Herbal tea of yellow bitter charm (Eurycoma longifolia Jack.) leaves and its potential analysis for commercial herbs drink

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Abstract. Yellow Bitter Charm (YBC) has been widely known as medicinal plants in Dipterocarps forest ecosystem. Common utilization of YBC is roots and stems, causing problems with its sustainability. In this study, some active compounds contained in YBC leave were identified due to antioxidant of YBC leaf is higher than root and stem. Thus, efficacious diversification products from it leaves as herbal tea was introduced. YBC leaves were taken from Samboja (East Kalimantan), Kotawaringin Timur and Katingan (Central Kalimantan). Chemical compounds showed that YBC leaves contained some important compounds, namely: canthin (anti-cancer), ethanol (aphrodisiac), anti-malaria (eurycomanone), and anti-HIV (quainoid), while other active compounds were saponins, tannins and alkaloids are very good for improving blood circulation. Herbal tea products from YBC leaves are made into 3 flavors, namely: original, jasmine and lime aroma. Organoleptic tests of these products including color, aroma and taste were tested on 58 respondents from different age classes showed that teenager respondents preferred herbal tea with lime aroma, adult respondents preferred jasmine aroma, while elderly respondent was very fond of original due to efficacy considerations. Antioxidant tests on these products proved that aroma additional might cause a decrease in antioxidant activity.

1. Introduction
Yellow Bitter Charm (Eurycoma longifolia Jack.) belongs to the family Simaroubaceae which is known as Pasak Bumi is commonly found in Asian countries such as Malaysia, Indonesia, Thailand, Myanmar, and Cambodia. This plant is famously known for its various pharmacological activities [1]. This plant is also used traditionally by local folks for the treatment of other ailments such as bleeding, cough, fever, antimalarial treatment, ulcer, high blood pressure, fevers, etc. and all these unique activities performed by E. longifolia is as a result of the presence of very important bioactive compounds known as quassinoid [2,3]. However, those bioactive compounds of this plant are mostly contributed by the root part and stem.

E. longifolia may have sufficient evidence that elicit benefits on endurance performance and physiological responses in high dosage and longer supplementation on herbal beverages [4]. Herbal tea is a functional drink derived from natural ingredients such as flowers, leaves, seeds, roots, or dried fruit and contains natural antioxidants that can keep as anti-body from free radical attacks. Herbal products in tea bag packaging were chosen due to its more effective and efficient, and were more easily accepted by consumers.
Considering the potential and benefits of pasak bumi that have been widely proven, it is necessary to innovate the development of diversified products as well as testing so that the product is worth selling. For this reason, a diversification of YBC products in the form of herbal teabags was introduced, which utilize leaf part for its sustainability consideration in nature.

2. Material and Methods

2.1. Organoleptic test and YBC herbal tea making process

Based on observing, touching, and sniffing senses, the organoleptic analysis was carried out by a human panel [5]. Organic evaluation of herbal as drugs by means of the organ of sense (skin, eye, tongue, nose, and ear) or macroscopic evaluation of herbal drugs by color, odor, taste, size, shape, and special feature, like touch, texture, etc [6]. In this study, organoleptic analysis were conducted by comparing three kinds of YBC herbal tea as follows: 1) leaf powder without aroma ingredients (as a control), 2) leaf powder + lime leaf aroma ingredients, and 3) leaf powder + jasmine flower aroma ingredients.

The ingredients used for making herbal tea was included YBC leaves, lime leaves and jasmine flowers. Fresh YBC leaves dried at room temperature (+ 25°C) for 2 weeks. Then it was advance dried in the oven at a temperature of 50°C for 24 hours. Lime leaves and jasmine flowers are also dried in the oven at 50°C for 6-7 hours. YBC leaves, lime leaves and jasmine flowers are mashed with a blender to become a coarse powder. The composition of YBC leaf powder used as much as 0.5g and aroma ingredients (lime leaves or jasmine flowers) as much as 0.2g. The organoleptic test of herbal tea was conducted on 58 respondents including 28 teenagers (ages 14-17 years), 9 adults (ages 20-24 years), 21 elderly (ages 41-70 years) to assess the color, aroma and taste of these products. The respondents were men or women who were taken randomly and were willing to test the preference for organoleptics. Assessment of sensory use and preference level scales were carried out from numbers 1-5, namely: 1 = dislike; 2 = neutral; 3 = rather like; 4 = likes and 5 = very like. After each respondent tasted 3 tea products offered (control, orange aroma and jasmine aroma), then asked to rank the preferred choice (1 = the most preferred choice among the other / most preferred tea product choices, 2 = the second choice of tea products preferred, 3 = the choice of the three preferred tea products). The YBC herbal tea products most favored by respondents can be seen in the highest number of choices.

2.2. Antioxidant assay

Three YBC herbal tea products (control, orange aroma and jasmine aroma) were tested for antioxidants. 2g of YBC leaf powder is brewed with 200 ml of hot water for 15 minutes and left to cool. The same process is also done for YBC herbal tea products with the aroma of oranges and jasmine, only by adding orange leaves or jasmine flowers as much as 0.5g. The sample was put into cuvette as much as 33 μl, added 467 μl ethanol, and 500 μl DPPH. Mixing is sufficient if the sample volume has reached 1000 μl (1 ml). Samples were incubated for 20 minutes in indoor with minimum light. Antioxidant Activity (AA) was determined by decolorization of DPPH with a wavelength of 514 nm using a spectrophotometer. The scavenging activity was calculated as a percentage of DPPH decoloration relative to a negative control using the following equation [7]:

Free-radical scavenging activity (%) = ((Control-Sample)/Control) x 100

2.3. Phytochemical

Extracts were tested for the presence of active principles such as flavonoids, saponins, steroids, tannins, terpenoids, alkaloids and carbohydrate by using some following standard procedures [8,9].

Alkaloids Determination: 5ml ethanolic extract was reacted with 2 drops Potassium bismuth iodide solution reagents in test-tubes. Development of creamy and an orange color respectively indicated a positive result.

Flavonoids Determination: About 1 ml of ethanolic extract was shaken with 1 ml of dilute
ammonia solution. The layers were allowed to separate and the yellow color in the ammonical layer (bottom layer) indicates the presence of flavonoids.

**Saponins Determination:** 5 ml of the filtrate was diluted with 20 ml of water and shaken vigorously (15 minutes). A stable froth (foam) up on standing indicates the presence of saponins.

**Tannins Determination:** Test solution (5 ml ethanolic extract) with sodium hydroxide solution (1%) gives yellow to red precipitate within short time indicates the presence of tannins.

**Steroids Determination:** 1 ml of ethanolic extract of each sample is boiled with 10 ml chloroform, cooled, 1 to 2 drops of concentrated sulfuric acid were added slowly through the wall of the tube. Shake well and allow standing for some time, red color appears at the lower layer indicates the presence of Steroids.

**Triterpenoid Determination:** 1 ml of ethanolic extract of each sample is boiled with 10 ml chloroform, cooled, 1 to 2 drops of concentrated sulfuric acid were added slowly through the wall of the tube. Shake well and allow standing for some time, reddish-purple color appears at the lower layer indicates the presence of Triterpenoids.

**Carbohydrate Determination:** Extract hydrolyzed with HCl in the water heater. Then, it was added with 1 ml of pyridine and a few drops of a solution of sodium nitroprusside into the hydrolyzate, after it was etched with an alkaline solution of sodium hydroxide. The formation of a pink to red color indicates the presence of glycosides.

**Carotenoids:** 1 ml of extract is mixed with 5 ml of chloroform in a test tube, then shaken then filtered and then added 85% sulfuric acid. If blue or green is formed above the surface, it shows the presence of carotenoids.

**Kumarine:** as much as 1 ml of extract is mixed with a few drops of NaOH and then added alcohol if the yellow color is formed, indicating the presence of coumarin.

2.4. **GC-MS analysis**

GC-MS testing procedure followed [10]. Gas Chromatography combined with mass spectrometry (GC–MS) was used for identification of component [9]. 1 mg/mL (μL) of yellow bitter charm leaf extracted with ethanol solvent was taken to be analyzed using GC-MS equipment’s by Shimadzu QP 2010: RTX – column type is 5 ms, Restek Corp (30 m length). The injector and detector temperatures were both maintained at 250°C, while operation temperature at 50-300°C. The column temperature was programmed at 50-120°C, with 4°C increase per min which was maintained for 1 min. Then it was programmed at 120-300°C, with 6°C increase per min and held on for 5 min, with retention time (Rt) totaled 60 min. Helium was used as a carrier gas is 50-500 atomic mass unit (AMU). The identification of the compounds and structure determination were based on the comparison of mass spectra and their fragmentation profiles using published data, Wiley, NIST (National Institute of Standards Technology) data base library.

3. **Results and discussions**

3.1. **Organoleptic of YBC herbal tea**

Owing to medicinal properties attributed to an herb, it is necessary to maintain its quality and purity in the commercial market [11]. In order to maintain quality, purity, potency, safety, and efficacy of herbal drugs (product of medicinal plants) needs sensory/organoleptic analysis as consumer acceptance [11, 12]. The organoleptic test results of YBC tea are presented in Tables 1, 2 and 3. Based on Table 1, it was shown that teenagers assess YBC tea original (control) for color (rather like), aroma (neutral) and taste (dislike); for YBC tea with lime aroma for color (rather like), aroma (rather like) and taste (dislike); and YBC tea with jasmine aroma for color (rather like – like), aroma (neutral) and taste (rather like). Generally, teenagers, respondents have shown rather like for color, aroma, and taste of YBC tea for all variants due to a bitter perception. The most preferred choice for them was YBC with jasmine aroma. YBC with jasmine aroma has a less bitter taste compared to both of YBC original and YBC with lime aroma. Another study showed that the addition of natural substance in some
beverages tends to improved organoleptic test results. As an example, the use of Mentha arvensis extract on whet-based pineapple mint beverages can improve the color, taste, appearance and acceptability of the respondents [13].

Table 1. Organoleptic test of YBC tea of teenagers respondents.

| Respondent | Tea variant | Score | Color | Aroma | Taste | Σchoice 1 |
|------------|-------------|-------|-------|-------|-------|----------|
| 28 teenagers | K           | 1     | 6     | 4     | 9     | 3        |
|             | 2           | 8     | 14    |       | 8     |          |
|             | 3           | 10    | 6     | 5     |       |          |
|             | 4           | 4     | 4     | 4     |       |          |
|             | 5           | 0     | 0     | 2     |       |          |
|             | J           | 1     | 5     | 4     | 11    | 7        |
|             | 2           | 8     | 6     | 8     |       |          |
|             | 3           | 10    | 11    | 6     |       |          |
|             | 4           | 5     | 4     | 2     |       |          |
|             | 5           | 0     | 3     | 1     |       |          |
|             | M           | 1     | 6     | 1     | 2     | 8        |
|             | 2           | 6     | 13    | 9     |       |          |
|             | 3           | 8     | 7     | 11    |       |          |
|             | 4           | 8     | 5     | 3     |       |          |
|             | 5           | 0     | 2     | 3     |       |          |

Remarks: 1 = dislike; 2 = neutral; 3 = rather like; 4 = likes and 5 = very like,
K = YBC tea original (control), J = YBC tea with lime aroma, M = YBC tea with jasmine aroma

While, organoleptic test of adult respondents acceptance shown in Table 2. It can be shown they like YBC with lime aroma. The addition of lime leaves to YBC tea gives a more concentrated color, a refreshing aroma, and a more bitter taste compared to the control. Substances or components that affect the color of steeping tea are usually from flavonoids and tannins [14]. From the results of phytochemicals on YBC leaf extract for water and ethanol solvents containing flavanoids and tannins. The refreshing aroma of lime leaves can treat the bitter taste that is in YBC tea. The addition of 40% lemon zest can mask the unpleasant aroma of dragon fruit rind teabag [15]. Kaffir lime leaves are efficacious as stimulants, fresheners, and aromatherapy. Lime is one of the food additives that functions as an acid or neutralizer and is beneficial to the body because it contains limonene, lanolin acetate, geranly acetate.

Many elderly respondents were chosen tea variant which is they like most, directly. Thus, the assessment of color, aroma, and taste were ignored (Table 3). They preferred YBC tea original comparing than the others. They assume that bitter taste more important and more valuable benefits of herbal tea.
Table 2. Organoleptic test of YBC tea of adult respondents.

| Respondent | Tea variant | Score | Color | Aroma | Taste | Σchoice 1 |
|------------|-------------|-------|-------|-------|-------|-----------|
| 9 adults   | K           | 1     | 0     | 0     | 4     | 1         |
|            | 2           | 0     | 1     |       | 2     |           |
|            | 3           | 0     | 5     |       | 3     |           |
|            | 4           | 6     | 2     |       | 3     |           |
|            | 5           | 3     | 1     |       | 0     |           |
| J          | 1           | 0     | 0     |       | 4     | 5         |
|            | 2           | 0     | 0     |       | 2     |           |
|            | 3           | 3     | 1     |       | 2     |           |
|            | 4           | 4     | 4     |       | 1     |           |
|            | 5           | 2     | 4     |       | 0     |           |
| M          | 1           | 0     | 0     |       | 2     | 2         |
|            | 2           | 1     | 2     |       | 4     |           |
|            | 3           | 5     | 3     |       | 1     |           |
|            | 4           | 2     | 2     |       | 2     |           |
|            | 5           | 1     | 1     |       | 0     |           |

Remarks: 1 = dislike; 2 = neutral; 3 = rather like; 4 = likes and 5 = very like
K = YBC tea original (control), J = YBC tea with lime aroma, M = YBC tea with jasmine aroma

Table 3. Organoleptic test of YBC tea of elderly respondents.

| Respondent | Tea variant | Score | Color | Aroma | Taste | Σchoice 1 |
|------------|-------------|-------|-------|-------|-------|-----------|
| 21 elderly | K           | 1     | 1     | 0     | 3     | 6         |
|            | 2           | 1     | 3     |       | 3     |           |
|            | 3           | 4     | 3     |       | 3     |           |
|            | 4           | 0     | 1     |       | 3     |           |
|            | 5           | 0     | 0     |       | 1     |           |
| J          | 1           | 1     | 2     |       | 2     | 5         |
|            | 2           | 1     | 1     |       | 1     |           |
|            | 3           | 2     | 1     |       | 6     |           |
|            | 4           | 4     | 4     |       | 5     |           |
|            | 5           | 1     | 3     |       | 1     |           |
| M          | 1           | 1     | 1     |       | 4     | 3         |
|            | 2           | 2     | 2     |       | 1     |           |
|            | 3           | 0     | 2     |       | 3     |           |
|            | 4           | 4     | 4     |       | 5     |           |
|            | 5           | 0     | 0     |       | 0     |           |

Remarks: 1 = dislike; 2 = neutral; 3 = rather like; 4 = likes and 5 = very like
K = YBC tea original (control), J = YBC tea with lime aroma, M = YBC tea with jasmine aroma
3.2. Antioxidant assay of YBC herbal tea
Antioxidants play a major role in helping to protect our body from the formation of free radicals and prevent or delay the occurrence of lipid peroxidation [16]. Its have been able to destroy a single oxygen molecule and neutralize chemically active products of metabolism to protecting oxidative damage to cells, which cause several diseases such as cancer, aging, and diabetes [17, 18]. Antioxidants of YBC herbal tea without additional aroma ingredients and with additional aroma ingredients are presented in Table 4. Antioxidants of YBC herbal tea original (control) showed the highest antioxidants compared to YBC with lime aroma and YBC with jasmine aroma. The high value of antioxidant in YBC leaves tea predicted can improve immune for the inhibitory action of cancer cells. Almost 47% of anti-cancer drugs come from a natural product [19]. While; additional lime and jasmine flowers tend to decrease in antioxidant activity of YBC tea. Antioxidant activity of dragon fruit rind tea was decreased with additional lemon peel. Antioxidant activity without additional of lemon peel 48.02%, but antioxidant activity with the addition of lemon peel (as much as 15%, 27.5%, and 40%) reduced to 45.32%, 44.43%, and 42.57%. YBC leaf extract contained very strong antioxidants for ethanol solvent extract with IC50 values <50 ppm and moderate antioxidants for water solvent extract with IC50 value of 85 ppm.

Tabel 4. Antioxidant of YBC herbal tea.

| Sample (treatment)       | Reducing antioxidant capacity of DPPH (%) |
|--------------------------|------------------------------------------|
| YBC leaf (control)       | 42.35                                    |
| YBC with lime leaf       | 29.82                                    |
| YBC with jasmine flower  | 30.61                                    |

3.3. Phytochemical of YBC
The result showed that the qualitative screening of phytochemical compounds in YBC leaves ethanolic and water extracts revealed the presence of flavonoids, tannins, triterpenoid, carotenoid, kumarin, carbohydrate, and saponin (Table 5). Presence of alkaloids in Agarwood leaves extracts was of great justify for the plant as some pharmaceutical uses, such as antimalaria, analgesic, antispasmodic, bactericidal and others [20]. Further, present trends towards technologies and processes that increase the use of residues make carbohydrate (starchy) vegetal biomass an important alternative material in various applications. Starch is used as an excipient, a type of bonding agent to active drugs in the pharmaceutical industry [21].

Figure 1. YBC herbal tea (A = control, B = lime aroma, C = jasmine aroma).
Table 5. Summary of the phytochemical screening of YBC leaves by maceration and hot water extractions.

| Phytochemicals | Ethyl acetate | Ethanol | Water |
|----------------|---------------|---------|-------|
| Alkaloid       | -             | -       | -     |
| Flavonoid      | -             | +       | +     |
| Tannin         | -             | +       | ++    |
| Triterpen      | +++           | ++      | +     |
| Steroid        | -             | -       | -     |
| Carotenoid     | -             | +       | -     |
| Kumarin        | +             | +       | +     |
| Carbohydrate   | +++           | +++     | +++   |
| Saponin        | -             | -       | ++    |

Remarks: - : Absent; +: Slightly present; ++: Significant; +++: Very significant.
The classification was based on the extent of reaction during the quantitative study.

3.4. GC-MS

GC-MS test results extracts of single maceration of ethanol solvent on YBC leaves can be seen in Figure 2. Chemical content identified in ethanol solvent extract from YBC leaves as many as 100 chemical components. The highest chemical content in YBC leaf ethanol extract is 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- with an area percentage of 6.78% (Table 6).

![Figure 2. Results of GC-MS analysis of YBC leaf with ethanol maceration (X axes for area, Y axes for retention times).](image)

The GC-MS test results suspected that the ethanol leaf extract of YBC contains 3 (three) major compounds, ie 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (6.78%), 2-Cyclohexene-1-one, 4-hydroxy-3,5,6-trimethyl-4-(3-oxo-1-butenyl)- (6.46%), and Acetic acid, 2-(2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)-propenyl ester (5.61%). 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- has biological activity as anti-microbial, anti-inflammatory, and strong antioxidant capacity properties in glucose-histidine Maillard reaction products. It remains an open question in which way the Maillard reaction might be controlled during food technological processes to increase the oxidative stability of resulting products [22, 23]. In accordance with the previous findings, most of the identified compounds from this study has also been reported elsewhere in other species. 2-Cyclohexene-1-one, 4-hydroxy-3,5,6-trimethyl-4-(3-oxo-1-butenyl)-, and Acetic acid, 2-(2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)-propenyl ester have been reported to possess various biological activities such as anti-microbial, anti-cancer, anti-mutagenic, anti-peptic, anti-septic, anti-spasmodic, anti-adrenogenic, and hypocholesterolemic activities as summarized in Table 6.
Table 6. Chemical compound in YBC leaves extract based on GC-MS test result.

| No | Retention time | Area (%) | Chemical Compound | Molecular weigh (g/mol) | Molecular formula |
|----|----------------|----------|-------------------|------------------------|-------------------|
| 1  | 13.45          | 6.78     | 4H-Pyrane-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- | 144                  | C₆H₉O₄          |
| 2  | 30.72          | 6.46     | 2-Cyclohex-1-one, 4-hydroxy-3,5,6-trimethyl-4-(3-oxo-1-butyl)- | 222                  | C₁₃H₁₈O₃        |
| 3  | 30.26          | 5.61     | Acetic acid, 2-(2,2,6-trimethyl-7-oxa-bicyclo[4.1.0]hept-1-yl)-propenyl ester | 238                  | C₁₄H₂₂O₃        |
| 4  | 58.72          | 2.59     | 3-Ethoxy-1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane | 340                  | C₁₁H₃₂O₄Si₄     |
| 5  | 7.24           | 2.53     | Cyclopentane-1,2-diol | 102                  | C₅H₁₀O₂         |
| 6  | 59.33          | 1.68     | Pentasiloxane, 1,3,3,5,5,7,7,9,9-decamethyl- | 356                  | C₁₀H₃₂O₄Si₅     |
| 7  | 58.42          | 1.65     | Benzeneacetic acid, 3-methoxy-4-[(trimethylsilyl)oxy]-, ethyl ester | 282                  | C₁₄H₂₂O₄Si₅     |
| 8  | 7.94           | 1.32     | 1-Octene, 3-ethyl- | 140                  | C₁₀H₂₀          |
| 9  | 28.52          | 1.28     | Photocitral a      | 152                  | C₁₀H₁₆O        |
| 10 | 57.68          | 1.28     | Benzothiophene-3(2H)-one, 2-(3-nitrobenzyldeno) | 283                  | C₁₅H₉NO₃S      |

4. Conclusions

Generally, YBC tea with aroma addition is preferred by respondents both teenager (YBC with jasmine aroma) and adult (YBC with lime aroma). Meanwhile, the natural bitter impression of YBC tea (original) is more beneficial for most elderly respondents. The addition of other natural ingredients to YBC tea products makes a unique impression and taste, besides to enriching the taste as well also to improve its benefits. Enthusiastic respondents indicated that even the diversification of YBC tea products could be produced and accepted as a nutritious herbal beverage that has expended commercially.

A recent study identified that all variants of YBC leaves tea have antioxidants contained. Antioxidants of YBC herbal tea original (original) showed the highest antioxidants compared to YBC with lime aroma and YBC with jasmine aroma. The high value of antioxidant in YBC leaves tea predicted can improve immune for the inhibitory action of cancer cells. On the otherhand, this study showed that the qualitative screening of phytochemical compounds in YBC leaves ethanolic and water extracts revealed the presence of flavonoids, tannins, triterpenoid, carotenoid, kumarin, carbohydrate, and saponin. Present trends towards technologies and processes that increase the use of such as compound for active drugs in the pharmaceutical industry. The GC-MS test results suspected that the ethanol leaf extract of YBC leaves contains various biological activities such as anti-microbial, anti-cancer, antimutagenic, anti-peptic, anti-septic, anti-spasmodic, anti-adrenogenic, anti-inflammatory, and strong antioxidant capacity properties in glucose-histidine Maillard reaction products. Consistently, in our study, all of the variants of YBC leaves herbal tea can be accepted as an alternative medicinal beverage according to organoleptic test resulted. This study also provided an innovation of herbal medicine that it would be predicted in attracting consumers to the market to promote general positive image consumers for having more healthy beverages.

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