Exploration and preliminary analysis of *sengkubak* (*Pycnarrhena cauliflora* (Diels)) as natural food flavouring additive prospect

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Abstract. *Sengkubak* is a native plant to Southeast Asia which mainly grows in the Kalimantan and Sumatra Islands. The local community grows it as a natural flavour enhancer as historical heredity. This paper aims to find a deeper potential prospect, exploration, and preliminary testing of glutamic acid as a natural flavouring agent. This research used a hybrid approach through laboratory testing and literature studies related to user community information, economic prospects. The collecting process and drying were carried out at West Kalimantan Assessment Institute for Agricultural Technology, while extraction activities were carried out at ISMCRI. The glutamic acid analysis used Ultra Performance Liquid Chromatography (UPLC) at the Saraswanti Indogenetic laboratory. The results show that *sengkubak* has not been cultivated well and harvested wild. The yield value of the extract produced using water and ethanol solvent 96% were 21.23% and 17.43%, respectively. By water and ethanol solvent, the concentrated extract contained the glutamic acid 2,845.82 mg/kg and 1979.32 mg/kg, respectively. Further research is needed to create prototypes of derivative products from *sengkubak* extract with water solvent extracts to be used commercially.

Keywords: *sengkubak*, flavour enhancer, glutamic acid, natural MSG

1. Introduction

Monosodium glutamate (MSG) is one of the most abundantly found amino acids in nature. It is present in a heterogeneous group of foods as a flavour enhancer. In early 1900, MSG was discovered in Japan from seaweed by extraction methods. Later on, MSG was produced by fermentation involving bacteria which secrete glutamic acid through their cells. MSG is the sodium salt of glutamic acid (glutamic acid), widely consumed in the form of L-glutamic acid. The addition of MSG will make the food taste more delicious because MSG contains salt from sodium glutamic acid, which can function as a flavour enhancer and flavouring, especially in foods that contain protein [1].

The long-term usage of MSG will affect the health of those who consume it. MSG has been reported to trigger cancer, cardiometabolic disease (high blood pressure/hypertension), damage to the nervous system, and Chinese Restaurant Syndrome [2]. In addition, MSG can act as free radicals, excitotoxins and interfere with the work of several body organs, namely the heart, neurological, respiratory, gastrointestinal tract, muscles, genital and urinary tract, skin, and vision [3]. Even though The Food and...
Drug Administration (FDA) declared it safe for limited usage, several potential side effects are linked to increased MSG consumption [4].

Some research had been conducted to find alternative natural sources for flavour enhancers as a substitution for MSG. Sengkubak (Pycnarrhena cauliflora (Miers) Diels) is an endemic plant around Kayan Mentarang National Park, West Kalimantan. This plant has been used for generations as a flavour enhancer because it can give a savoury taste to dishes [5]. Sengkubak (Pycnarrhena cauliflora (Diels)) is a member of the Menispermaceae tribe and can grow in hilly areas above 500-1500 meters above sea level [6, 7]. However, the Indonesian people do not widely known sengkubak because it only grows in certain areas, such as in the forests of West Kalimantan and Jambi [8, 5]. In West Kalimantan, the sengkubak plant is commonly called saksang, while in Jambi people call it kemangi imbo [8].

Sengkubak leaf was used as a flavouring agent because this part gives a sweet taste to food, especially the young leaves, to eliminate the bitter taste of food. The Dayak communities carry out sengkubak leaf processing by pounding the fresh leaves or thinly sliced and mixed into cooking food. If the sengkubak is to be stored for a long time, then the leaves are thinly sliced, air-dried and stored in a container [5]. Besides as a flavouring agent, sengkubak is also used as a meat tenderizer because it contains protease enzymes [9].

Several previous studies related to the potential use of sengkubak as a natural flavouring have been carried out by [5, 10, 11, 12]. Setyasi had developed kemangi imbo leaves in powder products, and compared to MSG, according to the panellist, the products were acceptable [10]. Sengkubak contains C_{16}H_{26}O compounds that can play a role in the food and pharmaceutical fields, namely natural flavours and therapeutic agents [13]. In addition, sengkubak also contains C_{4}H_{11}O_{2}, acetic acid, butyl ester (CAS) (8.81%) compounds which also have functioned as natural flavours and sweetening agents [14].

Sengkubak contains other active ingredients, especially the roots and leaves, which can be anti-breast cancer and uterine cancer [11]. Sengkubak leaves can also be used as a headache medicine [15]. The methanol extract of sengkubak leaves has strong antioxidant activity with an IC_{50} value of 608.81 ppm (IC_{50} < 1000 ppm) and has the potential as an anti-cancer with an LC_{50} value of 248.75 ppm (LC_{50} < 1000 ppm) [16]. Cauliflora (Miers) species contain alkaloids with various biological activities, including antiprotozoal, antifungal, anti-plasmodial, and anti-bacterial [17].

This preliminary study was conducted to find the potential prospect of sengkubak as an alternative substitution for MSG as a flavouring agent. This research used a hybrid approach through laboratory analysis and literature studies.

2. Materials and methods
2.1. Time and place
These field activities and research was conducted from March to December 2020 at West Kalimantan Agricultural Institute for Assessment of Technology (AIAT) and Indonesian Spices and Medicinal Crops Research Institute (ISMCRI).

2.2. Materials and equipment
The materials used were sengkubak leaves from West Kalimantan, pure water, and ethanol 96% pa. Meanwhile, the equipment used in this research were Erlenmeyer, spatula, filter paper, cup, oven, grinder, round bottom flask, homogenizer, rotary vacuum evaporator, and Ultra Performance Liquid Chromatography (UPLC).

2.3. Procedure
The research procedure consisted of 3 stages. The first stage is exploration and domestication of sengkubak from the forest, the second stage is the drying process of fresh leaves, the extraction process using the maceration method, and the third stage is glutamic acid quantification analysis.
2.3.1. Drying process of sengkubak leaves
The fresh sengkubak leaves were washed under running water, cut into thin pieces, and then dried in the oven at 40°C for 14 hours. The dried leaves were then grounded using a grinder and then sieved with a 40 mesh to get a fine powder.

2.3.2. Extracts production and glutamic analysis
The sample was weighed as much as 500 grams (125 leaves) and added by 2,500 ml solvent, as shown in Table 1. The sample was put into a macerator and stirred continuously using a homogenizer for 3 hours at room temperature. The macerate was allowed to stand for 24 hours and then filtered using filter paper. The filtrate obtained is then put into a round bottom flask to be concentrated by evaporation technique using a rotary vacuum evaporator with a rotation speed of 50 rpm and a pressure of 200 mbar. The evaporation process condition for ethanol solvent was 5 hours at a temperature of 50°C, and the water solvent was 24 hours at a temperature of 70°C. Finally, the viscous extract obtained was weighed to calculate the yield of each solvent.

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\text{Extract Yields} = \frac{\text{Weight of extract}}{\text{Weight of sample}} \times 100\%
\]

| Treatments | Ethanol 96% (ml) | Water (ml) | Dried Leaves (gr) |
|------------|-----------------|------------|-------------------|
| I          | 2500            | 0          | 500               |
| II         | 0               | 2500       | 500               |

Sengkubak leaf extract was analysed to determine the content of glutamic acid compounds using Ultra Performance Liquid Chromatography (UPLC) at PT. Saraswati Genetech, Bogor. The working principle of UPLC is almost the same as HPLC (High-Pressure Liquid Chromatography), which is the separation of secondary metabolites using pressure. However, UPLC has the advantages of shorter time, more efficient solvents, higher sensitivity, and resolution [18].

3. Results and discussion
3.1. Sengkubak exploration
Exploration activities aim to gather sources of genetic diversity available in nature by collecting information such as morphology and plants genetics from several areas, then will be carried out in the collection process [19]. The exploration is the initial step to get superior varieties of sengkubak. This exploration was based on the obtained information from the community who live near the forest in West Kalimantan. The exploration found that the sengkubak plant was spread growing in the forests in each regency of West Kalimantan province. However, sengkubak has not been cultivated yet by the community. Most of the people still gather sengkubak leaves from the forest, and several of them started to plant one or two sengkubak trees in their yards by taking saplings that grow in the forest. However, it has not been cultivated properly. West Kalimantan Assessment Institute for Agricultural Technology (West Kalimantan AIAT) tried to do in situ conservation by the community and carry out ex-situ conservation by collecting them in the greenhouse.

West Kalimantan AIAT has collected by planting as many as ten accessions of sengkubak plants obtained from exploration activities. Sengkubak plants were planted in polybags using ultisol soil and RYP soil (red yellow podzolic) with the addition of manure and NPK fertilizer (Figure 1.)
3.2. Extraction and analysis

The fresh sengkubak leaves were collected and sorted to obtain good and high-quality samples. Then it was washed with running water to separate the sample from the adhering dirt, then dried. The dried samples were then cut into smaller sizes to facilitate the drying, packing, and milling processes [20]. Sengkubak leaves are dried using an oven. Drying using an oven is carried out considering that the process of reducing water content can be in large quantities in a short time, and the temperature can be adjusted [21]. The drying process is carried out until the moisture content is constant. According to the Indonesian Herbal Pharmacopeia, the maximum water content of traditional medicine simplicial is 10% [22]. The high-water content in simplicial can be a medium for the growth of moulds and fungi. Besides that, enzymatic reactions can also decompose the active substances in simplicial [23]. The dried sample was ground using a grinder and then sieved 40 mesh to obtain a finer powder. The quality of simplicial of sengkubak can be seen in Table 2.

| Parameter                  | Value     | Methods                                      |
|----------------------------|-----------|----------------------------------------------|
| Water content (%)          | 8.54      | SNI 01-3709-1995, 6.2                       |
| Ash content (%)            | 2.32      | SNI 01-3709-1995, 6.3                       |
| Water-soluble content      | 7.61      | MMI Edition VI, 1995 page 325 appendix 9    |
| Alcohol soluble content    | 4.22      | MMI Edition VI, 1995 page 325 appendix 9    |
| Phytochemical tests        |           |                                              |
| - Alkaloid                 | +         |                                              |
| - Saponin                  | +         |                                              |
| - Tannin                   | +         |                                              |
| - Phenolic                 | +         |                                              |
| - Flavonoid                | +         |                                              |
| - Triterpenoid             | -         |                                              |
| - Steroid                  | +         |                                              |
| - Glucoside                | +         |                                              |

Note: (+) Detected (-) Not Detected

The extraction process of sengkubak using the maceration method with a ratio of 1:5, where 500 grams of sengkubak leaf mixed with 2,500 ml of solvent and stirring continuously for 3 hours (Figure 2). The advantages of the maceration method are simple, short time, and the active substance will be obtained optimally. The most important thing is that there is no heating process to prevent damage or loss of the active substances during the extraction process [24].

Figure 1. Ex-situ conservation in West Kalimantan AIAT; (a) using RYP soil, and (b) ultisol soil.
The extraction process of sengkubak using maceration method (a), filtration (b), evaporation process using rotary vacuum evaporator (c).

The filtration process was carried out to separate the extract from the liquid and then concentrated using a rotary vacuum evaporator with a rotation speed of 50 rpm and a pressure of 200 mbar (Figure 2). The setting for the evaporation process using ethanol 96% solvent was 5 hours at a temperature of 50°C, and for using the water, extraction was 24 hours at a temperature of 70°C. The quantity of extract yield of sengkubak by ethanol and water can be found at Table 3.

Table 3. Extract yield of sengkubak leaves by ethanol and water solvent.

| Solvent | Sample weight (gram) | Extract weight (gram) | Yield (%) |
|---------|----------------------|-----------------------|-----------|
| Water   | 500                  | 106.17                | 21.23     |
| Ethanol | 500                  | 87.13                 | 17.43     |

Extract yield is the ratio between the weight of the extract obtained and the weight of the dried leaf. The higher the yield value obtained, the higher the amount of extract produced [25]. From Table 3 it can be seen the yield of sengkubak leaf extract from each type of solvent. The yield of the extract using water as a solvent was 21.23%, while the ethanol 96% solvent was 17.43%. The value of sengkubak leaf extract using water as a solvent was greater than that of 96% ethanol because water is an excellent polar solvent for dissolving ionic compounds. Besides that, ethanol is a polar solvent that can form hydrogen bonds and evaporate faster during the evaporation process [24]. The high yield value indicates the high bioactive content in it, and the extract yield is influenced by the type of solvent, the extraction method used, and the required extraction time [26, 27, 28]. Each of these extracts then being analysed for glutamic acid content using UPLC (Table 4).

Table 4. Quantitative analysis of glutamic acid using UPLC.

| Solvent  | Parameter       | Value (mg/kg) | Method                               |
|----------|-----------------|---------------|--------------------------------------|
| Water    | L-glutamic acid | 2845.82       | 18-5-17/MU/SMM-SIG, UPLC             |
| Ethanol  | L-glutamic acid | 1979.32       | 18-5-17/MU/SMM-SIG, UPLC             |

Analysis of glutamic acid content from sengkubak leaves showed 2845.82 mg/kg for water solvent and 1979.32 mg/kg for 96% ethanol solvent. The value of glutamic acid using water as a solvent is higher than that of 96% ethanol. This content is in line with the yield of sengkubak leaf extract obtained. The content of L-glutamic acid in sengkubak leaf extract has the same polarity as water solvent, so water is more effective in dissolving than 96% ethanol solvent. The bioactive content in plants will dissolve in solvents that have the same polarity [29]. Therefore, the type of solvent affects the results of the bioactive content in the extract obtained [27]. From the research results, it was found that sengkubak
leaves were scientifically proven to act as natural flavours as has been done from generation to generation traditionally.

*Sengkubak* leaves contain glutamic acid, which can strengthen food taste and give rise to a savoury taste (umami) [30]. Glutamic acid is widely found in natural ingredients such as fish, mushrooms, algae (spirulina), *bekai* plants, *sengkubak* plants so that these natural ingredients can replace MSG sold in the market. In addition, *sengkubak* leaves have many benefits for human health, such as antioxidants, anti-cancer, anti-acne and anti-bacterial; this has been reported by [11, 16, 31]. *Sengkubak* can be used as an alternative to the use of natural flavour enhancers so that the potential of this plant is very large as a natural flavouring in the future [31]. Of course, it must be supported by further research to obtain high yields and glutamic acid content. The extraction process is necessary as the first step to separate chemical components that can be further analysed.

The bioactive plant content can be obtained through extraction by pulling chemical compounds from within the plant. Several studies have been conducted to determine the bioactive content of *sengkubak* plants using the maceration method [11, 16, 31]. Based on this, this study refers to the research above by using the maceration method. However, selecting an extractor for the maceration process will provide high effectiveness by paying attention to the solubility of the natural solvent compound [32]. Therefore, a study was conducted on the maceration extraction method using various water and 96% ethanol solvents to determine and compare the yield and glutamic acid content of *sengkubak* leaves. It was as well as other natural resources such as shiitake mushroom extract, a natural ingredient. Its extract can be used as a natural flavour enhancer to develop healthier products (owing to a reduction in sodium) with preserved sensory quality and will meet consumers’ requirements for the minimal use of chemical additives in food.

### 3.3 Economic prospect development

From the economic perspective, *sengkubak* has a potential alternative for substituting the MSG (monosodium glutamate) industry, which needs approximately 270 thousand tons of sugar in Indonesia annually. Moreover, Indonesia is still not sufficient producing sugar even though some government effort has been conducted, such as creating superior varieties, intensifying, and extending sugar plantation. Finding an alternative raw bio material industry of natural flavour enhancer could lessen the domestic sugar consumption. The simplicial of *sengkubak*, extract or the other new innovative products could be scale up in industry level. All involved actors should pay attention and make a collaboration conducting practical research so the process of industrialization and commercialisation for creating a new business model.

The domestication and cultivation of *sengkubak* have a prospect of becoming a sustainable agroforestry concept. That will generate income for the community near it. People can cultivate it using a multi-cropping system in which *sengkubak* is planted under the shade of annual plants without destroying the forest with a minimum tillage system. The community also can utilize their yard and idle land around the forest for cultivating this plant.

### 4. Conclusion

In summary, people still gather *sengkubak* leaves from the forest, which has not been cultivated yet. However, the ex-situ conservation was initiated by west Kalimantan AIAT, which has ten accessions for candidates of superior variety selection. Then the extraction of *sengkubak* leaves using water and ethanol 96% as solvents showed different yields. The yield of water solvent is 21.23%, and the ethanol solvent yield is 17.43%. The glutamic acid content in *sengkubak* leaf extract using water solvent is higher (2845.82 mg/kg) than using ethanol 96% solvent (1979.32 mg/kg). In economic prospect, not only can it be the niche income for society near the forest, but it also can be developed for the medium and micro-industry of natural flavour enhancer.
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