE

Figure 5 exhibits the effect of ultrasonic time on extraction yield at different time ranges from 5 to 45 min, under constant conditions of 300 W for microwave power, 90 seconds for irradiation time and 40°C for ultrasonic temperature. When the time increased from 5 to 10 min, extraction yield increased from 30.53 to 44.53%. Then increasing time from 10 to 45 min, leads to decreasing extraction yield from 44.53 to 10.12% and then remained constant. In longer times, structures of flavonoids such as quercetin have been destroyed. Also mass transfer between propolis powder and extraction solvent decreased.

3. 5. Effect of Ultrasound Temperature on Extraction Yield by MUAE

In this section, temperature variations from 30 to 70°C were investigated at constant condition of 300 W and 90 seconds for microwave, 10 minute for sonication time. Figure 6 shows increasing temperature from 30 to 40°C lead to rise of extraction yield from 23.45 to 44.53%. Ultrasonic waves produce bubbles, which increases solid and solvent contact area; therefore, solvent permeability into

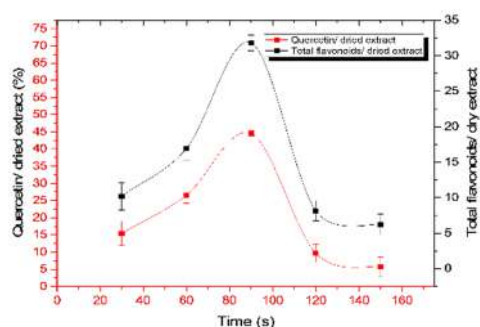


Figure 4. Effect of microwave time on extraction using MUAE

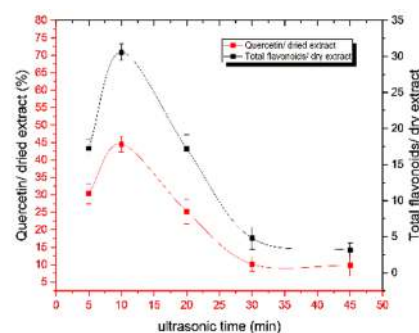


Figure 5. Effect of ultrasound time on extraction using MUAE

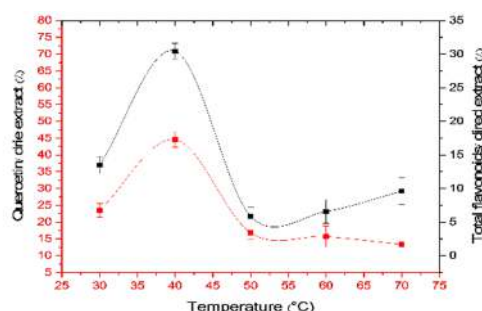


Figure 6. Effect of ultrasound temperature on extraction using MUAE

cell has increased. However, increasing temperature from 40 to 70 °C caused decreasing extraction yield from 44.53 to 13.34%. It may have been due to the reduction of sonochemical effect of bubbles which have been reduced around the boiling point of solvent [18]. Because, bubbles do not produce enough shear force to collapse the cell structure. As a result, the extraction efficiency was reduced about 50 °C, which is close to the boiling point of solvent (64.7 °C). Also, as shown in Figure 6, trend of increasing and decreasing of extraction yield is similar from 30 to 50 °C for quercetin and total flavonoids. But variation of total flavonoids yield has been reversed from 50 to 70 °C. This could be due to extraction of another flavonoid compound except quercetin, which caused an increase in extraction yield of total flavonoids. This could be verified by the HPLC diagram shown in Figure 7. This figure shows in addition to the quercetin peak at 3.71 minutes, another peak appears at 5.13 minutes, belonging to another type of flavonoid compound. Consequently, with the above information and the comparison of the extraction yield at the optimal point with literature, we found out that this method was able to extract the active compounds present in propolis with reasonable efficiency. For instance Biscaia et al. [24] have used supercritical fluid extraction method for propolis and the highest yield was reported 24.8%; while, in this study, the highest extraction yield of quercetin from propolis was 44.53%.

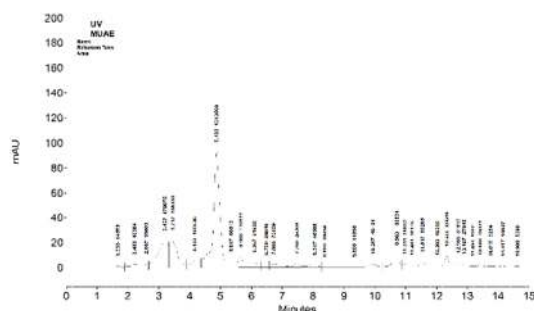


Figure 7. HPLC diagram for optimization of ultrasound temperature and presence of another flavonoid compound

3. 6. The Fourier Transform Infrared (FTIR)

FTIR analysis is used for identification of flavonoids in the propolis extract (Figure 8). This figure shows comparison of the spectra of propolis extract and standard quercetin. The broad band at 3410 cm^{-1} is a stretching bond correspond to O-H. The observed peaks at 2925 , 2847 , 1641 , 1447 and 1097 cm^{-1} , were related to C-H vibration in aromatic ring, C=O carbonyl group, vibrational tensile bond C=C in the aromatic group and C-O tensile bond, respectively. These bonds were common in the standard quercetin and propolis extract; thus, demonstrated the presence of quercetin in propolis extract.

3. 7. Radical Scavenging Activity of Propolis Extract

According to Figure 9, inhibition of free radical in propolis extract was investigated based on the inhibitory percent of DPPH free radicals. Antioxidant activity was tested at various concentration of propolis extract of 100, 250, 500, 1000 and 2000 ppm. Then it was compared with antioxidant activity of ascorbic acid. At concentration of 100 ppm, ascorbic acid exhibited activity of 75.5%. The extracts also had activities of 45.48, 83.18, 93.29, 93.6 and 93.7 at concentrations of 100, 250, 500, 1000 and 2000 ppm, respectively. Figure 9 shows there is no significant change in the inhibitory percentage at concentration above 1000 ppm. In

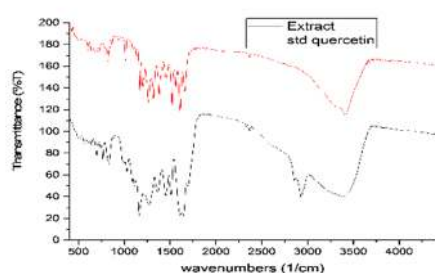


Figure 8. FTIR spectra of the standard and extracted quercetin

concentrations less than 250 ppm of extract, inhibitory effect is similar to ascorbic acid at 100 ppm. Antioxidant activity of the propolis extract caused by the components such as quercetin. Therefore, addition of propolis extract into food and medicine has many health benefits.

3. 8. Antibacterial Activity of Propolis Extract

In this study, disk diffusion method was employed to evaluate the antibacterial properties on two kinds microorganisms were examined. In both figures, the number of discs from 2 to 5 is referred to different concentrations of propolis extract from 2000 to 8000ppm, respectively. As it can be observed from Figure 10, the Gram-positive bacteria of *Staphylococcus aureus* (Figure 10.a) was more inhibited than the Gram-negative bacteria of *Escherichia coli* (Figure 10.b). Also, with increasing the concentration of extract, inhibition zone has increased. This may be attributed to lipopolysaccharides in the outer membrane of Gram-negative bacteria, which is inherently resistant to external factors such as antibiotics.

4. CONCLUSION

According to the above, the microwave- ultrasound assisted extraction method has better yield than other methods. This method can be considered as a low cost, fast and energy saving. The optimum conditions were obtained 300W and 1.5 min for microwave, $40\text{ }^{\circ}\text{C}$ and 10

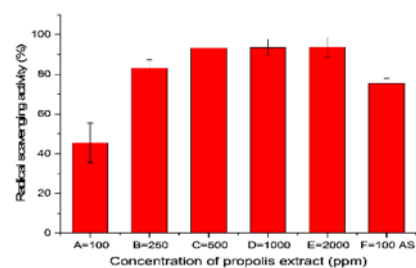


Figure 9. Antioxidant activity of propolis extract and ascorbic acid, AS= Ascorbic acid

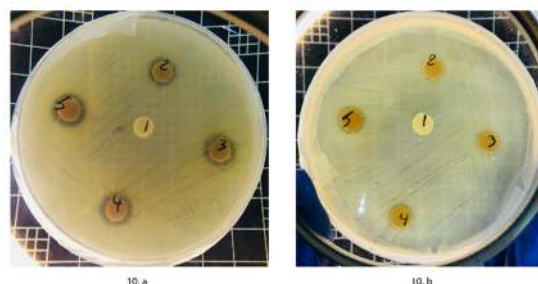


Figure 10. Antibacterial activity of propolis extract on (10.a) *S. aureus* and (10.b) *E. coli*

min for ultrasonic. This method seems to be a beneficial for extracting propolis, and has shown good efficacy against the other extraction methods. Also soxhlet extraction yield as the basis was obtained 67.85% for quercetin. Propolis extract showed acceptable antioxidant and antibacterial effect. Extract has a good inhibitory effect on Gram positive bacteria, but this lower for Gram negative bacteria due to the type of cell wall membrane that are more resistant.

5. REFERENCES

- Kasote, D.M., Pawar, M.V., Bhatia, R.S., Nandre, V.S., Gundu, S.S., Jagtap, S.D. and Kulkarni, M.V., "Hplc, nmr based chemical profiling and biological characterisation of indian propolis", *Fitoterapia*, Vol. 122, (2017), 52-60.
- Ristivojević, P., Dimkić, I., Guzelmeric, E., Trifković, J., Knežević, M., Berić, T., Yesilada, E., Milojković-Opsenica, D. and Stanković, S., "Profiling of turkish propolis subtypes: Comparative evaluation of their phytochemical compositions, antioxidant and antimicrobial activities", *LWT*, Vol. 95, (2018), 367-379; <https://doi.org/10.1016/j.lwt.2018.04.063>.
- Kunrath, C.A., Savoldi, D.C., Mileski, J.P.F., Novello, C.R., Alfaro, A.d.T., Marchi, J.F. and Tonial, I.B., "Application and evaluation of propolis, the natural antioxidant in italian-type salami", *Brazilian Journal of Food Technology*, Vol. 20, (2017); <http://dx.doi.org/10.1590/1981-6723.3516>.
- Popova, M., Giannopoulou, E., Skalicka-Woźniak, K., Graikou, K., Widelski, J., Bankova, V., Kalofonos, H., Sivolapenko, G., Gawel-Bęben, K. and Antosiewicz, B., "Characterization and biological evaluation of propolis from poland", *Molecules*, Vol. 22, No. 7, (2017), 1159; <https://doi.org/10.3390/molecules22071159>.
- Cook, N. and Samman, S., "Flavonoids—chemistry, metabolism, cardioprotective effects, and dietary sources", *The Journal of Nutritional Biochemistry*, Vol. 7, No. 2, (1996), 66-76.
- Havsteen, B.H., "The biochemistry and medical significance of the flavonoids", *Pharmacology & Therapeutics*, Vol. 96, No. 2-3, (2002), 67-202.
- Nijveldt, R.J., Van Nood, E., Van Hoorn, D.E., Boelens, P.G., Van Norren, K. and Van Leeuwen, P.A., "Flavonoids: A review of probable mechanisms of action and potential applications—", *The American Journal of Clinical Nutrition*, Vol. 74, No. 4, (2001), 418-425.
- Pellati, F., Prencipe, F.P., Bertelli, D. and Benvenuti, S., "An efficient chemical analysis of phenolic acids and flavonoids in raw propolis by microwave-assisted extraction combined with high-performance liquid chromatography using the fused-core technology", *Journal of Pharmaceutical and Biomedical Analysis*, Vol. 81, (2013), 126-132.
- Pujirahayu, N., Ritonga, H. and Uslinawaty, Z., "Properties and flavonoids content in propolis of some extraction method of raw propolis", *International Journal of Pharmacy and Pharmaceutical Science*, Vol. 6, (2014), 338-340.
- Luo, C., Zou, X., Li, Y., Sun, C., Jiang, Y. and Wu, Z., "Determination of flavonoids in propolis-rich functional foods by reversed phase high performance liquid chromatography with diode array detection", *Food Chemistry*, Vol. 127, No. 1, (2011), 314-320.
- Aminimoghadamfarouj, N. and Nematollahi, A., "Propolis diterpenes as a remarkable bio-source for drug discovery development: A review", *International Journal of Molecular Sciences*, Vol. 18, No. 6, (2017), 1290; <https://doi.org/10.3390/ijms18061290>.
- Pietta, P., Gardana, C. and Pietta, A., "Analytical methods for quality control of propolis", *Fitoterapia*, Vol. 73, (2002), S7-S20.
- Azmir, J., Zaidul, I., Rahman, M., Sharif, K., Mohamed, A., Sahena, F., Jahurul, M., Ghafoor, K., Norulaini, N. and Omar, A., "Techniques for extraction of bioactive compounds from plant materials: A review", *Journal of Food Engineering*, Vol. 117, No. 4, (2013), 426-436.
- Nourbakhsh Amiri, Z., Najafpour, G., Mohammadi, M. and Moghadamnia, A., "Subcritical water extraction of bioactive compounds from ginger (*Zingiber officinale* Roscoe)", *International Journal of Engineering, Transactions C: Aspects*, Vol. 31, No. 12, (2018), 1991-2000.
- Safdar, M.N., Kausar, T., Jabbar, S., Mumtaz, A., Ahad, K. and Saddozai, A.A., "Extraction and quantification of polyphenols from kinnow (*Citrus reticulata* L.) peel using ultrasound and maceration techniques", *Journal of Food and Drug Analysis*, Vol. 25, No. 3, (2017), 488-500.
- Sahne, F., Mohammadi, M., Najafpour, G.D. and Moghadamnia, A.A., "Extraction of bioactive compound curcumin from turmeric (*Curcuma longa* L.) via different routes: A comparative study", *Pakistan Journal of Biotechnology*, Vol. 13, No. 3, (2016), 173-180.
- Marić, M., Grassino, A.N., Zhu, Z., Barba, F.J., Brnčić, M. and Brnčić, S.R., "An overview of the traditional and innovative approaches for pectin extraction from plant food wastes and by-products: Ultrasound-, microwave-, and enzyme-assisted extraction", *Trends in Food Science & Technology*, Vol. 76, (2018), 28-37.
- Chemat, F., Rombaut, N., Meullemiestre, A., Turk, M., Perino, S., Fabiano-Tixier, A.-S. and Abert-Vian, M., "Review of green food processing techniques. Preservation, transformation, and extraction", *Innovative Food Science & Emerging Technologies*, Vol. 41, No. 1, (2017), 357-377.
- Lu, L.-C., Chen, Y.-W. and Chou, C.-C., "Antibacterial activity of propolis against *Staphylococcus aureus*", *International Journal of Food Microbiology*, Vol. 102, No. 2, (2005), 213-220.
- Apaydin, H. and Gümüş, T., "Inhibitory effect of propolis (bee gum) against *Staphylococcus aureus* bacteria isolated from instant soups 1", *Journal of Tekirdag Agricultural Faculty*, Vol. 15, No. 1, (2018), 67-75.
- Trusheva, B., Trunkova, D. and Bankova, V., "Different extraction methods of biologically active components from propolis: A preliminary study", *Chemistry Central Journal*, Vol. 1, No. 1, (2007), 13; <https://doi.org/10.1186/1752-153X-1-13>.
- Anđelković, B., Vujisić, L., Vučković, I., Tešević, V., Vajs, V. and Godevac, D., "Metabolomics study of populus type propolis", *Journal of Pharmaceutical and Biomedical Analysis*, Vol. 135, (2017), 217-226.
- Chang, C.-C., Yang, M.-H., Wen, H.-M. and Chern, J.-C., "Estimation of total flavonoid content in propolis by two complementary colorimetric methods", *Journal of Food and Drug Analysis*, Vol. 10, No. 3, (2002), 178-182.
- Biscaia, Danielle, and Sandra RS Ferreira. "Propolis extracts obtained by low pressure methods and supercritical fluid extraction." *The Journal of Supercritical Fluids*, Vol. 51, No. 1, (2009), 17-23.

Microwave Ultrasound Assisted Extraction: Determination of Quercetin for Antibacterial and Antioxidant Activities of Iranian Propolis

G. Heidari^a, G. D. Najafpour^a, M. Mohammadi^a, A. A. Moghadamnia^b

^a Department of Chemical Engineering, Babol Noshirvani University of Technology, Babol, Iran

^b Department of Pharmacology and Physiology, School of Medicine, Babol University of Medical Science, Babol, Iran

PAPER INFO

چکیده

Paper history:

Received 13 May 2019

Received in revised form 21 June 2019

Accepted 05 July 2019

Keywords:

Propolis

Extraction

Total Flavonoids

Microwave-ultrasound-assisted Extraction

Quercetin

بره موم از سالیان گذشته به عنوان مصارف دارویی، لوازم آرایشی و نوشیدنی کاربرد داشته است. مهمترین ترکیبات فعال در بره موم، ترکیبات فنولی و فلاونوئیدها هستند که در دسته رزین قرار دارند. هدف از این مطالعه، اندازه گیری میزان کل فلاونوئیدها و کوئرستین در بره موم شمال ایران و مقایسه آن با روش های مختلف استخراج می باشد. برای مقایسه بازده استخراج، روش های سوکسله، خیساندن و استخراج با کمک مایکروویو-اولتراسونیک (MUAE) انجام شد. مقدار کل فلاونوئیدها توسط اسپکتروفتومتری و مقدار کوئرستین با استفاده از سیستم کروماتوگرافی مایع با کارایی بالا (HPLC) تعیین شد. در این تحقیق، بهترین عملکرد استخراج برای کل فلاونوئیدها ۷۰/۸۸ درصد و برای کوئرستین ۴۴/۵۳ درصد در ۳۰۰ وات، ۱/۵ دقیقه برای مایکروویو و ۱۰ دقیقه و ۴۰ درجه سانتیگراد برای اولتراسونیک به دست آمد. همچنین عملکرد استخراج کوئرستین برای سوکسله (به عنوان روش مرجع) ۶۷/۸۵٪ و برای خیساندن ۱۳/۹٪ می باشد. برای تعیین عملکرد استخراج و ساختار شیمیایی ترکیبات فعال، از UV-vis، HPLC و FTIR استفاده شد. سپس اثر آنتی اکسیدانی و ضد باکتری ترکیبات عصاره مورد بررسی قرار گرفت و نتایج به دست آمده با مطالعات پیشین مقایسه شد.

doi: 10.5829/ije.2019.32.08b.01