The Effects of Different Ethanol Concentration on Total Phenolic and Total Flavonoid Content In Malaysian Propolis

IOP Conf. Series: Materials Science and Engineering **991** (2020) 012033 doi:10.1088/1757-899X/991/1/012033

The Effects of Different Ethanol Concentration on Total Phenolic and Total Flavonoid Content In Malaysian Propolis

N Yusof[1], M S Abdul Munaim[1*] and R Veloo Kutty[1]

[1] Faculty of Chemical & Process Engineering Technology, Universiti Malaysia Pahang,26300, Gambang, Pahang, Malaysia.

*Corresponding Author: mimi@ump.edu.my

Abstract. As compared to honey, propolis was considered 'less explored' in active compounds characterization study. The abundance of phytochemicals in propolis contributes to various medicinal properties such as antimicrobial, anticancer, wound healing and antioxidative. The most common antioxidant groups found in propolis are phenolic acids and flavonoids. Hence, in the current study, we investigated the relationship between different ethanol concentrations on the total phenolic content (TPC) and total flavonoid content (TFC) extracted from propolis. Crude propolis was crushed and sieved before ultrasonic extractions using different ethanol concentrations (20, 50, 80%). The determination of total phenolic content was obtained from the reaction of propolis extract with Folin- Ciocalteu reagent. The result of the total phenolic content was obtained from gallic acid calibration curve and represented in gallic acid equivalent (GAE). Meanwhile, quantification of total flavonoid content was obtained from the reaction of the extract with methanolic solution of aluminum chloride ($AlCl_3$). The result of the total flavonoid content of the propolis was acquired from quercetin calibration curve and represented in quercetin equivalent (QE). The highest TPC and TFC were recorded at 80% ethanol being 8.898 mg GAE/ml and 0.034 mg QE/ml respectively. From the experimental data, it is shown that TPC and TFC are directly propotional to ethanol concentration.

1. Introduction

Propolis, has been an attractive material for researchers recently due to the abundance of therapeutic active compounds that could be a potential natural product development. Approximately, propolis is composed from 80% resinuous materials, 10% aromatic and essential oils and the remaining 10% comprises of pollen and different types of organic compounds [1]. There are several applications of propolis extract such as cosmetics, nutraceuticals, dental, topical creams and food supplements [2]. The mixture of wax, bee enzymes and resinous material collected by bees from plants' buds and exudates largely make up the propolis composition [3]. It has distinctive and diverse chemical constituents and the variation of the composition is closely related to its origins. Up to this time, there are over 300 compounds that have been identified in propolis [4]. The major compounds classification can be divided to flavonoids, xanthones, phenolic acids, lignans and stilbenes. [5]. In the last 10 years, there are some identified compounds that possess anti- inflammatory properties such as caffeic acid, caffeic acid phenethyl ester (CAPE), naringenin, quecertin, salicyclic acid, ferulic acid, galangin and apigenin [6]. With the ability to reduce oxidative stress and prevent oxidation in larger molecular level, these compounds are known to be the good candidates for drug discovery in reducing degenerative diseases

likelihood of occurrence [7]. The awareness among scientists has been increasing on phenolic compounds due to its anti- inflammatory, oral hypoglycaemic agent, lipid lowering or antimicrobial, attributes but most researchers reported on the antioxidant property of these compounds [8]. The extraction of phenolics from samples is done using water or organic solvents such as alcohols, ketones and solvent water solutions. Of all these solvents, ethanol and ethanol water mixtures were the most favored solvent because it usually extracted an abundant amount of phenolic acids and flavonoids.

There has been an increase interest in naturally- derived antioxidants since some believe that synthetic antioxidants are unstable and possibly carcinogenic [9]. There are several methods being applied to study antioxidant activities such as total phenolic content (TPC) analysis, total flavonoid content (TFC) analysis, 2,2-Diphenyl-1 picrylhydrazyl (DPPH) radical scavenging activity, ferric reducing ability of plasma (FRAP) assay, oxygen radical absorbance capacity (ORAC) study, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging activity and hydrogen peroxide scavenging assay.

The major downside of applying only one method for antioxidant activity evaluation is that the results may not be relevant to the action mechanism of the antioxidant when tested in vivo [10]. In common practice, researchers would conduct more than one antioxidant assay to support the findings they obtained from in vitro experiments. Since the phytochemicals are varied geographically and largely depending to the respective plants in a particular area, we discovered that there is a lack of information on the propolis in Kuantan. Besides that, the use of solvent also plays an important role in chemical compounds extraction. Hence, this experiment was designed to investigate the relationship between ethanol concentrations and total phenolic content (TPC) and total flavonoid content (TFC) extracted from Malaysian propolis.

2. Materials and method

2.1 Material

A local resident in Bukit Goh, Malaysia provided us with the crude propolis which was freshly harvested from a stingless bee farm. The propolis sample was kept in a properly- sealed glass container and being stored at 4°C chiller until further use. The chemicals and reagents used were absolute methanol (Merck, USA), absolute methanol (Merck, USA), gallic acid (Sigma, USA), quecertin (Merck, USA), aluminum chloride (Sigma Aldrich, USA), sodium carbonate (Merck, USA), Folin and Ciocalteu's phenol reagent (Sigma Aldrich, USA), and ultrapure water. The filter paper used was Whatman No. 1. All chemicals and solvents used were of analytical grade.

2.2 Methodology

2.2.1 Preparation of Propolis Extract. The crude propolis was grounded and sieved. Half a gram of crude propolis was soaked in five ml of ethanol with different ethanol concentrations (20, 50 and 80% v/v) in different test tubes, assisted with ultrasonic for 20 minutes, in room temperature of 25°C. The extraction solution was filtered by using filter paper to separate the propolis extract from the propolis solid mass. The propolis extract was stored in 4°C chiller.

2.2.3 Quantification of Total Phenolic Content (TPC). The measurement of total phenolic content was done by allowing the sample solution to react with Folin- Ciocalteu reagent. The method was done according to [20] with slight modification. 2N Folin- Ciocalteu reagent (FCR) was made and to provide an alkaline environment to this reaction; 6% of sodium carbonate (Na_2CO_3) solution was prepared beforehand. 0.1 ml of propolis extract of each ethanol concentration was mixed with 1 ml distilled water and 0.2 ml diluted Folic Ciocalteu reagent. The mixtures were shaken and left to stand for 5 minutes. The Na_2CO_3 solution was pipetted into the mixtures and they were incubated for 30 minutes in the absence of light. The absorbance was read at 760 nm by using UV- Vis spectrometer. The amount of

the total phenolic content of the propolis were obtained from gallic acid standard calibration curve and represented in gallic acid equivalent (GAE) as depicted in (Figure 1).

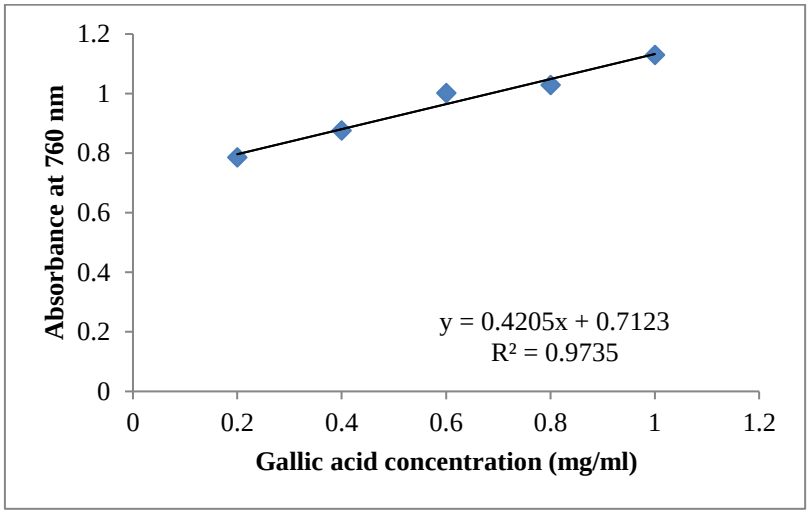

Figure 1. Standard calibration curve of gallic acid

2.2.4 Quantification of Total Flavonoid Content (TFC). Total flavonoid content extracted from propolis was obtained from the reaction of the extract with methanolic solution of aluminum chloride ($AlCl_3$), as per implemented by [21]. A methanolic solution of aluminium chloride of 20 mg/ml was prepared prior to the experiment. 500µl of propolis extract was mixed with the prepared 0.5 ml aluminium chloride solution. The mixture was then homogenized by using a vortex mixer to let both liquids to be well-mixed. The mixture was located in a dark space in the laboratory for 30 minutes incubation and the absorbance was read at 415 nm afterwards. The results of the total flavonoid content of the propolis were obtained from quecertin calibration curve and represented in quecertin equivalent (QE) as shown in (Figure 2).

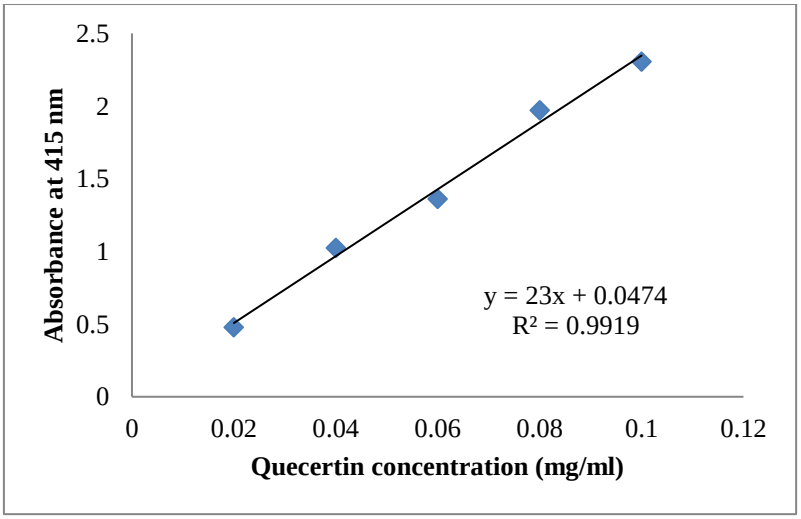

Figure 2. Quecertin standard calibration curve

3. Results and discussion

Table 1 shows the amount of TPC and TFC of propolis. The amount of TPC extracted from propolis for 80% of ethanol escalated for nearly 8 times of its amount for 20% ethanol; from 1.456 mg GAE/ml to

8.898 mg GAE/ml. The ethanol- water mixture provided a more polar environment for the solutes compared to the 100% ethanol. Of all the three solvent concentrations, 20% of ethanol was the most polar as it contained the most water, whereas 80% was the least polar. It is obviously seen that phenolic compounds has an affinity towards the least polar extraction solvent as this condition had enhanced the solubility of phenolic acids and thus increasing the extraction efficiency. Zuorro et al., (2019) mentioned that phenolic compounds exhibit a wide range of solubility in single mixture components and are often more soluble in solvents less polar than water.

Table 1. Total phenolic content and total flavonoid content of propolis extract at different ethanol concentrations

Ethanol Concentration (%)	Total Phenolic Content (mg GAE/ml)	Total Flavonoid Content (mg QE/ ml)
20	1.456 ± 0.0025	0.010 ± 0.19
50	3.851± 0.036	0.028 ± 0.0335
80	8.898 ± 0.008	0.034 ± 0.1875

Meanwhile, the least flavonoid content was obtained at the lowest ethanol concentration of 20%; 0.010 mg QE/ml while the highest was found to be at 80% ethanol; 0.034 mg QE/ml. As flavonoids are very less likely to be soluble in highly polar extraction solvent, that explains why 20% ethanol only yielded 0.010 mg QE/ml. The amount of TFC increased dramatically with 0.018 mg QE/ml difference, with the increasing ethanol concentration of 30%. Flavonoids are non- polar and to improve the extraction efficiency, it is suggested to use solvents that have low polarity. This data is parallel to total phenolic content as the amount of TPC increased with the increased ethanol concentration or less polar extraction environment. When the samples were exposed a better affinity solvent, the solvent could penetrate the solid matrix more easily, improving the efficiency of solutes and thus improving the extraction efficiency.

4. Conclusion

This study proves that increased ethanol concentration would have positive effects on both total phenolic and flavonoid content extracted from propolis. To have a more efficient extraction process, it is good for the researchers to have some basic knowledge on the solvent polarity and the affinity of targeted compounds towards the solvents. This study provides some preliminary data for researchers who are interested to conduct the study on antioxidant assay in the future.

References

[1] Ramnath S., Venkataramegowda S. 2016. *Int. J. Pharmacol., Phytochem. Ethomed.*. **5** 79
[2] Biscaia D, Sandra R, Ferreira S 2009 *Journal of Supercrit Fluid* **51** 17
[3] Sforcin J M, Bankova V. *J. Ethnopharmacol*. **133** 253
[4] Revilla I. Vivar- Quintana A M, González- Martín I, Escuredo O, Seijo C 2017 *Microchem. J.* **134** 211
[5] Weber F et al., 2014 *Polyphenols in Plants* (Arizona: Academic Press) chapter 15 pp 293 296
[6] Askari V H, Rahimi V B, Zamani P, Fereydouni N, Rahmanian-Devin P, Sahebkar A H, Rakhshandeh H 2018 *Biomed. Pharmacother*. **99** 346
[7] Ares A M, Valverde S, José L B, Nozal M J, Bernal J 2017 *J. Pharm. Biomed. Anal.*
[8] Mohdaly A AA, Mahmoud A A, Roby M H H, Smetanska I, Ramadan M F 2015 *J. Food Biochem*. **39** 538
[9] Chandra S, Khan S, Avula B, Lata H, Min HY, ElSohly M A, Khan I A 2014 *Evid. Based Complementary and Altern. Med.*

[10] Nemzer B et al., 2018 Sprouted Grains: Nutritional Value, Production and Applications (Elsevier) chapter 3 pp 55- 68
[11] Mouhoubi- Tafinine Z, Ouchemoukh S, Tamendjari A 2016 Ind. *Crop and Prod*. **88** 85
[12] Andrade J K S, Denadai M, Oliviera C S, Nunes M L, Narain N 2017 *Food Research International*
[13] Zuorro A, Iannone A, Lavecchia R 2019 *Processes* **7** 126