Characterization of bitter leaf (*Vernonia amygdalina* Delile) extract, lemon (*Citrus limon* Osbeck), and honey in functional beverages making

Melanie Cornelia [1*], *Irene* Agustini[1], and *Marcelia* Sugata[2]

[1]Department of Food Technology, Universitas Pelita Harapan, Tangerang, Indonesia
[2]Department of Biology, Universitas Pelita Harapan, Tangerang, Indonesia

Abstract. Bitter leaves (*Vernonia amygdalina*) are renowned for their health benefits, largely attributed to their polyphenol and flavonoid content, which contribute to their antioxidant properties. Traditionally, these leaves are consumed as a decoction to treat fever. In this study, the antioxidant compounds of bitter leaves were extracted using three different solvents—ethanol, ethyl acetate, and hexane—through maceration for 1, 2, and 3 days. The ethanol extract obtained after 2 days of maceration was selected for further analysis. To mitigate the inherent bitterness of bitter leaves (*Vernonia amygdalina*), complementary flavors and sweeteners such as lemon and honey were incorporated. The objective was to develop a functional beverage that is both palatable and retains antioxidant activity. Combinations of 5, 10, and 15 mL of 0.5% bitter leaf extract were mixed with lemon and honey ratios of 1:1, 1:3, and 1:5. The beverages were analyzed for their antioxidant activity, physicochemical characteristics (pH, color, total titratable acidity, and total soluble solids), and sensory properties through hedonic analysis. The selected formulation was the beverage with a lemon and honey ratio of 1:1 and 10 mL of bitter leaf extract, which exhibited an IC_{50} of 7.91%, total phenolic content of 13.11 mg GAE/100 mL, and total flavonoid content of 2.90 mg QE/100 mL. This study highlights the potential of utilizing local plants like *Vernonia amygdalina* in product development, which can contribute to the diversification of the beverage industry and support the use of indigenous resources.

1 Introduction

Bitter leaves (*Vernonia amygdalina* Delile) are herbs known for their physiological effects and health benefits. Traditionally, bitter leaves are consumed as a decoction [1]. These leaves contain various phytochemical compounds associated with their antioxidant activity. A previous study reported that the antioxidant activity of bitter leaf extracts had an IC50 value of 636.010 μg/mL [2]. However, there is limited research exploring the potential use of bitter leaves in functional beverages, creating a gap in knowledge regarding their broader applications in the food and beverage industry.

Lemon (*Citrus limon* Osbeck), a citrus fruit renowned for its high antioxidant activity, is also valued for its health benefits [3]. Its sour and slightly bitter taste, coupled with refreshing notes derived from flavanones, particularly naringenin, makes it a natural complement to other functional ingredients [4]. Honey, a natural syrup collected by honeybees from flower nectar, offers a sweet taste and numerous health benefits [5]. It serves as a source of carbohydrates and contains phenolics, flavonoids, tocopherols, and ascorbic acid, making it a popular natural sweetener [6].

Antioxidants play a crucial role in inhibiting oxidation by acting as electron donors, neutralizing free radicals in the body [7]. The antioxidant properties of bitter leaves are primarily attributed to their diverse phytochemical compounds [8].

In this study, antioxidant compounds from bitter leaves were extracted using different solvents, and their antioxidant activity was assessed separately. However, despite the known health benefits of bitter leaves, their bitter taste poses a challenge for consumer acceptance in beverage formulations. Therefore, this study also aims to address this challenge by developing a formulation based on antioxidant activity that is also organoleptically acceptable. By incorporating honey and lemon as complementary flavors, this research explores how the bitterness of *Vernonia amygdalina* can be balanced with natural sweetness and refreshing citrus notes. This approach not only promotes the diversification of the beverage industry but also encourages the use of indigenous resources, filling a significant gap in the current body of research on functional beverages.

2 Research Methodology

2.1 Materials and equipment

The main materials used in this study were bitter leaves dark green colored, with a length of 15 to 20 cm, yellow

* Corresponding author: melanie.cornelia@uph.edu

lemon with a diameter of 5 to 8 cm, commercial honey, and distilled water. The solvents used are food grade ethanol, ethyl acetate, and hexane. For the analysis used methanol, DPPH (2,2 diphenyl picrylhydrazyl), Folin Ciocalteu reagent, quercetin, Na2CO3, AlCl3, and gallic acid.

The equipment used in this study were cabinet dryer, dry blender, 35 mesh sieve, rotary evaporator, spectrophotometer, pH meter, chromameter, refractometer, vacuum Buchner, desiccator, furnace, Soxhlet, and Kjeldahl apparatus.

2.2 Procedures

2.2.1 Research Stage I

Research Stage I involved the extraction of bitter leaves using different solvents: ethanol, ethyl acetate, and hexane. The extraction duration was varied at 1, 2, and 3 days. The aim of this stage was to select an extract for Research Stage II, determined by yield and antioxidant activity (IC50). The extracts were also analyzed for moisture content, total phenolic content, and total flavonoid content.

Fresh bitter leaves were harvested, thoroughly washed, and cut into smaller pieces, approximately 2 cm in size. The leaves were dried in a cabinet dryer for 8 hours at a temperature of 40 ºC. The size of the dried bitter leaves was reduced using a dry blender, and the powder was then sieved through a 35-mesh sieve. About 15 grams of bitter leaf powder were weighed and placed into three Erlenmeyer flasks. The contents of each flask were macerated with 150 mL of solvent (ethanol, ethyl acetate, or hexane) for durations of 1 day, 2 days, and 3 days, and the mixtures were subsequently decanted into clean boiling flasks for concentration using a rotary evaporator. The selected extract was obtained, followed by phytochemical screening and toxicity analysis.

2.2.2 Research Stage II

Research stage II focused on selecting the formulation of a functional beverage made from bitter leaf extract, lemon, and honey. The formulation was evaluated based on sensory analysis (both scoring and hedonic methods) and antioxidant activity. Parameters assessed included color, total soluble solid, titratable acidity, pH, total phenolic content, and total flavonoid content.

The preparation of the functional beverage was based on Hardini [9], with modification. Lemon juice was prepared by washing the lemons, cutting them in half, and squeezing the juice using an orange squeezer. The juice was then filtered through a cloth to remove any pulp.

Lemon and honey were mixed with 70% water, using ratios of 1:1, 1:3, and 1:5, for lemon to honey to produce control samples (without extract). The selected bitter leaf extract from Stage I was weighed at 0.5 grams and dissolved in distilled water to a final volume of 100 mL, resulting in a stock solution with a concentration of 0.5%. Approximately 100 mL of each control was then mixed with 0.5% extract in volumes of 5, 10, and 15 mL). The parameters of the selected functional beverage were compared to those of the control and proximate analyses were conducted.

3 RESULTS AND DISUCSSION

3.1 Research Stage I

3.1.1 Drying of bitter leaves

The moisture content of fresh bitter leaves was 87.77%, which was reduced to 8.38% after the drying process. This result is consistent with the findings of Qing et al. [2], who reported a moisture content of 83.080% for *V. amygdalina* leaves. Drying is essential for reducing the water content, which helps inhibit the deterioration process and facilitates extraction. The yield after drying the bitter leaves was 15.89%.

3.1.2 Moisture content of bitter leaf extracts

As shown in Table 1, different solvent and extraction duration affected the moisture content of the extracts ($p<0.05$), and there was a significant interaction between both factors towards moisture content ($p<0.05$). Ethanol and ethyl acetate extracts exhibited lower moisture content compared to hexane. The components extracted by hexane could evaporate due to low boiling point [10]. Notably, ethyl acetate had the lowest moisture content. Ethyl acetate is not soluble in water, whereas ethanol is water-soluble; consequently, the ethanol extract had a higher water content [11]. Additionally, the hexane extract contained lower total solids than the ethanol and ethyl acetate extracts. The extracted components of bitter leaf in hexane may possess volatile properties, which could result in their loss during the moisture content analysis.

Table 1. Moisture content of bitter leaf extracts (%)

Solvent	Duration of Extraction		
	1 day	2 days	3 days
Ethanol	9.93 ± 0.82^a	9.69 ± 0.55^a	9.30 ± 1.18^a
Ethyl acetate	5.12 ± 0.15^a	5.11 ± 0.04^a	5.86 ± 0.99^a
Hexane	20.27 ± 7.24^a	56.30 ± 15.07^b	52.51 ± 7.66^b

Note: different letters in the same column indicate significant differences ($p<0.05$)

3.1.3 Yield of bitter leaf extracts

Different solvent and extraction duration affected the yield of extracts ($p<0.05$) and there was a significant interaction between both factors towards the yield of extraction (Fig. 1). The polarity sequence of the three solvents, from highest to lowest, is as follows: ethanol, ethyl acetate, and hexane. Bitter leaves are known to be more soluble in ethanol [10]. The yield of extraction using ethanol as a solvent showed that the bioactive compounds in bitter leaves, which have a similar polarity to ethanol, were present in higher concentrations compared to those extracted with ethyl acetate and hexane [11].

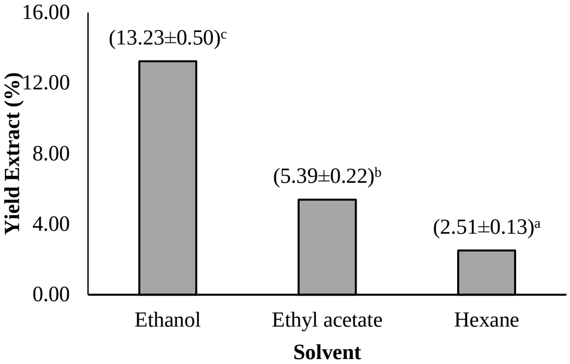

Fig. 1. Yield of bitter leaf extracts

3.1.4 Antioxidant activity of the extracts

Different solvent and extraction duration affected the yield of extracts ($p<0.05$) and there was a significant interaction between both factors towards antioxidant activity (Fig. 2). A lower IC50 value indicates greater antioxidant activity, as a higher extract concentration is required to inhibit free radicals [12]. The IC50 values, ranked from lowest to highest based on solvent type, were as follows: ethanol, hexane, and ethyl acetate. The differences in solvent polarity likely influenced the IC50 values due to the varying bioactive compounds solubilized by each solvent. The soluble bioactive components of bitter leaves in ethanol exhibited better antioxidant activity compared to those extracted with ethyl acetate and hexane [13]. Additionally, bitter leaves contain phenolic and flavonoid compounds that contribute to their antioxidant properties [14].

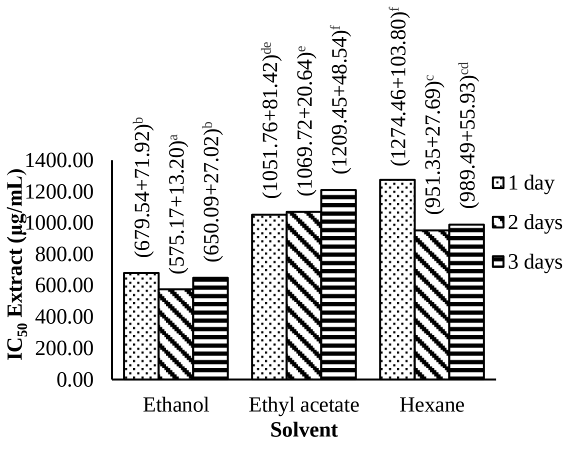

Fig. 2. Antioxidant activity of the bitter leaf extracts

The categorization of antioxidant activity is divided into four groups: very active groups (IC50 value less than 50 ppm), active (IC50 value between 50 and 100 ppm), less active (IC50 value between 100 and 1000 ppm), and not active (IC50 value greater than 1000 ppm) [15]. In this study, the ethanol extract exhibited an IC50 value of 575-679 µg/mL, categorizing it as a less active antioxidant. In contrast, the ethyl acetate and hexane extract generally had IC50 values exceeding 1000 ppm, classifying them as not active. The antioxidant activity of these extracts is primarily attributed to their phenolic compounds [16]

3.1.5 Total phenolic content of the extracts

Different solvent and extraction duration affected the yield of extracts ($p<0.05$) and there was a significant interaction between both factors towards total phenolic content (Fig. 3). The polarity of each solvent varies, resulting in differences in the quantity of compounds extracted. Ethanol extracts a greater concentration of phenolic compounds from bitter leaves compared to ethyl acetate and hexane. Additionally, increasing the duration of extraction enhances the total phenolic content, as the bitter leaf powder has a longer contact time with the solvent. Furthermore, the phenolic compounds extracted may possess different molecular weights depending on the solvent used [17].

3.1.6 Total flavonoid content of the extracts

Different solvent and extraction duration affected the yield of extracts ($p<0.05$) and there was a significant interaction between both factors towards total flavonoid content (Fig. 4). The effect of solvent type on total flavonoids content showed that extraction with ethyl acetate produced the highest total flavonoid levels, followed by ethanol and hexane extracts. The influence of extraction duration on total flavonoid content did not exhibit a consistent trend of increase or decrease. In the case of ethanol, longer extraction times resulted in higher total flavonoid content. Conversely, in ethyl acetate, there was a decrease in total flavonoids on the second day, followed by an increase on the third day. For hexane, the duration of extraction did not significantly affect total flavonoid content. This may be attributed to hexane's volatility, which could lead to a reduction in total flavonoid content due to evaporation and a subsequent decrease in solvent volume [18].

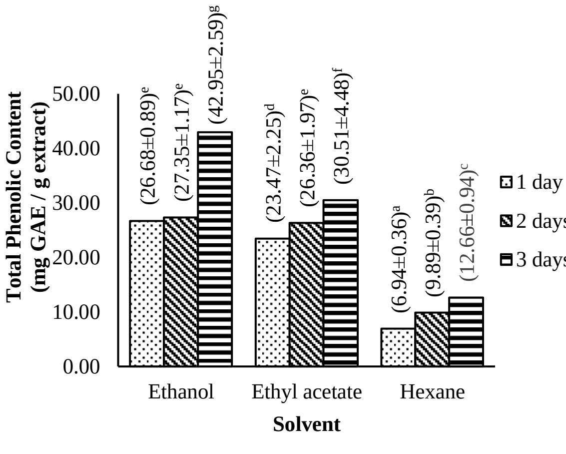

Fig. 3. Total phenolic content of the bitter leaf extracts

3.1.7 The correlation of antioxidant activity, total phenolic content, and total flavonoid content

The correlation between antioxidant activity was inversely related to total phenolic and total flavonoids (Fig. 4).

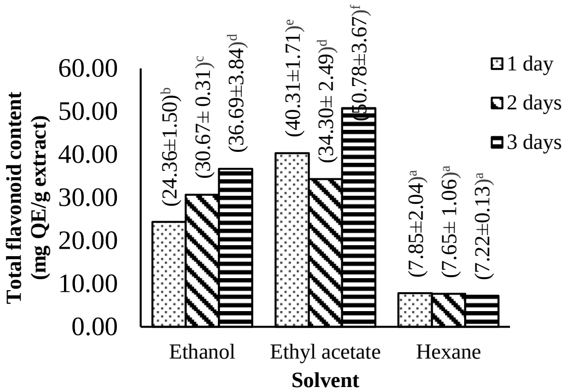

Fig. 4. Total flavonoid content of the bitter leaf extracts

In this research, a higher IC50 value and extended extraction duration might cause lower antioxidant activity. However, the observed inverse correlation reveals a discrepancy: as extraction time increased, both the IC50 value and the total phenolic and flavonoid content also increased. This could suggest that the phenolic compounds and flavonoids in the extract may not function as effective antioxidants, possibly because the free radicals were already inhibited.

The effect of an extended maceration duration may damage the extracted phytochemical components [18]. This damage can be indicated by a decrease in antioxidant activity, as reflected in an increasing IC_{50} value. Based on the highest antioxidant activity and yield, the extract selected was the ethanol extract obtained from maceration for 2 days (Table 2).

Table 2. Analysis result of the selected extract

Parameter	Value	Unit
Yield	13.18	%
Moisture content	9.69	%
IC_{50}	575.17	µg/mL
Total Phenolic	27.35	mg GAE/g extract
Total Flavonoid	32.80	mg QE/g extract

3.1.8 Phytochemical analysis of the selected extract

Table 3 shows the result of qualitative phytochemical, indicating that the selected extract contained phenolics, tannins, flavonoids, saponins, and steroids. Among the terpenoid compounds, sesquiterpene lactones, particularly octahydro-vernodalin, are known to significantly contribute to the bitter taste of bitter leaves [19]. However, this study did not identify such compounds in the bitter leaf extract. In addition to terpenoids, phenolic and flavonoid compounds, including saponins and tannins, also contribute to bitterness [12]. Tannins, specifically catechins and epicatechins, can further enhance the characteristic bitter taste [20]. Given that the bitter leaf extracts in this study contained tannins, it suggests that these extracts may exhibit bitter taste characteristics due to the presence of saponin and tannin.

3.1.9 Toxicity of the selected extract

The toxicity test of bitter leaf extract yielded an LC_{50} (Lethal Concentration) of 365.78 ppm, classifying it as low toxicity, as they fall within the range of 100–1000 ppm [21]. Many toxic compounds in plants often exhibit a bitter taste detectable by humans at micromolar concentrations, serving as a protective mechanism for the plants [12]. The toxicity categories are presented in Table 4.

Table 3. Phytochemical analysis of the selected extract

Compounds	Result
Alkaloid	-
Steroid	+
Terpenoid	-
Flavonoid	+
Saponin	+
Quinone	-
Tannin	+
Coumarin	-
Phenolic	+

Note: + detected, - not detected

Table 4. Toxicity categories

Category	LC_{50} (ppm)
Highly toxic	< 30
Toxic	30 – 100
Low toxic	100 – 1000
Not toxic	> 1000

Source: Juniarti, *et al.* [27]

3.2 Research Stage II

3.2.1 Color analysis

Different volumes of extract affected the °hue analysis of the functional beverage ($p<0.05$), while the ratio of lemon:honey did not show a statistically significant difference (Fig. 5). The hue degree of 0 ° (a *) was red colored, 90 ° (+ b *) yellow, 180 ° (-a *) green, and 270 ° (-b *) blue [22]. The functional beverages of bitter leaf extract generally had yellow color. Fig. 6 shows the increasing honey in the formulation will decrease the °hue, so the beverage color will be less green. Bitter leaf extract had a greenish yellow color thus the addition of bitter leaf extract causes °hue to increase and the color of the beverage was more greenish.

Fig. 7 shows that variation in lemon:honey ratio and extract volume affected the lightness (L*) of the functional beverage ($p<0.05$). However, there was no significant interaction between these two factors. An increased honey ratio in the formulation contributed to a decrease in L*, resulting in a duller and darker color. Lemon juice has a brighter color compared to honey; therefore, as more honey is added to the formulation, the beverage becomes darker. Although both lemon and honey exhibit yellow, lemon possesses higher °hue and

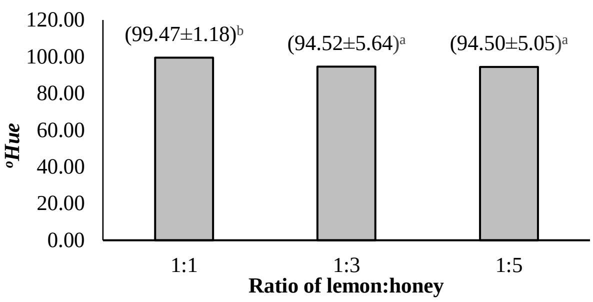

Fig. 5. °hue of the functional beverage

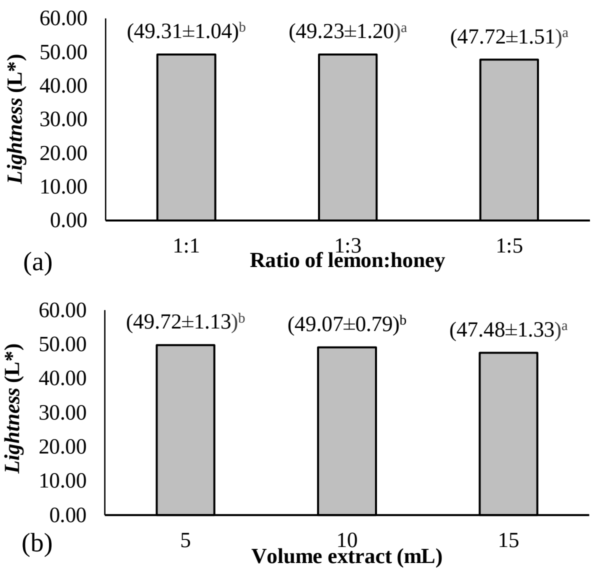

Fig. 6. Effect ratio of lemon:honey (a) and volume extract (b) towards L* of the functional beverage

L* values than honey. Additionally, an increase in the volume of the extract led to a reduction in L*, causing the product to appear darker, as the bitter leaf extract has a lower L* value.

3.2.2 Total soluble solid

Fig. 8 shows that variation in lemon:honey ratio and extract volume affected total soluble solids (TSS) of the functional beverage ($p<0.05$). However, there was no significant interaction between these two factors. An increase in the honey ratio in the formulation resulted in a higher °Brix value of the functional beverage. This is attributed to honey's sugar content, which elevates the total soluble solids. Honey typically has a high °Brix value of approximately 80° [23]. Conversely, the effect of extract volume decreases the total soluble solids. The sucrose content in the extract is significantly lower than that in the functional beverage. As the volume of the extract increases, the total sucrose content in the beverage decreases, leading to a reduction in total soluble solids.

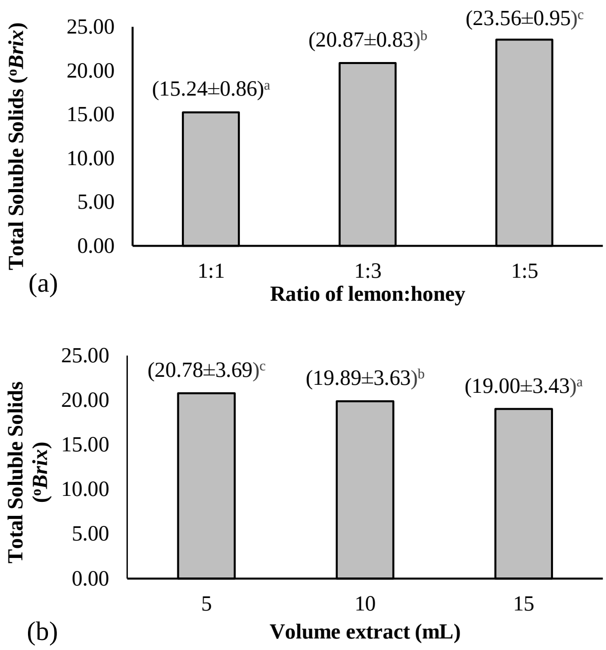

Fig. 7. Effect ratio of lemon:honey (a) and volume extract (b) towards total soluble solids of the functional beverage

3.2.3 Titratable acidity and pH

Fig. 9 showed that variation in lemon:honey ratio and extract volume had significant effects ($p<0.05$) on titratable acidity of the functional beverage, with a significant interaction observed between these two factors. An increased ratio of lemon, specifically at a 1:1 ratio and with a lower volume of extract, resulted in higher titratable acidity. Lemon significantly contributes to titratable acidity due to its organic acids, such as ascorbic and citric acid [24]. Citric acid serves as an acidulant, a chemical compound added to foods to enhance the acidic taste and suppress aftertaste [25].

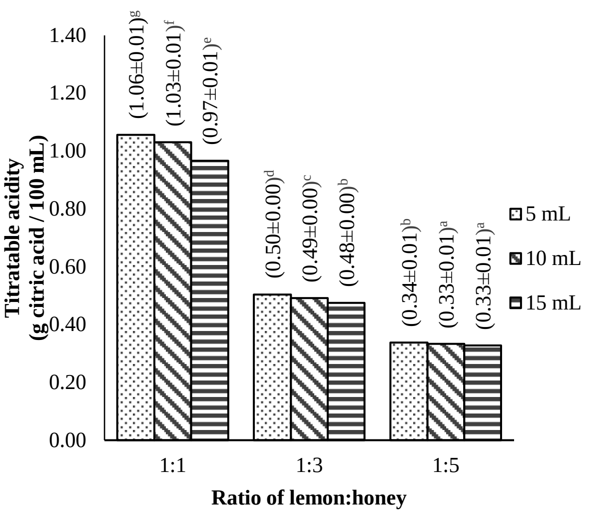

Fig. 8. Titratable acidity of the functional beverages

Fig. 10 shows that variation in lemon:honey ratio and extract volume had significant effects ($p<0.05$) on pH of the functional beverage. However, there was no significant interaction between these two factors. Increasing the amount of lemon juice in the formulation resulted in a lower pH, making the beverage more acidic. The sour taste of this beverage can be attributed to lemon, which has a low pH of approximately 2.4, primarily due to its organic acids [24]. Conversely, a higher volume of extract in the formulation may lead to an increase in pH, as the 0.5% stock solution of the extract is close to neutral. Generally, the pH of functional beverages is acidic.

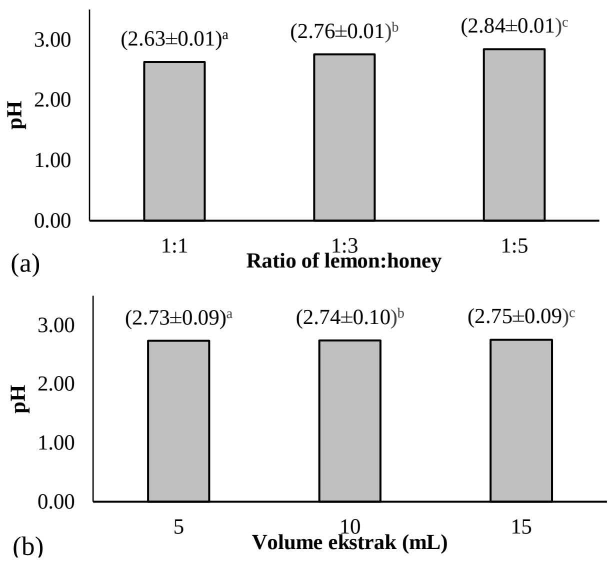

Fig. 9. Effect ratio of lemon:honey (a) and volume extract (b) towards pH of the functional beverage

Titratable acidity and pH are measured to assess the acidity of a sample; however, they are inversely correlated. Titratable acidity refers to the concentration of acids in a medium, while pH indicates the concentration of free hydrogen ions. Organic acids are classified as weak acids, as they partially ionize in water, in contrast to strong acids, which ionize completely. Therefore, pH does not necessarily provide a complete measure of acidity due to the presence of partially ionized organic acids [26].

3.2.4 Antioxidant activity of the functional beverages

Fig. 11 shows that variation in lemon:honey ratio and extract volume had significant effects ($p<0.05$) on antioxidant activity of the functional beverage, with a significant interaction between these two factors. As the honey content and extract volume increased, there was a corresponding decrease in antioxidant activity. The bioactive compounds present in bitter leaf extracts contributed to enhancing the antioxidant activity of the functional beverage, even at very low concentrations. Additionally, lemon juice exhibited a lower IC50 than honey; therefore, a higher amount of lemon in the formulation resulted in a lower IC50 value. Lemons contain phenolic compounds that contribute to the antioxidant activity of functional beverages.

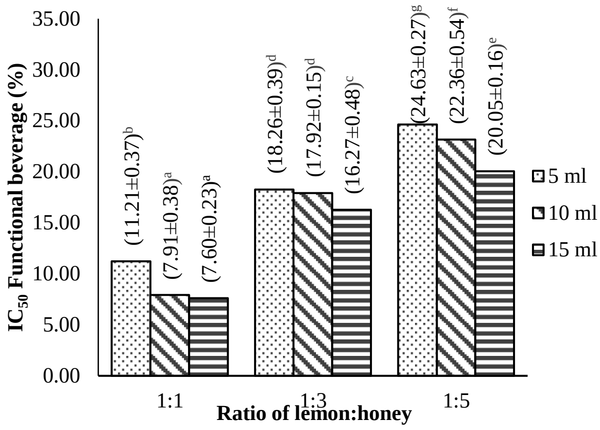

Fig. 10. Antioxidant activity of the functional beverages

3.2.5 Total phenolic content of the functional beverage

Fig. 11 shows variation in lemon:honey ratio and extract volume had significant effects ($p<0.05$) on total phenolic content of the functional beverage, with a significant interaction observed between these two factors. An increase in the amount of lemon in the formulation resulted in a higher total phenolic content in the functional beverage. Additionally, the total phenolic content varies between different types of phenolic compounds found in lemon and honey. There was an inverse correlation between the total phenolic content of the functional beverage and the IC50 value. Phenolic compounds are recognized for their potential as antioxidants [14].

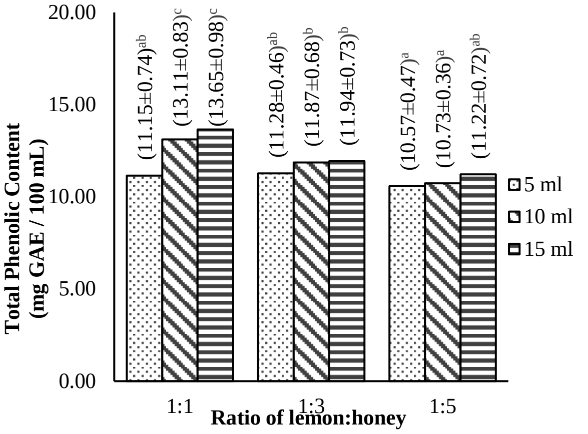

Fig. 11. Total phenolic content of the functional beverages

3.2.6 Total flavonoid content of the functional beverage

Fig.12 shows that variation in lemon:honey ratio and extract volume had significant effects ($p<0.05$) on total phenolic content of the functional beverage, with a significant interaction between these two factors. An increased amount of lemon in the formulation resulted in higher total flavonoid content in the beverage. It is known that lemons contain a greater total flavonoid content compared to honey. Additionally, increasing the extract volume also led to an increase in the total flavonoid content of the product. Flavonoid compounds, which are recognized as antioxidants, are directly correlated with total phenolic content. This relationship exists because flavonoids are a subset of the phenolic group and are known for their antioxidant potential [14].

The IC50 value was inversely correlated with both total phenolic and total flavonoid content. Data indicate that as the amount of lemon and the volume of extracts increased, the IC50 value decreased, signifying enhanced antioxidant activity. Consequently, both total phenolic and flavonoid content also increased.

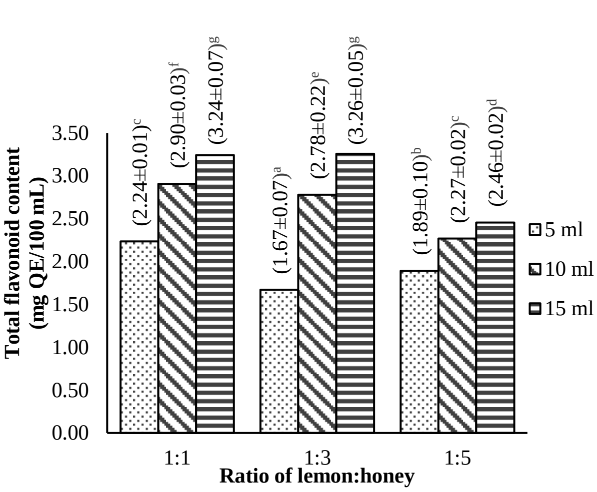

Fig. 12. Total flavonoid content of the functional beverage

3.3 Sensory analysis

3.3.1 Overall

Fig. 13 shows that variation in lemon:honey ratio and extract volume had significant effects ($p<0.05$) on hedonic value of color of the functional beverage. However, there was no significant interaction between these two factors. An increased amount of honey in the formulations enhances the overall preference for the beverage. The higher sweetness level observed in the lemon ratio of 1:5 is associated with both taste hedonic ratings and the °Brix value of the beverage. However, the effect of extract volume on overall hedonic ratings decreased the overall preference. Overall, the beverages were found to be acceptable, with ratings categorized as slightly disliked to neutral.

3.3.2 Selected functional beverage

The selected product was determined by its IC_{50} value, total phenolic content, total flavonoid content, and sensory analysis (scoring and hedonic). The highest antioxidant activity was observed in the formulation with lemon:honey ratio of 1:1 and 15 ml of extract, followed by lemon:honey ratio of 1:1 with 10 ml of extract. These two formulations were not significantly different; thus, the selected beverage was the lemon:honey ratio lemon:honey of 1:1 and 10 mL extract. The total phenolic content and total flavonoid content were inversely correlated to IC_{50} value, supporting the selection of the beverage with a lemon ratio of 1:1 and 10 mL of extract.

The overall hedonic analysis yielded an average of 3.29 on a scale of 1 to 7, indicating a preference near the

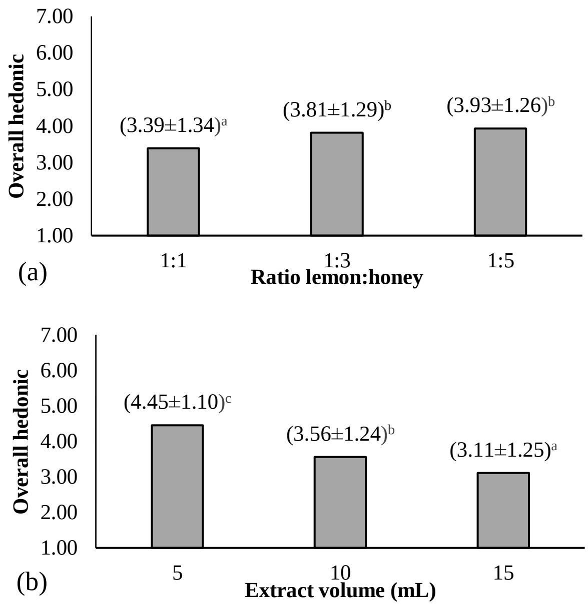

Fig. 13. Overall hedonic value based on ratio of lemon:honey (a) and volume extract (b)

Note: score 1-7 = extremely dislike - extremely like neutral category. The selected functional beverage was then compared to a control formulation with the same lemon:honey ratio of 1:1 but without any addition of extracts.

Table 5 shows that the selected functional beverage was significantly different from the control ($p < 0.05$) in term of °hue, lightness, total soluble solids, titratable acidity, pH, IC_{50}, total phenolic content, and total flavonoid content. The addition of the extract influenced the color analysis, resulting in a higher hue value that indicated a more greenish-yellow color, while the lower L* value suggested a darker yellow compared to the control. This change is attributed to the dark green color of the extract stock solution. Furthermore, the addition of the extract also led to a decrease in °Brix in the selected beverage. It affected titratable acidity as well, resulting in a lower value compared to the control. This may be due to the dilution of the stock solution, which can reduce both °Brix and titratable acidity. Additionally, the inclusion of the extract caused an increase in pH, making the functional beverage more alkaline compared to the control, as the extract stock solution had a higher pH value.

The addition of the extracts led to a decrease in the IC50 value, while also resulting in higher total phenolic and total flavonoid content compared to the control. This may be attributed to the antioxidant activity of the extract stock solution, which contains phenolic and flavonoid compounds. Consequently, this lowered the IC50 value while increasing the total phenolic and flavonoid content.

Table 5. The selected and the control of functional beverage

Parameters	**Unit**	**Value**	
		Selected	**Control**
Hue	°Hue	99.32±0.41^a	96.06±1.50^b
Lightness	L*	49.52±0.39^a	52.05±0.64^b
Total soluble solids	°Brix	15.20±0.69^a	16.63±0.71^b
Titratable acidity	g citric acid /100mL	1.02±0.01^a	1.22±0.01^b
pH		2.62±0.01^a	2.61±0.01^b
IC_{50}	%	7.91±0.38^a	14.03±0.59^b
Phenolic	mg GAE /100mL	13.11±10.83^a	10.46±0.36^b
Flavonoid	mg QE /100mL	2.90±0.03^a	1.55±0.06^b

Note: different letters in the same column indicate significant differences ($p<0.05$)

The proximate analysis of the selected functional beverage is presented in Table 6. The functional beverage, formulated with lemon:honey ratio of 1:1 and 10 mL extract, exhibited a moisture content exceeding 70%, reflecting the substantial amount of water used in the formulation. The protein and fat content in the beverage was less than 1%, likely due to the dilution of the extract in the functional beverage, as both lemon and honey are low in protein and fat [15]. Carbohydrate content was higher than that of the other parameters, primarily contributed by the honey, which serves as a sweetener in the beverage.

Table 6. Proximate analysis of the selected beverage

Parameter	**Results**
Moisture content (%)	84.10
Protein (%)	0.09
Total fat (%)	<0.02
Carbohydrate (%)	15.72
Ash content (%)	0.09

4 Conclusions

The study successfully addressed the impact of solvent polarity and extraction time on the antioxidant activity of bitter leaf extract. The results showed that ethanol, a polar solvent, extracted the highest antioxidant activity compared to ethyl acetate and hexane, with the differing polarities of the solvents influencing the extracted components. Furthermore, the research demonstrated that longer extraction times resulted in a greater quantity of extracted compounds, thereby enhancing the overall antioxidant potential of the extract. By incorporating honey and lemon into the formulation, the study not only mitigated the bitter taste of the leaves but also enhanced the antioxidant properties, making the beverage both organoleptically acceptable and nutritionally beneficial. Based on antioxidant activity analysis and sensory evaluation, the preferred formulation was a 1:1 lemon to honey ratio with 10 mL of a 0.5% extract.

References

1. S.K. Yeap, W.Y. Ho, B.K. Beh, W.S. Liang, H. Ky, A.H.N. Yousr, N.B. Alitheen, W. San Liang, W. Ho, G. Beh, A.H. Yousr, *Vernonia amygdalina*, an ethnoveterinary and ethnomedical used green vegetable with multiple bioactivities. J. Med. Plants Res. **4**(25), 2787-2812 (2010)
2. R.Q. Foo, M. Elumalai, G.A. Akowuah, Antimicrobial and antioxidant studies of *Vernonia amygdalina*. J. App. Pharm. **6**(4), 360-371 (2014). https://doi.org/10.21065/19204159.6.4.332
3. T. Turner, B.J. Burri, Potential nutritional benefits of current citrus consumption. Agric. **3**(1), 170-187 (2013). https://doi.org/10.3390/agriculture3010170
4. Y.Q. Liu, E. Heying, S.A. Tanumihardjo, History, global distribution, and nutritional importance of citrus fruits. Compr. Rev. Food Sci. Food Saf. **11**, 531-545 (2012). https://doi.org/10.1111/j.1541-4337.2012.00201.x
5. S.R. Utami, Kajian perbandingan sari daun jambu biji dengan sari salak bongkok dan penambahan madu pada produk minuman fungsional, Bachelor Thesis, Universitas Pasundan, Indonesia, 2017
6. E. Intar, P.S. Widyawati, D.W. Budianta, Pengaruh penambahan madu terhadap aktivitas antioksidan minuman beluntas-teh hitam dengan perbandingan 25:75% (b/b), Jurnal Teknologi Pangan dan Gizi. **15**(1), 13-18 (2016)
7. Z.M. Xu, L.R. Howard, Analysis of antioxidant-rich phytochemicals (Blackwell Publishing, UK 2012)
8. U. Usonubun, N.P. Okolie, Phytochemical, trace and mineral composition of *Vernonia amygdalina* leaves. J. Biol. Pharm. Res. **6**(5), 393-399 (2015)
9. Hardini, Aktivitas antibakteri minuman kulit kayu manis (*Cinnamomum burmanii* Blume) dengan penambahan jeruk lemon (*Citrus limon* Osbeck) dan madu, Bachelor Thesis, Universitas Pelita Harapan, Indonesia, 2017.
10. S.O. Isehunwa, E.B. Olanisebe, O.O. Ajiboye, S.A. Akintola, Estimation of the refractive indices of some binary mixtures. Afr. J. Pure Appl. Chem. **9**(4), 58-64 (2015). https://doi.org/10.5897/AJPAC2015.0613
11. Q.D. Do, A.E. Angkawijaya, P.L. Tran-Nguyen, L.H. Huynh, F.E. Soetardjo, S. Ismadji, Y.H. Ju, Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. J. Food Drug Anal. **22**(3), 296-302 (2014). https://doi.org/10.1016/j.jfda.2013.11.001
12. A. Drewnoski, C. Gomez-Carneros, Bitter taste, phytonutrients, and the consumer: a review. Am. J. Clin. Nutr. **72**(6), 1424–1435 (2000). https://doi.org/10.1093/ajcn/72.6.1424
13. U.B. Ekaluo, E.V. Ikpeme, E.E. Ekerette, C.I. Chukwu, In vitro antioxidant and free radical activity of some Nigerian medicinal plants: Bitter leaf (*Vernonia amygdalina* L.) and guava (*Psidium guajava* Del.). Res. J. Med. Plant. **9**(5), 215-226 (2015). https://doi.org/10.3923/rjmp.2015.215.226
14. O.A. Adesanoye, E.O. Farombi, In vitro antioxidant properties of methanolic leaf extract *Vernonia amygdalina* Del. Niger. J. Physiol. Sci. **29**, 91-101 (2014)
15. Prasetyorini, Moerfiah, S. Wardatun, Z. Rusli, Potensi antioksidan berbagai sediaan buah sirsak [*Anonna muricata* Linn]. Penel Gizi Makan **37**(2), 137-144 (2014)
16. S. Ariviani, R.H.R. Parnanto, Kapasitas antioksidan buah salak (*Salacca edulis* Reinw) kultivar pondoh, nglumut dan bali serta korelasinya dengan kadar fenolik total dan vitamin C. Agritech. **33**(3), 324-333 (2013). https://doi.org/10.22146/agritech.9555
17. W.L.F. Armarego, Purification of laboratory chemicals, 8th ed. (Butterworth-Heinemann, UK, 2017). https://doi.org/10.1016/B978-0-12-805457-4.50003-3
18. N. Kemit, I.W.R. Widarta, K.A Nocianitri, Pengaruh jenis pelarut dan waktu maserasi terhadap kandungan senyawa flavonoid dan aktivitas antioksidan ekstrak daun alpukat (*Persea Americana* Mil). Jurnal Ilmu dan Teknologi Pangan. **5**(2), 130-141 (2016)
19. N.J. Toyang, R. Verpoorte, A review of the medicinal potentials of plants of the genus *Vernonia* (Asteraceae). J. Ethnopharmocol. **146**(3), 681-723 (2013). https://doi.org/10.1016/j.jep.2013.01.040
20. J.L. Puech, A. Prida, S. Isz, Quality assessment of oenological tannins utilising global selectivity chemical sensors array ("Electronic Tongue"). S. Afr. J. Enol. Vitic. **28**(2), 101-106 (2007). https://doi.org/10.21548/28-2-1465
21. M.I. Iwo, S.L. Sjahlim, S.F. Rahmawati, Effect of *Vernonia amygdalina* Del. leaf ethanolic extract on intoxicated male Wistar rats' liver. Sci. Pharm. **85**(16), 1-7 (2017). https://doi.org/10.3390/scipharm85020016
22. S. Sahin, S.G. Sumnu, Physical Properties of Foods (Springer, USA, 2006)
23. E. Attard, A.B. Douglas, Physicochemical characterization of Maltese honey. In V.de A.A. de Toledo, Honey Analysis (IntechOpen, UK, 2017) http://dx.doi.org/10.5772/66330

24. M.A. Patil, P.A. Ubale, S.S. Karhale, V.B. Helavi, Lemon juice: An environmentally benign catalyst for synthesis of benzothiazoles, and benzoxazole derivatives in aqueous medium. Der Chemica Sinica **8**(1), 198-205 (2017)
25. C. Saragih, N. Herawati, R. Efendi, Pembuatan sirup ubi jalar ungu (*Ipomea batatas* L.) dengan penambahan sari lemon (*Citrus limon* L.). Jom Faperta Ur. **4**(1), 1-15 (2017)
26. S. Nielsen, Food analysis. 5th ed. (Springer, New York, 2017). https://doi.org/10.1007/978-3-319-45776-5
27. Juniarti, Osmeli, D., dan Yuhernita, Kandungan senyawa kimia, uji toksisitas (Brine Shrimp Lethality Test) dan antioksidan (1,1-diphenyl-2-*pikrilhydrazyl*) dari ekstrak daun saga (*Abrus* precatorius L.). Jurnal Makara Sains **13**(1), 50-54 (2009). https://doi.org/10.7454/mss.v13i1.378