Preference Level Of Bees Apis Mellifera L. To The Supplementary Feed Of Mixed Syrup And Paliasa Leaf Decoction And Physico-Chemical Characteristics Of Produced Honey

Aliyah, Elly Wahyudin, Cahyono Kaelan, Mappatoba Sila

Abstract: Supplementary feeding mixed with paliasa leaves (*Kleinhovia hospita* Linn.) to the honeybees *Apis mellifera L*. is expected to produce honey, which have properties of paliasa naturally. Four colonies of honeybees were fed a mixture of syrup and paliasa leaf decoction with four different concentrations, one for each colony, to produce honey essence of paliasa (HEP). In general the bees liked the given supplementary feed. Differences in color and aroma of HEP did not depend on the amount of feed consumed but were influenced by the concentration of paliasa in the supplementary feed given. The higher concentration of paliasa resulting in HEP with the darker color, the more real aroma of paliasa, and the more viscous consistency. The HEP had a sweet and sour sweet taste with pH between 4.90 and 5.15 and water content between 19.51 and 21.67%. The qualitative analysis showed that the HEP produced by bees fed without paliasa gave only positive reactions for alkaloids, while the HEP produced by bees fed with paliasa gave positive results for alkaloids, polyphenols and flavonoids. This indicated that the chemical components of paliasa were transformed by the bees into the HEP. The higher concentration of paliasa the higher level of total polyphenols and total flavonoids of HEP with the amount of total polyphenol contents was greater than the total flavonoid contents.

Keywords: *Apis mellifera* L*.,* supplementary feed, syrup, paliasa leaf

1. INTRODUCTION

One of natural ingredients that is widely used especially by people in South Sulawesi to treat hepatitis is paliasa leaves (*Kleinhovia hospita* Linn.). Paliasa leaves contain saponin, cardenolin, bufadienol, and anthraquinone compounds (Raflizar, *et a.l*, 2006) [1], scopoletin, keampferol, quercetin, cyanogenic compounds (Philippine Medicinal Plants, 2010) [2], and cycloartane triterpenoids (Li, *et al*., 2009) [3]. Such paliasa, honey is also one of the natural materials that has been known not only as ingredient in a diverse range of food products because of its rich nutrition and good taste but also as a traditional therapeutic material (Erguder, *et al*., 2008) [4]. Honey is able to treat a variety of diseases, such as to heal burns, anti-inflammatory (Simon, *et al*., 2009; Fiorani *et al*., 2006) [5],[6], hepatoprotective treatment (Mahesh *et al*., 2009) [7], and to modulate cell damage liver and kidneys (Halawa *et al*., 2009) [8]. Honey contains fructose, glucose, and antioxidants, such as phenolic compounds, chrysin, pinobanksin, vitamin C, catalase and pinocembrin (Lei, *et al*., 2000; Nagai, *et al*., 2006), [9],[10], and flavonoid compounds such as luteolin, quercetin, apigenin, fisetin, kaempferol, isorhamnetin, acacetin, tamarixetin, chrysin, and galangin (Erguder, *et al*., 2008) [4].

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Because of the similarity in efficacy both paliasa and honey in treating heart disease, it is important to produce honey product by feeding supplementary feed that is a mixture of syrup and a decoction of the paliasa leaves to the honey bee *Apis mellifera* L. This product is expected to contain polyphenolic and flavonoids compounds and synergistic properties of honey and paliasa naturally. The purposes of this study were to determine the preference of bees to the supplemantary feed and physico-chemical characteristics of honey produced include color, aroma, taste, pH, viscosity, water content, and the content of total polyphenols and total flavonoids.

2. MATERIALS AND METHODS

Materials used in this study were honeybees Apis mellifera L., paliasa leaves (Kleihovia hospita Linn.), sugar, distillated water. acetic acid, $AlCl_3$, n-butanol, ethanol, and $FeCl_3$.

2.1 Preparation of paliasa leaf decoction and syrup

Paliasa leaves selected were old enough, then washed with water, drained, and cut into small pieces. Paliasa leaf decoction was made with three concentrations, i.e 20% w/v, 40% w/v and 60% w/v in distilled water at 90^oC for 15 minutes. Syrup made from sugar solution with a concentration of 85% w/v.

2.2 Production of honey essence of paliasa

Four honeybee *Apis mellifera* L colonies with a uniform condition, each consists of seven frames bees were placed in a row with equal distance in one room. Each colony was given a different treatment. Before treatments were assigned, the bee colonies were adapted to the room for one week. A bee feeder contained supplementary feed was placed in each bee colony stup next to bee frames. The treatments were: Colony I was given a mixture of distilled water and syrup with a ratio of 2 : 3 (without paliasa). Colony II was given a mixture of paliasa leaf decoction with concentration of 20% w/v and syrup with a ratio of 2 : 3. Colony III was given a mixture of paliasa leaf decoction with concentration of 40 % w/v and syrup with a ratio of 2 : 3. Colony IV was given a mixture of paliasa leaf decoction with concentration of 60% w/v and syrup with a ratio of 2 : 3. Thus, paliasa concentrations in the feed were 0%, 8%, 16%, and 24%. Supplementary feeds with a predetermined weight were performed three times a week for three weeks. Any replacement of supplementary feed, bee preferences to the supplementary feed were measured by weighing the remaining supplementary feed. After three weeks of supplementary feeding, the honey produced by each bee colony was harvested. Furthermore, bees were rested for one week in a way not given supplementary feed but still be able to feed outside. Treatments were repeated in the same way three times. The produced honey was so-called honey essence of paliasa (HEP). The term HEP is given to distinguish the paliasa-honey which are produced from nectar of paliasa flower collected by bees from forests or plantations that are dominated by paliasa plants. Honey produced by the colony I called HEP(A), colony II called HEP(B), colony III called HEP(C), and colony IV called HEP(D).

2.3 Organoleptic determination and physical properties testing

Organoleptic determination consist of color, aroma and taste determination of HEP. HEP color determination was done using the Munsell criteria, while aroma and taste examination were conducted by five volunteers. Physical properties testing of HEP consist of pH, viscosity and water content. The pH measurement was done using a pH meter to a solution of HEP 10% w/w in distilled water (Ahmed, et al., 2007; Ouchemoukh, et al., 2007) [11],[12]. HEP viscosity was determined using a Brookfield viscometer with spindle no. 6 with a speed of 50 rpm. Read numbers were multiplied by the correction factor corresponding to the spindle and rpm used. HEP water content was determined using a refractometer.

2.4 Analysis of phenolic and flavonoid compounds

Qualitative analysis of phenolic and flavonoid compounds made with chemical reagents and thin layer chromatography. Chemical reagents used in the analysis of phenolic compounds are $FeCl_3$. Tested positive outcome in the event of a blue green, dark green, or blue-black color, while for flavonoids done Wilstater method, ie by adding a few drops of concentrated HCl and magnesium powder. Stated positive results in case of red, pink, or yellow. Analysis of phenolic and flavonoid compounds by thin layer chromatography (TLC) was done by diluting the sample HEP and leofilisat paliasa leaf decoction with 96% ethanol, and the supernatant was taken and concentrated, then spotted on TLC plates. Used as a mobile phase mixture of n-butanol: acetic acid: water (4: 1: 3). Spot formed observed under 366 nm UV light, and with 10% $AlCl_3$ reagent spray. Samples that showed positive results in qualitative analysis were followed by quantitative analysis using visible spectrophotometer. Quantitative analysis of total polyphenol compounds were calculated using the Folin-Ciocalteu (Kaskoniene, *et al*., 2009) [13], while the quantitative analysis of total flavonoid compounds were calculated using Chang methods.

3. RESULT AND DISCUSSION

3.1 Preference of bees to the given supplementary feed

Level of preference of bees to the feed was the tendency of bees to consume a certain type of supplementary feed which was measured by the number of supplementary feed consumed by the bees.

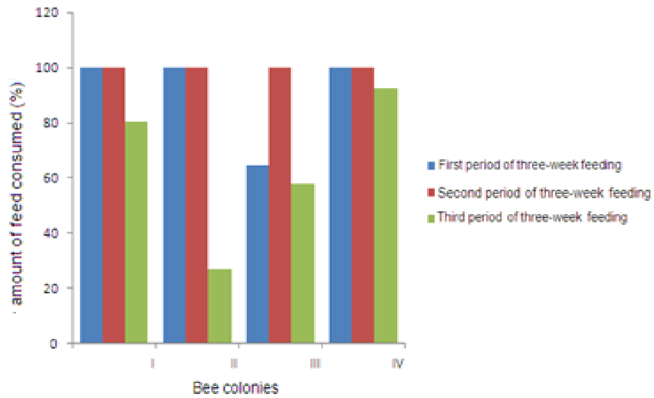

Fig 1. Diagram preference of bees to supplementary feed

Figure 1 shows that in general the bees liked the given feed. This is seen in the first three weeks of treatment, except the colony III who only consumed 64.68% of the supplementary feed given, all the supplementary feed given to the colonies I, II, and IV were consumed. In the second round of three weeks of treatment, all the colonies were able to consume all the feed given. But in the third round of treatment, almost all colonies showed the decreasing of ability to consume feed, except for colony IV. The possibility of these phenomena was because of the effects of weather, temperature and humidity. During this period, the room temperature was becoming decreased and the humidity had been raised to 74% as a result of heavy rain, so the bees were less appetizing to consume feed. Another possibility was the worker bees were dying, because the worker bees age ranged from 20-40 days (Winston, 1987) [14], therefore a lot of bees in the colony looked young and small, so the ability to eat was not optimal.

3.2 Organoleptic determination

HEP colors produced by honey bees can be seen in Figure 2.

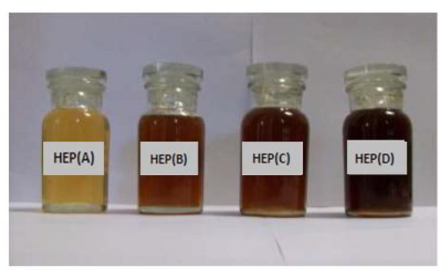

Fig 2. Photo honey essence of paliasa produced by *Apis mellifera* L. fed with the supplementary feed a mixture of syrup and paliasa leaf decoction with various concentration

Table 1. The results of organoleptic determination of honey essence of paliasa

Evaluation	HEP (A)	HEP (B)	HEP (C)	HEP (D)
Color	yellow	yellowish red	red	blackish red
Aroma	honey	less aroma leaves	aroma leaves	clear aroma leaves
Taste	sweet	sweet and sour	sweet and sour	sweet and sour
Average pH (10%b/b)	4.90	5.15	5.08	4.95
Average Viscosity (cps)	2733.3	5233.3	7966.7	11666.7
Average Water content (%)	20.00	21.67	19.51	19.71

Naturally, color, aroma and taste of honey are determined by the source where the honey is obtained. Table 1 showed that HEP had different color, aroma, and taste. HEP color varied from yellow on the HEP (A) to blackish red on the HEP (D). This color variation depends on the composition of the dye contained in the honey. Dyes that make up the color of honey include xantopil, carotene and chlorophyl (Sarwono, 2001) [15]. HEP (A) (without paliasa) had a yellow color, sweet taste with aromas of honey in general, while the HEP produced by bees fed with paliasa supplementary feed had yellowish red to blackish red color, sour sweet taste and aroma of paliasa leaf decoction. Differences in color, aroma and taste of the HEP were influenced by the concentration of the paliasa supplementary feed. This is consistent with that shown in Figure 2, there was a tendency that the higher the concentration of the paliasa supplementary feed given, the darker the color of the HEP. Table 1 also showed that the higher concentration of the paliasa supplementary feed, the closer the aroma of HEP to the aroma of paliasa leaves. Thus, there was a close relationship between the color and the aroma of HEP, the darker the color of HEP, the closer the aroma of HEP to the aroma of paliasa leaves. This fact is consistent with the statement of Sumoprastowo and Suprapto (1993) [16], which says that the honey aroma closely associated with color. The darker the color of honey, the harder or sharper the aroma.

3.3 Physical characteristics of honey essence of paliasa

Table 1 shows that all of HEP were acidic with an average pH ranged from 4.90 to 5.15. According to Ahmed et al. (2007) [11] and Nanda et al. (2003) [17], the acidity of honey is due to the content of organic acids, mainly gluconic acid and inorganic ions of phosphate and chloride. Those types of acids include formic acid, acetic acid, citric acid, lactic acid, butyric acid, oxalic acid, and succinic acid (Bogdanov, 2009) [18]. The average water content of HEP ranged from 19.51 to 21.67%. These results meet the Indonesian National Standard criteria for honey (SNI 01-3545-2004), ie less than 22%. According to Gojmerac (1983) [19] and Nanda *et al*. (2003) [17], the water content of honey is affected by moisture of the surrounding air, the climate and the season. There is a tendency that the higher the concentration paliasa in feed, the more viscous the HEP. The viscosity of honey depends on the composition of honey, especially the water content. In addition, the viscosity is also influenced by temperature. When the honey temperature increase, honey viscosity will decrease.

Chemical characteristics of honey essence of paliasa

The chemical compunds of HEP to be tested were polyphenols, flavonoids, and alkaloids. Flavonoids are a group of polyphenolic compounds found in most plants, including found in leaves. According to Raflizar *et al*. (2006) [1], paliasa leaves contain saponins, cardenolin, bufadienol and anthraquinone, while Philippine Medicinal Plants (2010) [2] mentioned that chemical compounds of paliasa leaves were scopoletin, keampferol, and quercetin which are flavonoid compounds. Taebe (2004) [20] stated that paliasa leaves contained group of alkaloids and flavonoids compounds. The qualitative analysis of phenolic and flavonoid compounds using chemical reagents indicated that HEP(A) gave only positive results for alkaloids, whereas HEP(B), (C), and (D) gave positive results for polyphenols, flavonoids, and alkaloids. TLC chromatogram under UV light 366 nm (Figure 3a) showed the presence of a glowing blue spot for HEP (B), (C), and (D) samples. Flavonoid compounds when observed under 366 nm UV light will result in a dark spot or fluoresce yellowish, greenish, or bluish spots depend on the structure and the types of flavonoids. Observations of chromatogram using spray reagent $AlCl_3$ (Figure 3b) showed a yellow spot for HEP(B), (C), and (D). The yellow color was formed by $AlCl_3$ which binds to the flavonoids in the ortho-hydroxyl group or in the hydroxyl group which bonds to the carbonyl group (Markham, 1982) [21].

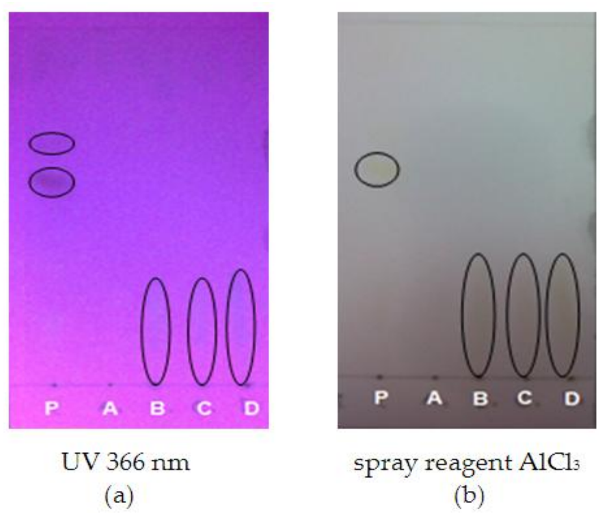

Fig. 3. Photo of thin layer chromatogram of paliasa, HEP(A), (B), (C), and (D) with visualization 366 nm UV light and spray reagent $AlCl_3$

Figure 3 shows that paliasa, HEP(B), (C), and (D) positively contained flavonoids, whereas HEP(A) did not contain flavonoid compounds because it did not show the spot, neither on observation of chromatogram TLC under UV light at 366 nm nor by using a spray reagent $AlCl_3$. These results indicated that there was a flavonoid compound from the paliasa leaves transformed by the bees that produced the honey. The quantitative analysis was only performed on the HEP(B), (C), and (D), because HEP(A) did not contain phenolic compounds and flavonoids. Results of quantitative analysis showed that HEP(B), (C), and (D) had total polyphenol contents which was calculated as gallic acid, respectively 0.301 ± 0.023%, 0.332 ± 0.005%, and 0.359 ± 0.006% and total flavonoid content which was calculated as quercetin, respectively 0.093 ± 0.07%, 0.110 ± 0.003%, and 0.132 ± 0.002%. These results indicated that there was a tendency, the greater paliasa content in the feed given to the bees, the greater total polyphenol contents and total flavonoid contents in HEP. Relationship between the content of paliasa in the feed to total polyphenol and flavonoid contents of HEP produced by honey bees can be seen at Figure 4.

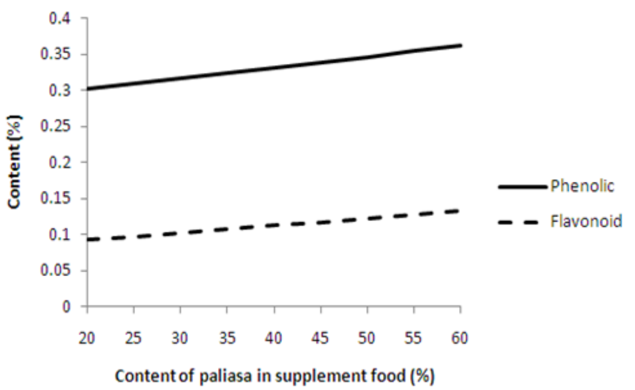

Fig 4. Relationship between the content of the paliasa in the feed to the total polyphenol and total flavonoid contents

Figure 4 explains that the higher the paliasa content in feed given to honey bees, the greater the content of total polyphenols and flavonoids of HEP produced with total polyphenol contents greater than the total flavonoid contents. In other words, the higher the content of total polyphenols in the HEP produced, the higher the total flavonoid contents. This indicates a positive correlation between the total polyphenol contents and the total flavonoid contents.

4. CONCLUSION

The supplementary feed in the form of paliasa leaf decoction with various concentrations mixed with syrup with a ratio of 2: 3 were accepted by the honey *bee Apis mellifera* L. Color and aroma of the resulting HEP did not depend on the amount of feed consumed by the bees but they were influenced by the concentration of the paliasa leaf decoction in supplementary feed. The higher concentration of paliasa leaf decoction, resulting in HEP with the darker color and the sharper aroma of paliasa leaves. All HEP meets the physical characteristic requirements of the Indonesian National Standar (SNI) with the viscosity increased by increasing concentrations of paliasa leaf decoction. The honey produced by bees fed without paliasa in the supplementary feed only gave positive reactions for alkaloids, while the HEP produced by bees fed with paliasa in the supplementary feed gave positive results for alkaloids, polyphenols and flavonoids. The higher paliasa concentration in the supplementary feed, the hihger total polyphenols and total flavonoids in HEP, where the total polyphenol contents was greater than the total flavonoid contents.

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