Propolis Extraction Using Vacuum Resistive Heating Method

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Abstract. Indonesian raw propolis as herbal medicine is produced by many farms but the bioactive content is not yet known. An innovative propolis extractor based on vacuum ohmic heating emerged. Vacuum ohmic heating is an extraction technology that uses an electric current that is carried out in a vacuum. This study aims to determine the effect of the origin of propolis on chemical characteristics extract using propolis extractor machine and compared with commercial propolis. This study used completely randomized design method with the origin of propolis variations. The extraction process was carried out in two stages. The results were compared with commercial product. The highest total phenol and flavonoid in vacuum resistive heating method was obtained in Makassar propolis while the lowest was obtained from Bali. In conclusion, the origin of the propolis area affects the results of propolis extraction using vacuum resistive heating technology to the parameters of total phenol and total flavonoids. By knowing the difference in the yield of bioactive compounds in propolis, it can be seen which propolis is best for use in producing herbal medicine propolis as a source of antioxidants.

1 INTRODUCTION

Indonesia is a country rich in fauna that are beneficial to man one of them is a bee. Products produced by bees include honey, pollen, royal jelly, beeswax and propolis [1]. Propolis is a bee product that has a fairly high economic value [2]. Bee propolis or glue is a type of bee resin that is collected from various plant sap mixed with saliva and bee enzymes to be used to seal holes in the hive. The existence of this cover can protect the entrance against intruders and prevent the decomposition of foreign organisms [3]. So far, propolis is known to the people of Indonesia and even the world as a panacea for various types of diseases. This is because the active ingredients contained in propolis flavonoids include antioxidant potential as a source of high level produced by honeybees. The antioxidant activity in propolis ranked first in strength against oxidants and free radicals in comparison with the results of other bee products [4].

Propolis great potential for development still has many obstacles in its production technology. This is related to the propolis market, which is still dominated by imported products, especially Brazilian production [5]. It is recognized that the domestic production of raw propolis is quite but still face difficulties in processing because the limitation of technology. In addition, propolis spread in Indonesia has different characteristics but unknown specific compounds contained therein. Information
about the specifications of propolis from various regions in Indonesia is still low and research on this propolis is needed.

Propolis usage cannot be in a raw form (raw propolis), but must be purified first. One of the most important methods of producing propolis is extraction. Extraction is a method of separating an active part of the component by using a certain solvent to obtain a pure product [6]. Extraction of propolis aims to eliminate beeswax removes inactive components such as balsam resin compound [7]. So far, the technology most commonly used in propolis extraction is batch maceration with a time of more than 72 hours [8]. Maceration widely used in the industry but is not effective and efficient way to extract the bioactive compounds because it takes a long time and the amount of solvent that many.

Extraction technology continues to develop, one of which is the vacuum ohmic heating technology. Ohmic technology is an extraction technology by utilizing alternating electric currents (alternating currents) passed through the material [9], the process is carried out in a vacuum to reduce the temperature used in the extraction. This new technology is expected to be able to optimize the extraction of propolis from various regions in Indonesia. Resistive heating vacuum extractor machine is new method of propolis extraction. This study aims to determine the effect of the origin of propolis on chemical characteristics extract using propolis extractor machine and compared with commercial propolis.

2 METHOD

2.1 Tools and Materials

The tools needed in this study include a set of vacuum ohmic heating, UV-Vis spectrophotometer (Shimadzu Brand), transformer, multi meter (Sanwa brand), vortex, refrigerator, digital scales and glassware (measuring flask, beaker, measuring cup, funnel, glass stirrer, test tube, measuring pipette, dropper pipette). The materials needed include raw propolis obtained from the Joyo Batu Flower Farm, Denpasar Nusantara Bee Farms, and livestock in Makassar, technical ethanol solvent, distilled water, ethanol pro analysis (MERCK brand), standard gallic acid, fine filter paper, Folin ciocalteau, Na2CO3 (MERCK Brand), standard quercetin, NaNO2 (MERCK Brand), AlCl3 (MERCK Brand), and 1 M NaOH.

2.2 Extraction Procedure

The preparation of the ingredients begins with propolis which is frozen at -18°C for 24 hours in order to avoid propolis from mushrooms or other things that can decrease the quality of the propolis and facilitate the next step, namely grating using cheese surrender. Propolis is put in the refrigerator at 5°C. Raw propolis is weighed according to a certain ratio. In this study, 20 grams of raw propolis were used which were measured using a digital scale with an accuracy of 0.001 grams and using 200 ml of solvent which was measured using a measuring cup with a volume of 100 ml twice.

This study used completely randomized design method with the origin of propolis variations. The extraction process is carried out in two stages, starting with extraction using distilled water then filtering and extracting it, then the propolis waste is extracted again using 70% ethanol solvent. The two processes were carried out on three different types of propolis from the region in a stable condition with control variables in the form of vacuum pressure and temperature. The first process is distilled water in a stable state at a pressure of 16.6 KPa, a voltage of 100V, and a holding temperature of 58°C. While the second process is 70% ethanol solvent in a stable state at a pressure of 16.6 KPa, a voltage of 220V, and a holding temperature of 37°C. The treatment was done 3 repetitions were then compared with control propolis maceration method 50 °C for 24 hours and 2 brands commercial propolis.

2.3 Moisture content

The water content of raw propolis or analysed using a thermogravimetric moisture analyser. Moisture analyser is used to measure the water content in the presence of thermogravimetric balance [10]. Infrared in the tool will evaporate the water in the material then the water loss will be calculated as a percentage of water content. The accuracy of the thermogravimetric method is ± 0.1%. Weighed as much as 3 grams of raw propolis and lighted tool to calculate the water content.

2.4.2. Total phenol (TP)
Total phenol was analysed using gallic acid as standard and Folin – Ciocalteu as reagent [11]. Furthermore, the absorbance of the solution is measured using a spectrophotometer.

2.4.3 Total Flavonoids

Total flavonoid content was determined by the literature with modifications [12]. Tests are carried out using AlCl₃ was measured by the spectrophotometric method.

3 RESULT AND DISCUSSION

3.1 Characteristics of Propolis

Raw materials used in this research that propolis from several regions in Indonesia, namely Batu, Bali and Makassar. Propolis was obtained from the Joyo Batu Flower Farm, Denpasar Nusantara Bee Friend Farm, and a farm in Makassar. Raw Propolis, which is still in the form of chunks and solid, is then ground to become raw propolis powder.

![Figure 1: Raw Propolis from the Batu before extracted](image)

| Origin of Propolis | Aroma       | Characteristics | Moisture Content (%) |
|-------------------|-------------|-----------------|----------------------|
| Makassar          | Strong (++++)| Brownish Yellow | 3.23 ± 0.21a         |
| Batu              | Strong (+)  | Black           | 3.66 ± 0.08b         |
| Bali              | Strong (+++) | Dark Brown      | 10.95 ± 0.41b        |

Information: 1) Each data set is the mean of 3 replications ± standard deviation  
2) Different notations show significant differences in the DMRT test (α=0.05)

Based on Table 1 above, there is a relationship between aroma and colour where the stronger the aroma, the brighter the colour. The characteristics of propolis with a stronger aroma and brighter colour are owned by Makassar propolis. In contrast, the weaker the aroma, the darker (black) colour found in propolis from Batu, East Java and Bali propolis. Propolis is a sticky solid at room temperature and will become denser, brittle and fragile at temperatures below 15 °C. All propolis samples became denser and more easily destroyed in the second hour of storage at -18 °C. Different physical appearances can represent different chemical compositions which can cause different biological activities [13].

The characteristic of water content in the raw material shows a difference in the values that are in the average range between 3.23% - 10.95%. Makassar's propolis water content was in the lowest position, namely 3.23%, followed by Batu propolis with a water content of 3.66%, then the highest water content came from Bali propolis with a water content of 10.95%. Difference in moisture content Based on the literature, propolis from the southern Spanish region has a moisture content in the range of 2.35–22.6% [14]. In addition, the guidelines issued by the São Paulo State Beekeepers Association and the International Honey Commission stipulate a maximum water content of 8% propolis [15]. Thus, the analysis results obtained are within the range contained in the literature. The water content of Batu and Makassar's propolis meets the guidelines issued by the São Paulo State Beekeepers Association and the International Honey Commission while the water content of Bali's propolis is still above the prescribed standard.

The difference in water content is influenced by storage conditions, propolis handling, and propolis origin [16]. The storage process that does not pay attention to humidity will affect the moisture
content of propolis which should be stored dry and not humid. Storage in humid areas results in increased moisture content. Handling of propolis is cleaning raw propolis by washing it through a process of immersion in water. If the next drying step is not carried out properly then the moisture content in the final product will be affected. In addition, differences in water content are also influenced by the origin of the propolis region which has geographic specifications [17].

3.2 Total Phenolic Content

The highest mean phenolic content from propolis extract with differences in the origin of the propolis area using the vacuum ohmic heating method is found in Makassar propolis which is worth 41.58 mg GAE / g for the 1st stage extraction with distilled water and 45.72 mg GAE / g for the 2nd stage extraction with ethanol 70%. These data indicate that the phenolic content propolis from Makassar is higher than the total phenol propolis from Batu and Bali. The mean phenolic content of propolis extract with the effect of variations in the origin of propolis can be seen in Table 2.

| Origin of Propolis | Stage 1 (Distilled) | Stage 2 (Etanol 70%) |
|-------------------|---------------------|---------------------|
| Makassar          | 41.59 ± 0.48        | 45.72 ± 0.94        |
| Batu              | 34.17 ± 0.82        | 36.17 ± 0.58        |
| Bali              | 14.49 ± 0.32        | 15.61 ± 0.54        |

Information: 1) Each data set is the mean of 3 replications ± standard deviation

Table 2 shows that the highest phenolic content in the vacuum ohmic heating method was obtained in Makassar propolis stage 2 (70% ethanol) of 45.72 mg GAE / g while the lowest total phenol was obtained in Bali propolis stage 1 (distilled) of 41.59 mg GAE / g.

The phenolic content obtained from the study had a higher value than the results of the research which states that propolis from Batu and Mojokerto has a phenolic content of 15.170 mg GAE / g and 9603 mg GAE / g [18]. Then the research which is evaluate the phenol content of propolis from several regions in Indonesia, it is known that there are differences in the total phenol in each region in Indonesia, namely West Nusa Tenggara, South Sulawesi, West Java and West Kalimantan with an average phenolic content value of 39.9 – 97.4 mg GAE / gr [19]. In addition, there are variations in the phenolic content values of propolis from various countries. Propolis from the central region of Portugal has a phenolic content of 151 mg GAE / g). The amount is higher than propolis from Brazil which has a phenolic content of 120 mg GAE / g [20] and Thai propolis with a phenolic content of 31.2 mg GAE / g [21].

Based on these data, it can be seen that there are differences in the number of phenolic compounds in each propolis which is influenced by differences in regional origin. The area of origin determines the physical properties and chemical composition, as well as the bioactive potential of propolis [22]. Other influences that affect the diversity of polyphenols in propolis are season, regional vegetation, and the state of propolis (fresh or old). The bioactivity capacity of propolis is highly dependent on its phenolic content, including phenolic acids, flavonoids, anthocyanins and some aromatic acids and esters. Differences in phenolic content due to regional factors of origin are related to the potential of different plants in each region as a source of nutrition for bees. Based on this, the difference in total phenol between Makassar, Batu and Bali propolis is caused by differences in the location of the bee farms that produce propolis. Makassar's propolis is obtained from breeders who live in Sulawesi Island who tend to have warmer and drier conditions, this affects the vegetation in the area. Propolis Batu is obtained from breeders in Java Island, more precisely in East Java Province. Batu City is a cool area surrounded by mountains and forests, many trees and types of plants that live in the Batu area, so the source of phenolic compounds obtained by bees can be more diverse. Balinese propolis is obtained from the beekeeper on the island of Bali which has a unique flora. The differences in the islands of the three samples resulted in variations in the nutritional sources of the bees being bred. As a result, bees produce phenolic compounds in varying quantities and variations.
3.3 Total Flavonoids

The highest total flavonoids using the vacuum ohmic heating method are found in Makassar propolis. The highest total flavonoid value in the figure is found in Makassar propolis with a value of 15.11 mg QE / g for phase 1 extraction with distilled water and 15.19 mg QE / g for stage 2 extraction with 70% ethanol, while the total flavonoid value from the original propolis extract Batu and Bali lower. Average total flavonoid extract of propolis with the effect of variation of propolis origin can be seen in Table 3.

Table 3 Average Total Flavonoids from Propolis Extract by Vacuum Ohmic Heating Method and Maceration Treatment as Control

| Origin of Propolis | Total Flavonoid (mg QE/g) |
|--------------------|--------------------------|
|                    | Stage 1 (Distilled) | Stage 2 (Etanol 70%) |
| Makassar           | 15.11 ± 0.31          | 15.19 ± 0.42          |
| Batu               | 8.17 ± 0.42           | 10.47 ± 0.36          |
| Bali               | 7.27 ± 0.66           | 8.01 ± 0.47           |

Based on Table 3 regarding the average total flavonoids of propolis extract from various regions of the vacuum ohmic heating method and maceration treatment as a control, it is known that the highest total flavonoids in the vacuum ohmic heating method were found in Makassar propolis stage 2 (70% ethanol) of 15.19 mg QE / g while the lowest total flavonoids were obtained in Bali propolis stage 1 (distilled) of 7.27 mg QE / g. Based on these data, it can be seen that the existence of each propolis with a different origin will result in a difference in the number of flavonoid compounds.

Total flavonoids from propolis originating from five regions in Indonesia have a range between 0.024-0.046 mg QE / g [23]. The flavonoid content of propolis originating from several regions in Indonesia, namely West Nusa Teggara, South Sulawesi, West Java and West Kalimantan, has different results ranging from 3.12 mg QE / g to 20.22 mg QE / g [24]. The levels of flavonoids from propolis in Indonesia have various values, as well as foreign propolis. The value of flavonoid content in different seasons, namely 1.10-1.71 mg QE / g in winter and 1.12-1.56 mg QE / g in spring [25].

Based on these data, it is known that there is a diversity of total flavonoid values from various regions, both in Indonesia and abroad. Propolis has a complex chemical composition and depends on the flora in the breeding area of the propolis-producing bees. Another thing that affects the bioactive components in propolis is the propolis harvest season. A study comparing the chemical compound propolis collected at four seasons in Taiwan. The highest flavonoid content is found in propolis which is harvested in the summer months of May to July and the lowest is in the winter months, namely October to December [26]. When applied in Indonesia, which has two seasons, namely the test season and the dry season, there is a difference in the yield of bioactive compounds in the form of flavonoids in Makassar, Batu and Bali propolis which is influenced by the harvesting time between the two seasons. If it is related to water content, Bali propolis has high water content compared to propolis from other regions so that its flavonoid content is relatively low.

3.4 Vacuum Resistive Heating Compared to Commercial Propolis

Development of technology to make propolis not only sold in the form of crude or raw propolis, but also in the form of extracts with various brands. Propolis extract is sold in various forms ranging from liquid, capsule, and syrup. In this study, the commercial propolis used as a comparison with the content of propolis extraction results using ohmic heating vacuum that future is expected to be an alternative method of extracting propolis industrial scale. The commercial propolis used consists of two brands, namely propolis A and propolis B. Propolis A is obtained from the Medison Pharmacy in Malang City, while propolis B is obtained from one of the UKM in Batu City. The mean total phenols and flavonoids of the best treatment with the vacuum ohmic heating and commercial propolis methods can be seen in Figure 2.
Figure 2 shows the difference in total phenol and total flavonoids between commercial propolis and vacuum ohmic extraction results. Commercial propolis A has a total phenol of 135.71 mg GAE / g and a total flavonoid of 57.04 mg QE / g. Commercial propolis B has a total phenol of 122.38 mg GAE / g and a total flavonoid of 42.35 mg QE / g. While the best treatment of propolis with vacuum ohmic heating method has a total phenol of 45.72 mg GAE / g and a total flavonoid of 15.19 mg QE / g.

Based on this, it is known that commercial propolis and propolis from vacuum ohmic heating have differences. This can occur due to differences in raw propolis origin and differences in final treatment of propolis extract. Commercial propolis has gone through the evaporation process using a rotary evaporator to a more concentrated concentration so that the bioactive content in commercial propolis is more. In addition, the majority of commercial propolis claims that the propolis used in its production comes from Brazil and uses the latest technology. The technology used by commercial propolis may vary, but there is one brand that claims that propolis produced with supercritical extraction technology has a flavonoid of 59 mg QE / g. Supercritical fluid extraction (SFE) is an extraction process with the help of supercritical fluid or commonly uses CO₂ as the extraction solvent. This technology is effective for extracting both solids and liquids but the use of supercritical fluid extraction is still limited due to the high investment costs compared to other extraction technologies [27]. One of the commercial
propolis located in Indonesia and claims to be made from raw Brazilian propolis has a total phenol of 376.3 mg GAE / g and a total flavonoid of 42.56 mg QE / g. The various values of propolis content lead to the importance of standardizing propolis products for distribution in the market [28].

Propolis has become a bee product that has attracted consumer interest in the last decade. The demand for propolis continues to increase along with the various benefits provided. Efforts made to protect consumers are through product standardization. Unfortunately, in Indonesia there is no SNI for propolis. Currently there are several standards for propolis, namely the outdated Russian standard and the Argentine standard. The International Honey Commission conducts research on analytical methods, general composition criteria for the quality of propolis. Meanwhile, researchers from Japan, Korea, China and Taiwan developed propolis standards for their countries [29]. The International Honey Commission recommends standard values for bioactive compounds for the two most widespread types of propolis, namely European poplar (Poplar type) and Brazilian green propolis (Baccharis type). Poplar propolis has a total phenol standard of 21% of the dry weight of raw materials, while Brazilian Green Propolis has a total phenol standard of 5% and a total standard of flavonoids of 0.5% of the dry weight of raw materials [30]. Argentina has the same phenolic standards as Brazil, namely 5% and total flavonoid standards of 1% of the dry weight of raw materials [31]. In addition, there is a law from Brazil which describes the phenolic standard of propolis at least 0.5% and the propolis flavonoid standard at least 0.25% [32]. Based on the data above, it is known that the propolis standard from each country is different depending on the propolis that country produces.

The best treatment in the vacuum ohmic heating method is obtained from Makassar propolis which has the highest total phenol and total flavonoid values. The total phenol propolis in Makassar is 4.6% so that it suitable with the Brazilian propolis standard. Meanwhile, the total flavonoid propolis in Makassar is 1.5% so that it suitable with the propolis standards of Argentina, Brazil and the International Honey Commission.

4 CONCLUSIONS
The origin of the propolis area affects the results of propolis extraction using vacuum resistive heating technology to the parameters of total phenol and total flavonoids. The highest phenol content was 45.72 mg GAE / g and the highest flavonoid level was 15.9 mg QE / g obtained from propolis from Makassar area. By knowing the difference in the yield of bioactive compounds in propolis, it can be seen which propolis is best for use in producing herbal medicine propolis as a source of antioxidants.

REFERENCES
[1] Haryanto B., Hasan Z., Kuswandi and Artika. 2012. Penggunaan Propolis Untuk Meningkatkan Produktivitas Ternak Sapi Peranakan Ongole (Po). Jurnal Ilmu Ternak Dan Veteriner Vol. 17 No 3.
[2] Artdiyasa N., A Chaidir., E K Wirawati., and T Susanti. 2010. Trigona: Lebah Penghasil Propolis. Trubus Online. Http://Www.Trubus-Online.Co.Id [24 January 2020].
[3] Jaya Firman. 2017. Produk Produk Lebah Madu Dan Hasil Olahanannya. Malang: UB Press.
[4] Manach C, Scalbert A. Morand C, Remesy L. Dan Jimenez L. 2004. Polyfenols Food Sources and Bioavailability. American Journal of Clinical Nutrition 79 (1): 727-747
[5] Halim Eliza. 2012. Kajian Bioaktif Dan Zat Gizi Propolis Indonesia Dan Brazil. Jurnal Gizi Dan Pangan 7(1): 2-3
[6] Handa S S., Khanuja S P S, Longo G and Rakesh D D. 2008. Extraction Technologies For Medicinal And Aromatic Plants. International Center For Science And High Technology 2 (1): 21-25.
[7] Lofty M. 2006. Biological Activity Of Bee Propolis In Health And Disease. Asian Pac J Cancer Prev 7:22-31.
[8] Lutpiatina L. 2015. Efektivitas Ektrak Propolis Lebah Kelulut (Trigona Spp) Dalam Menghambat Pertumbuhan Salmonella Typhi, Staphylococcus Aureus dan Candida Albicans. Jurnal Skala Kesehatan 6(1)
[9] El Darra N., Grimi N., Vorobiev E., Louka N., and Maroun R. 2013. Extraction Of Polyphenols From Red Grape Pomace Assisted By Pulsed Ohmic Heating. Food And Bioprocess Technology 6(5)
[10] Zhu, Y., Zou, X., Shen, T., Shi, J., Zhao, J., Holmes, M., dan Li, G. 2016. Determination Of Total Acid Content And Moisture Content During Solid-State Fermentation Processes Using Hyperspectral Imaging. Journal of Food Engineering 174

[11] Sharma G.N. 2011. Phytochemical Screening and Estimation Of Total Phenolic Content in Aegle marmelos Seeds. International Journal of Pharmaceutical and Clinical Research. 2(3): 27-29.

[12] Li W., Dai R.J., Yu Y H., Li L., Wu C M., Luan W W., Meng W W., Zhang X S. & Deng Y L. 2007. Antihyperglycemic Effect of Cephalotaxus sinensis Leaves and GLUT4 Translocation Facilitating Activity Of Its Flavonoid Constituents. Biology Phamcy Bulletin 30(6): 1123-1129.

[13] Yuliana N D., Wijaya C H., dan Nasrullah N. 2013. Classification Of Trigona Spp Bee Propolis From Four Regions In Indonesia Using Firr Metabolomics Approach. In 13th Asean Food Conference

[14] Bonvehí J S., and Bermejo F O. 2013. Element Content Of Propolis Collected From Different Areas Of South Spain. Environmental Monitoring And Assessment, 185(7), 6035-6047.

[15] Bankova V., Bertelli D., Borba R., Conti B J., Da Silva Cunha I B., Danert C.,Papotti G. 2019. Standard Methods For Apis Mellifera Propolis Research. Journal Of Apicultural Research, 58(2), 1-49.

[16] Cunha I., Sawaya A C., Caetano F M., Shimizu M T., Marcucci M C., Drezza F T., and Carvalho P D O. 2004. Factors That Influence The Yield And Composition Of Brazilian Propolis Extracts. Journal Of The Brazilian Chemical Society, 15(6), 964-970.

[17] Rosyidi D., Radiati L E., Minarti S., Mustakim M., Susilo A., Jaya F., and Azis A. 2018. Perbandingan Sifat Antioksidan Propolis Pada Dua Jenis Lebah (Apis Mellifera Dan Trigona Sp.) Di Mojokerto Dan Batu, Jawa Timur, Indonesia. Jurnal Ilmu Dan Teknologi Hasil Ternak (Jitek), 13(2), 108-117

[18] Yuliana N D., Wijaya C H., and Nasrullah N. 2013. Classification Of Trigona Spp Bee Propolis From Four Regions In Indonesia Using Firr Metabolomics Approach. In 13th Asean Food Conference

[19] Choi Y M., Noh D O., Cho S Y., Suh H J., Kim K M., Kim J M. 2006. Antioxidant And Antimicrobial Activities Of Propolis From Several Regions Of Korea. Food Science Technology 39: 756–761.

[20] Kumazawa S, Hamasaka T, Nakayama T. 2004. Antioxidant Activity Of Propolis Of Various Geographical Origins. Food Chemistry. 84:329–339.

[21] Chinh H., Asadov A., Dan Kolayli S. 2015. Phenolic Profile And Antioxidant Potential Of Propolis From Azerbaijan. Mellifera, 15(1), 16-28

[22] Hasana A E Z., Mangunwidjajad D., Sunartic T C., Suparno O., and Setiyono A. 2014. Investigating The Antioxidant Dan Anticytotoxic Activities Of Propolis Collected From Five Regions Of Indonesia Dan Their Abilities To Induce Apoptosis. Emirates Journal Of Food Dan Agriculture, 26(5), 390– 398

[23] Miguel M G., Nunes S., Dandlen  S A., Cavaco A M., and Antunes M D. 2010. Phenols Dan Antioxidant Activity of Hydro-Alcoholic Extracts Of Propolis From Algarve, South Of Portugal. Food and Chemical Toxicology, 48(12), 3418–3423.

[24] Chen, Y.-W., Wu, S.-W., Ho, K.-K., Lin, S.-B., Huang, C.-Y., dan Chen, C.-N. 2008. Characterisation Of Taiwanese Propolis Collected From Different Locations And Seasons. Journal Of The Science Of Food And Agriculture 88(3)

[25] Conde-Hernández L. A., Espinosa-Victoria J. R., Trejo A., and Guerrero-Beltrán J A. 2017. CO2 -Supercritical Extraction, Hydrodistillation And Steam Distillation Of Essential Oil Of Rosemary ( Rosmarinus Officinalis ). Journal Of Food Engineering, 200, 81–86.

[26] Yuliana N D., Wijaya C H., dan Nasrullah N. 2013. Classification Of Trigona Spp Bee Propolis From Four Regions In Indonesia Using Firr Metabolomics Approach. In 13th Asean Food Conference
[29] Stan L., Mărghităș L A., and Dezmirean D. 2011. *Quality Criteria For Propolis Standardization*. Scientific Papers Animal Science And Biotechnologies, 44(2), 137-140.

[30] Bankova V., Bertelli D., Borba R., Conti B J., Da Silva Cunha I B., Danert C., Papotti G. 2019. *Standard Methods For Apis Mellifera Propolis Research*. Journal Of Apicultural Research, 58(2), 1-49.

[31] Instituto Argentino De Normalization. 2004. *Esquema I De Norma Iram-Inta 15935-1.Productos Del Noroeste Argentino: Propoleos*

[32] Da Silva J F M., De Souza M C., Matta S R., De Andrade M R., Dan Vidal F V N. 2006. *Correlation Analysis Between Phenolic Levels Of Brazilian Propolis Extracts And Their Antimicrobial And Antioxidant Activities*. Food Chemistry, 99(3), 431-435.