

## CHEMICAL AND ORGANOLEPTIC PROPERTIES OF BEKAI (*Pycnarrhena tumefacta* Miers) LEAVES FOR FLAVOURING AGENT (BIO-VETSIN)

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CHEMICAL AND ORGANOLEPTIC PROPERTIES OF BEKAI (*Pycnarrhena tumefacta* Miers) LEAVES FOR FLAVOURING AGENT (BIO-VETSIN). The "tasty" cuisine tends to use chemical flavour agent containing monosodium glutamate (MSG). Utilization of MSG, in long-term, may cause health problems, especially triggering cancer cells. Therefore, it is necessary to introduce and increase a natural flavouring agent to eliminate those health problem, such as beikai leaf. Bekai (*Pycnarrhena tumefacta* Miers) is familiar as a natural flavour agent (bio-vetsin) in cuisine for forest communities in Nyapa Indah Village, Berau, East Kalimantan, Indonesia. However, until now there has been no proper analysis support for widespread utilization of beikai leaf. This paper studies the presence of phytochemicals, antioxidant and GC MS analysis from beikai leaf extracts, as well as five hedonic classifications of organoleptic test, to reinforce the need for a better understanding of consumers reaction in terms of possible acceptance of additional beikai leaves applied in soup as bio-vetsin. Present study showed that the qualitative screening of phytochemical compounds in beikai leaves ethanolic extracts revealed presence of alkaloids, flavonoids, tannins and steroids. Antioxidants of beikai leaves using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method showed that concentrated extract has 80.1%, which was predicted can improve immune for inhibitory action of cancer cells. GC MS analysis suspected that beikai leaf extract contained five major compounds, i.e. oxirane dodecyl, gamma sitosterol, vitamin E ( $\alpha$  tokoferol), 9.12-Octadecadienoic acid (Z,Z)- (natural linoleic acid), and 3-Tetradecanoic acid (myristic acid). These chemical compound in related with their phytochemical were predicted to contain strong antioxidant activities and some of them are commonly used as flavour agent in cuisine for some food industries. Meanwhile, results of organoleptic tests presence in three soup variant showed that soup with additional beikai leaves has best acceptance in the children's perception due to unique smell, tasty and no colour changing compared with added MSG and control. Thus beikai leaf can be used as an innovation for healthy food and new market opportunities for MSG substitutes.

Keywords: Bekai leaf, phytochemicals, antioxidants, GC-MS, flavouring agent, organoleptic

*KARAKTERISTIK KIMIA DAN ORGANOLEPTIK DAUN BEKAI (*Pycnarrhena tumefacta* Miers) UNTUK PENYEDAP ALAMI (BIO-VETSIN). Masakan "enak" cenderung menggunakan bahan penyedap kimia yang mengandung monosodium glutamat (MSG). Pemanfaatan MSG dalam jangka panjang dapat menyebabkan gangguan kesehatan, terutama pemicu sel kanker. Oleh karena itu perlu dilakukan pengenalan dan penambahan zat penyedap alami untuk mengatasi gangguan kesehatan tersebut, seperti daun beikai. Bekai (*Pycnarrhena tumefacta* Miers) dikenal sebagai penyedap alami (bio-vetsin) dalam masakan oleh masyarakat di Desa Nyapa Indah, Berau, Kalimantan Timur, Indonesia. Namun hingga saat ini belum ada dukungan analisis yang tepat untuk pemanfaatan daun beikai secara luas. Penelitian ini menentukan keberadaan fitokimia, anti oksidan dan analisis GC MS dari ekstrak daun beikai, serta lima klasifikasi hedonik uji organoleptik, untuk memperkuat perlunya pemahaman yang lebih baik tentang reaksi konsumen dalam hal kemungkinan penerimaan penyedap asal daun beikai yang ditambahkan dalam sup sebagai bio-vetsin. Penelitian terbaru*

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menunjukkan bahwa skrining kualitatif senyawa fitokimia pada ekstrak etanol daun bekai menunjukkan adanya alkaloid, flavonoid, tanin dan steroid. Anti oksidan daun bekai dengan metode 2,2-difenil-1-pikrilhidrol (DPPH) menunjukkan ekstrak pekat memiliki kandungan 80,1% yang diperkirakan dapat meningkatkan kekebalan tubuh untuk menghambat aksi sel kanker. Analisis GC MS diduga ekstrak daun bekai mengandung 5 (lima) senyawa utama yaitu oksiran dodesil, gamma sitosterol, vitamin E (a tokoferol), 9.12-oktadekadienoat (Z, Z) - (asam linoleat alami), dan 3-Tetradekanoat (asam miristat). Senyawa kimia yang terkait dengan fitokimianya diduga mengandung aktivitas antioksidan yang kuat dan beberapa di antaranya biasa digunakan sebagai agen penyedap masakan pada beberapa industri makanan. Sedangkan dari hasil uji organoleptik keberadaan tiga varian soto menunjukkan bahwa dengan tambahan daun bekai paling diterima persepsi anak-anak karena memiliki bau yang khas, gurih dan tidak berubah warna dibandingkan dengan penambahan MSG dan kontrol. Dengan demikian daun bekai dapat digunakan sebagai inovasi untuk makanan sehat dan peluang pasar baru untuk pengganti MSG.

*Kata kunci:* Daun bekai, phytochemical, anti-oksidan, GC-MS, penyedap alami, organoleptik

## I. INTRODUCTION

People choose high quality food that provide health benefits by adding certain ingredients (additives) in food (Sauceda, Martinez, Rodriguez, Aguilar, & Zavalal, 2016). An increase in economic rate raises the population's awareness of food additives usages (Kumar, Singh, Chandra & Samsheer, 2017). These additive materials have begun to shift from factory synthesis materials, such as MSG (Monosodium glutamate) to natural ingredients. This is because to MSG utilizations, in long-term, can become a toxic and possibly threat to public health, especially triggering cancer cells (Niaz, Zaplatić & Spoor, 2018).

Indonesia is one of the countries with megabiodiversity and some plants are potentially used for food spices and herbs (Supartono, Sukartiko, Yuliando, & Kristanti, 2015). Some spices and herbs are known to be used as cuisine ingredients such as ginger and galangal. Ginger (*Zingiber officinale*) is extensively used around the world in cuisine as a spice (Ghosh, Banerjee, Mullick, & Banerjee, 2011). While, *Alpinia galanga* (galangal) is rhizome used as spice and flavouring agent and its leaves and inflorescence are consumed as vegetable (Wong, Lim, & Omar, 2008). There are many other potentials of herbal plants in Indonesia that has a function as natural flavouring agent and can be used as an MSG substitute.

One of the potential herbaceous plants as natural flavouring agent is bekai (*Pycnarrhena*

*tumefacta* Miers) from Menispermaceae family. Hereditary, local community of Nyapa Indah Village, Berau District, East Kalimantan, Indonesia planted bekai both on local yard and under forest tree stands (Figure 1). Usually bekai leaves are added into cuisine which meat as basic ingredients, while for other cuisine has never been tried. Besides for accelerating process of meat into well-done, bekai leaves could also be used as natural flavouring agent. However, there has been no research publication available on the content of chemical compounds and also organoleptic tests on cuisine or food with additional bekai leaves (*Pycnarrhena tumefacta* Miers). As a comparison in other genera with different species, namely sengkubak (*Pycnarrhena cauliflora* (Miers) Diels) in West Kalimantan, the community have used it as a basic ingredient for accelerating process of meat and savoury flavours in food. *Pycnarrhena cauliflora* (Miers) Diels has roots that are predicted to play a role as cytotoxic and pro-apoptotic activities in human breast cancer (Masriani, Mustofa, Jumina, Sunarti, & Enawaty, 2014). Further, Masriani, Mustofa, Jumina and Sunarti (2013) stated that the ethanolic extracts from root, stem and leaves of *P. cauliflora* inhibited the growth of HeLa cells (cervical cancer cell).

Development of novel foods by adding functional component to carrier food provides new market opportunities for manufacturers (Pestoric et al., 2015). In order to be successful on the market a product or product category

needs to both benefit from positive general image and offer product qualities that match or surpass consumers' expectations (Almli, Verbeke, Vanhonacker, Naes, & Hersleth, 2011). Thus, a considerable proportion of product failure can be attributed to a mismatch between sensory properties and consumer needs or expectations (Kemp, Hollowood, & Hort, 2009). The positive general image of consumer through human senses include colour, odour, taste, and others special feature were necessary to maintain reproducible efficacy and safety of herbal product as rational drug (Bisla, Choudhary, & Chaudhary, 2014).

In the present study, some chemical properties of bekai leaves were determined as a natural flavouring agent (bio-vetsin), especially for others cuisine (i.e. soup). This study also provided organoleptic data of its cuisine products to reinforce the need for a better understanding of consumers reaction in terms of possible acceptance of additional bekai leaves as an innovation for healthy food and new market opportunities for MSG substitutes.

## II. MATERIAL AND METHOD

### A. Materials

Fresh leaves of bekai (*Pycnarrhena tumefacta* Miers) were collected from Custom Forest of Nyapa Indah Village (around Labanan Forest

Research), Berau District, East Kalimantan, Indonesia (N 01°52 '48.2 "and E 117 ° 18' 03.2"). Bekai leaves were dried in the laboratory with air conditioned (A.C.) set for 25°C for 3 days and ground to a fine powder using crusher and sieved through 40–60 mesh. The powder samples were kept at A.C. room in a covered glass container to protect them from humidity and light prior to extraction. Then these samples were prepared for further analysis.

### B. Methods

#### 1. Maceration

Five grams dried powder of bekai leaves were exhaustively extracted by maceration in 200 ml ethanolic solvent for 24 hours at room temperature (28±2°C). Whereas, each extraction was concentrated from 200 ml into 10 ml concentrated crude ethanolic extracts, dried in oven at 50°C to give dark green extracts (Maharani et al., 2016; Zhang et al., 2018). Further, these extracts were used for phytochemical and antioxidant analysis (5 ml), and GC-MS analysis (5 ml).

#### 2. Antioxidant Assay

In this antioxidant test, 100% of 5 concentrated samples were grouped into 200 ppm, 100 ppm, 50 ppm and 25 ppm times of dilution, respectively. Further, 1 mg of vitamin C was weighed, then dissolved in 5000 µl of



Figure 1. Bekai planted in forest community area (a), and bekai leaf used for natural flavouring agent (b)

distilled water and regarded as a positive control. While, negative control was used its solvent (distilled water) (Fitriana, Istiqomah, Ersam, & Fatmawati, 2018), 100 µl sample was mixed in cuvette with 400 µl of distilled water was added, and 500 µl of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. Mixing was stopped when the sample volume has reached 1000 µl (1 ml). Samples were incubated for 20 minutes indoor with minimum light. The antioxidant activity was determined by decolorization of DPPH with a wavelength of 517 nm using a spectrophotometer. The scavenging activity was calculated as a percentage of DPPH decolouration relative to a negative control using equation 1 (Maheswari, Reena, & Sivaraj, 2017).

### C. Analysis

#### 1. Preliminary Phytochemical Analysis

Bekai extracts were tested for active compound such as flavonoids, saponins, steroids, tannins, terpenoids, alkaloids and carbohydrate using some following standard procedures (Keo et al., 2017; Jaradat, Hussien, & Ali, 2015).

#### 2. Flavonoids Determination

About 1 ml of ethanolic extract was mixed and 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 (Jaradat et al., 2015).

#### 3. Saponins Determination

Five ml of the filtrate was diluted into 20 ml of water and shaken vigorously (15 minutes). A stable froth (foam) upon standing indicates the presence of saponins (Keo et al., 2017).

#### Steroids Determination

One ml of ethanolic extract of each sample is boiled with 10 ml of chloroform and it was cooled accordingly. Then, 1 to 2 drops of concentrated sulfuric acid were added slowly through the wall of the tube. The mixtures were then shook well and it was allowed to stand for some time. The red color appears at the lower

layer indicates the presence of steroids (Keo et al., 2017).

#### 4. Tannins Determination

Test solution of 5 ml ethanolic extract with 1% sodium hydroxide solution (1%) provides yellow to red precipitation within short time indicates the presence of tannins (Keo et al., 2017).

#### 5. Triterpenoid Determination

One ml of ethanolic extract of each sample is boiled in the mixture of 10 ml chloroform and cooled down. One to two drops of concentrated sulfuric acid were added slowly through the wall of the tube. Shake tube well and allow standing for some time, reddish-purple colour appears at the lower layer indicates the presence of Triterpenoids (Keo et al., 2017).

#### 6. Alkaloids Determination

Five ml of ethanolic extract was reacted with two drops of potassium bismuth iodide solution reagents in test-tubes. The development of creamy and orange colour respectively indicated positive result (Keo et al., 2017).

#### 7. Carbohydrate Determination

The extract was hydrolyzed with HCl in the water heater. Then, it was added with 1 ml of pyridine and a few drops of sodium nitroprusside solution into the hydrolyzate, after it was etched with an alkaline solution of sodium hydroxide. The formation of pink to red colour indicates the presence of glycosides (Keo et al., 2017).

#### 8. Gas Chromatography-Mass Spectrometry Analysis (GC-MS Analysis)

Gas Chromatography-Mass Spectrometry (G.C.-M.S.) analysis was carried out for the ethanolic extracts. The analysis was performed according to the GC-MS equipments by Shimadzu Q.P. 2010: R.T.X. - column type is 5 ms, Restek Corp (30 m length). The injector and detector temperatures were both maintained at 250°C, while operation temperature was set at 50–300°C. The column temperature was programmed at 50–120°C, with 40°C increment per minute which was maintained

for one minute. Then, it was programmed at 120–300°C, with 60°C increment per min and held for five minutes, with retention time (Rt) of 60 minutes. Helium was used as a carrier gas is 50–500 atomic mass unit (A.M.U). The compounds of each extract were identified by using computer searchers in commercial libraries of NIST (Maharani, Fernandes, Turjaman, Lukmandaru & Kuspradini, 2016). Furthermore, the structure of chemical compounds associated with phytochemical and antioxidant tests determined will be drawn by using chemical office software.

### 9. Organoleptic or Macroscopic Evaluation Analysis

Based on observing, touching and sniffing senses, organoleptic analysis was carried out by a human panel (Xu et al., 2018). In this study, organoleptic analysis were conducted by comparing soup with becai leaves addition (9 airdried leaves for 2 L), soup with MSG addition (half-one teaspoon for 2 L), and soup without any additional flavour (as a control). Organoleptic analysis of soup were carried out on 20 children in average 10-12 years old as panelist one by one by using Hedonic scale with 5 classifications (very dislike=1, dislike=2, neutral=3, like=4, and very like=5). They were decided on their favorite soup for sensory attributes of odor, flavour/taste and color.

## III. RESULT AND DISCUSSION

### A. Phytochemical Test of Bekai Leaf

Qualitative screening of becai (*Pycnarrhena tumefacta* Miers) leaves ethanolic phytochemical compounds showed the presence of alkaloids, flavonoids, tannins and steroids. Previous study of other *Pycnarrhena* genus stated that *Pycnarrhena longifolia* leaves contained alkaloid (Masriani et al., 2013) and flavonoids (Mohammed et al., 2020). Meanwhile, others

*Pycnarrhena manillensis* included Philippine endemic medicinal plant, it leaves contained alkaloids and steroids (Ragasa, Tepora, & Rideout, 2009). Another genus from West Kalimantan, Indonesia known locally as sengkubak root (*Pycnarrhena cauliflora*) has been determined for its alkaloid content which is identified as an anticancer compound (Masriani et al., 2014).

### B. Antioxidant Test of Bekai Leaf

The result of the antioxidant test of becai leaf using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method showed that concentrated extract had DPPH absorption reduction of 44.5% in 200 ppm dilution, 68.1% in 100 ppm dilution, 80.1% in 50 ppm dilution, and 83.2% in 25 ppm dilution (Table 1). DPPH is used to test the ability of the compound to act as a free radical trap or hydrogen donor and evaluate the antioxidant activity (Devi & Ganjewala, 2011). Antioxidant properties are very important in counteracting the deleterious role of free radicals in foods and biological systems (Pirbalouti, Firoznejhad, Craker, & Akbarzadeh, 2013).

Antioxidant tested of becai leaves using DPPH method showed that concentrated extract was 80.1%, almost equivalent to high dose of Vitamin C of same genus, Tubu (*Pycnarrhena longifolia*) leaves that it had 87% antioxidant activity (Mohammed et al., 2020). The interpolation was calculated from 0 (zero) to the optimum concentration, which is between 0 - 83.2%. The optimum reading of antioxidants is at a concentration of 25 ppm. As comparison, vitamin C 100 ppm has an antioxidant of 97.1% and 96.1% in 50 ppm. The colour of sample can affected reading, a high concentration of sample colour is getting stronger or darker. In this study, becai samples had a dark green blackest extracted colour. Sometimes the sample that containing phenolic

Table 1. Antioxidant test of becai leaf with DPPH method

	200 ppm	100 ppm	50 ppm	25 ppm
Bekai (%)	44.5	68.1	80.1	83.2
Vit C (%)	-	97.1	96.1	-

group such as anthocyanin, could have colour interference of the DPPH assay. The samples leads to under-estimation of antioxidant activity (Choong et al., 2007). Other study stated that a limitation of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability (DRSA) due to the presence of pigments and colours in the extracts of plant-based foods was addressed. This fact could interferes the elaborate absorbance readings of DPPH radicals (Yeo & Shahidi, 2019).

The high value of antioxidant of bekai leaves could improve human immune for inhibitory action of cancer cells, as well as almost 47% of anticancer drugs, come from the natural product (Jabeen, Hanif, Khan, & Qadri, 2014). Consuming of foods rich in natural antioxidants, as well as processed foods enriched with them, provides the desired supply antioxidant and prevent potential diseases (Hardy, 2000; Jukic, Hmjica, & Aldžic, 2015). Meanwhile, the evaluation of antioxidants with complex compounds are cannot be done singly. The DPPH method is based solely on removal of electrons which are replaced by hydrogen atoms from DPPH and is influenced by the polarity of compound (Gangwar et al., 2014).

### C. A Chemical Compound in Bekai Leaves Based on GC-MS Test

The GC-MS test results (Figure 2, Table 2) indicated that the ethanol leaf extract of bekai contains five major compounds, ie 17.1% Oxirane dodecyl, 14.1% gamma sitosterol, 11.3% vitamin E ( $\alpha$  tokoferol), 10.7%

9.12-Octadecadienoic acid (Z,Z)- (natural linoleic acid), 7.5% 3-Tetradecanoic acid (myristic acid). As well as major compound in bekai leaf, Gazzola (2016) stated that sodium dodecyl sulphate contained in fresh leaves of some natural products have been significant source of anticancer agents because of its antioxidant activity.

According to phytochemical tests, bekai leaves contained flavonoids, which are predicted to be able to provide various flavours in many food (Tanwar & Modgil, 2012). Flavonoids belong to low molecular weight with phenolic compounds (Panche, Diwan, & Chandra, 2016). Strong antioxidant activities in medicinal plants are due to the presence of phenolic compounds (Karau, Njagi, Machocho, Wangai, & Nthinga, 2015). Flavonoids in bekai leaves was Phenol, 2,4-bis(1,1-dimethyl ethyl)-. Phenol, 2,4-bis(1,1-dimethyl ethyl)- ( $C_{14}H_{22}O$ ) detected in the third peak, 2.0% (Figure 3.). These components have same antioxidant properties as the major compound, oxiran dodecyl, which has flavouring agent properties due to include in same dodecyl group. This group is included in the list of chemical flavouring substances allowed for manufacturing of edible flavours (Customs Union Commission, 2011).

Bekai leaves were also contained alkaloids based on the phytochemical test. The alkaloid in food represented the bitter gustatory sensation (Astray, Rio, Mejuto, & Pastrana, 2007). Alkaloids are low molecular weight, and nitrogen-containing compound (Matsuura & Neto, 2015). Alkaloids in bekai leaves were

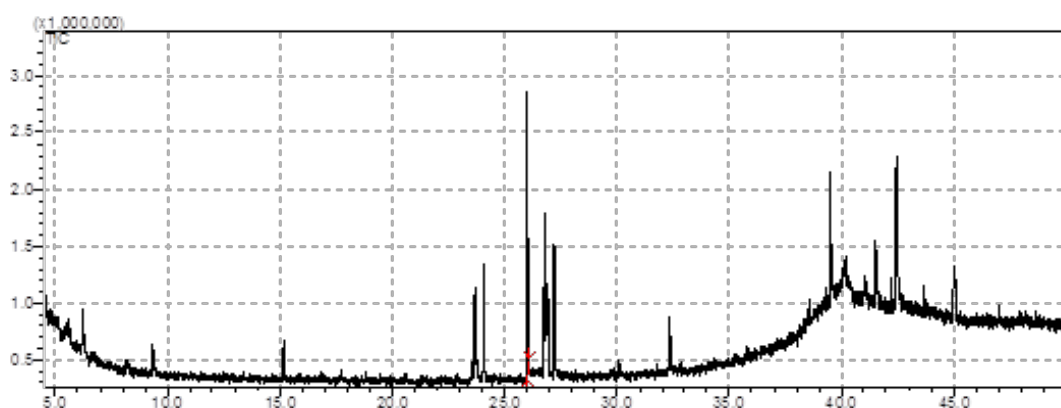
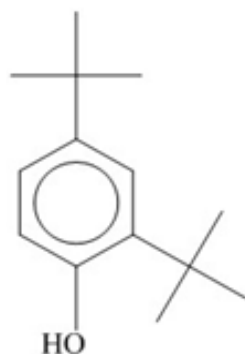


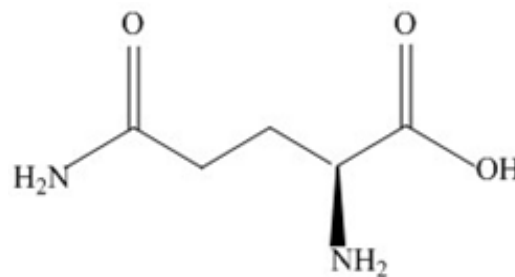
Figure 2. GC-MS analysis of bekai leaves

Table 2. A chemical compound of bekai leaves extract based on GC-MS test result

Peak#	Ret.Time	Name	Mol.Form	Area (%)	Similarity index (%)
1	6.231	Nonane, 2-methyl-	C <sub>10</sub> H <sub>22</sub>	2.8	90
2	9.349	Glutamine	C <sub>5</sub> H <sub>10</sub> N <sub>2</sub> O <sub>3</sub>	1.1	93
3	15.166	Phenol, 2,4-bis(1,1-dimethylethyl)-	C <sub>14</sub> H <sub>22</sub> O	2.0	91
4	23.681	Phthalic acid, dibutyl ester	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	5.3	91
5	24.062	Palmitic acid, ethyl ester	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	6.2	96
6	26.023	Oxirane, dodecyl-	C <sub>14</sub> H <sub>28</sub> O	17.1	92
7	26.784	9,12-Octadecadienoic acid (Z,Z)-	C <sub>21</sub> H <sub>38</sub> O <sub>4</sub>	10.7	95
8	26.86	9-Octadecenoic acid, ethyl ester	C <sub>20</sub> H <sub>38</sub> O <sub>2</sub>	4.2	94
9	26.899	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	C <sub>20</sub> H <sub>34</sub> O <sub>2</sub>	4.2	91
10	27.228	3-Tetradecanoic acid	C <sub>14</sub> H <sub>24</sub> O <sub>2</sub>	7.5	90
11	32.357	Phthalic acid, mono-(2-ethylhexyl) ester	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	3.5	90
12	39.521	Vitamin E (alpha tocopherol)	C <sub>29</sub> H <sub>50</sub> O <sub>2</sub>	11.3	96
		Pregn-5-en-3.beta.-ol, 20.alpha.-			90
13	41.526	[(1R,2R)-2-(1R)-1,2-dimethylpropyl-2-methylcyclopropyl]-	C <sub>30</sub> H <sub>50</sub> O	4.7	
14	42.465	.gamma.-Sitosterol	C <sub>29</sub> H <sub>50</sub> O	14.1	97
15	45.018	9,19-Cyclolanost-25-en-3-ol, 24-methyl-, (3.beta.,24S)-	C <sub>31</sub> H <sub>52</sub> O	5.4	90



Phenol, 2,4-bis(1,1-dimethylethyl)-



Glutamine

Figure 3. Phenol, 2,4-bis(1,1-dimethyl ethyl)- and glutamine suspected in fresh leaves of some natural products, as well as bekai leaves

identified as glutamine (C<sub>5</sub>H<sub>10</sub>N<sub>2</sub>O<sub>3</sub>). Glutamine detected in the second peak, 1.1%. Glutamine is a conditionally essential amino acid widely used in sports nutrition due to NH<sub>2</sub> (NH<sub>2</sub> functional group) presences (Coqueiro et al., 2019). Furthermore, glutamine from snakehead fish could be increased glutathione, which has antioxidant activity in the human body and brain (Sunarno, 2015).

Steroids contained in bekai leaves were gamma sitosterol and  $\alpha$ -tocopherol (Figure 4).  $\alpha$ -tocopherol detected in the 12<sup>th</sup> peak, 11.3% considered to be one of the most powerful antioxidants (Fritsche, Wang, & Jung, 2017). Kuchan et al. (2018) determined that  $\alpha$ -tocopherol plays an important role in protecting cell membrane. Meanwhile, gamma sitosterol detected in the 14<sup>th</sup> peak, 14.1%, and

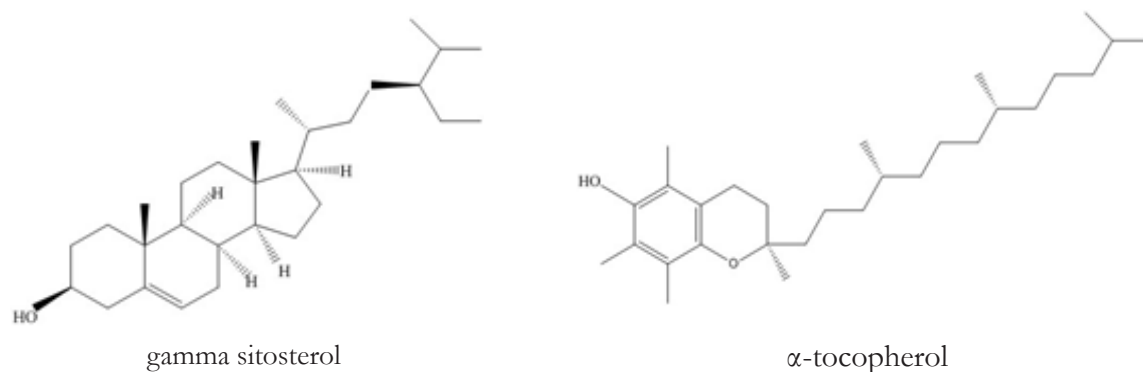
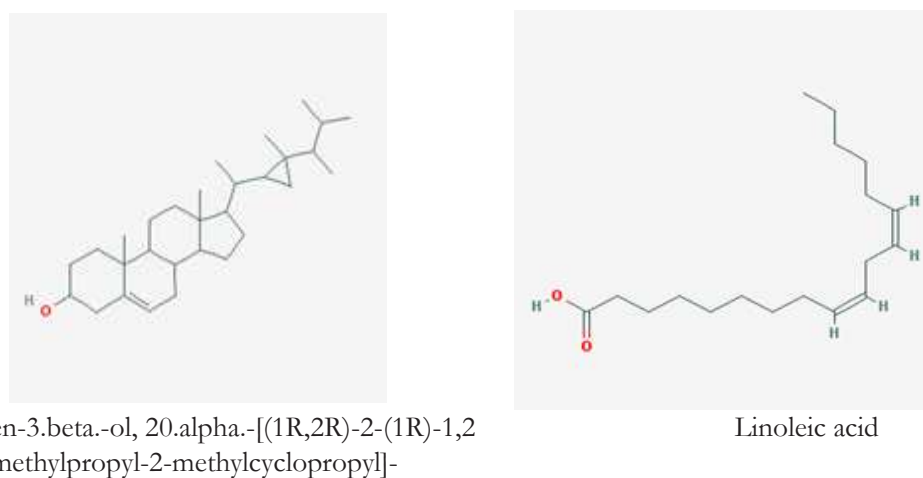


Figure 4. Gamma sitosterol and vitamin E ( $\alpha$  tocoferol) suspected in fresh leaves of some natural products, as well as bekai leaves



Pregn-5-en-3.beta.-ol, 20.alpha.-[(1R,2R)-2-(1R)-1,2 dimethylpropyl-2-methylcyclopropyl]-

Linoleic acid

Figure 5. Pregnen-20-acetate and linoleic acid suspected in fresh leaves of some natural products, as well as bekai leaves

it can be considered as hypolipidemic agent (Balamurugan, Stalin, Aravinthan, & Kim, 2014) and antihyperglycemic activity (Sirikhansaeng, Tanee, Sudmoon, & Chaveerach, 2017).

In addition, other chemical compounds that are included in the steroid group and contained in bekai leaves were Pregnen-20-acetate or known as gorgosterol detected in the 13<sup>th</sup> peak, 4.69% (Figure 5). Pregnen-20-acetate that belong to pregnen compound. Cheenpracha et al. (2017) stated that Pregnen compound belong to the steroid group due to has a C-cyclic bond that showed its strong antioxidant activities.

It can be shown in Table 2, some fatty acids presented linoleic and myristic acid (9,12-Octadecadienoic acid (Z,Z)- (natural linoleic acid) detected in the 7<sup>th</sup> peak, 10.7%). Linoleic acid consumption can be used as optimal dietary human health (Jandacek, 2017). Linoleic acid has antioxidant activities, including free radical scavenging capacity (Ali et al, 2012). 3-Tetradecanoic acid (myristic acid) detected in 10<sup>th</sup> peak, 7.49%. Myristic acid can be used as flavour agent in cuisine and commonly used in some food industries (Burdock & Carabin, 2007). Myristic acid has antioxidant activities in vitro and hepatoprotective effects against carbon tetrachloride-induced acute liver injury (Liu, Yuan, Ramaswamy, Ren, & Ren, 2019).



On the other hand, esters were major components of flavour widely distributed in nature (Bayout et al., 2019). Bekai leaves contained esters, i.e. palmitic acid ethyl ester and 9-Octadecenoic acid, ethyl ester. Palmitic acid ethyl ester detected in the 5<sup>th</sup> peak, 6.2% (Table 2). Palmitic acid ethyl ester known as hexadecanoic acid ethyl ester, was reported as flavour activity (Gideon, 2015). Other biological activities were antioxidant, hypercholesterolemic, nematocidal, pesticide, antiandrogenic flavour, haemolytic and alpha-reductase inhibitor (Sudha, Chidambarampillai, & Mohan, 2013). 9-Octadecenoic acid, ethyl ester detected in the 8<sup>th</sup>, 4.17%. 9-Octadecenoic acid, ethyl ester had biological activity as hepatoprotective, anti-histaminic, antieczemic and hypocholesterolemic (Arora, Kumar, & Meena, 2017).

#### D. Organoleptic Test of Cuisine (soup) with Additional of Bekai Leaves Compared with MSG

In order to maintain quality, purity, potency, safety, and efficacy of herbal drugs or product of medicinal plant needs sensory/organoleptic analysis as consumer acceptance (Patil et al., 2013; Vanhonacker et al., 2013). A sensory

experience is described as an individual's perception of goods or services or other essentials in a service process as an image that challenges the human mind and senses. Thus, this sensory marketing is defined as a way of measuring and explaining consumer emotions as well as spotting and capitalizing on new market opportunities, and finally ensuring long-lasting product success (Randhir et al., 2016).

Results of organoleptic tests presence in three soup variants can be shown in Table 3, as flavour agent for children's a flavour/taste perception.

Generally, most children preferred best (very like) in taste, colour and odour of soup with an additional of bekai leaves compared to MSG added and control (Table 3). Soup with an additional of bekai leaves was offered a unique odour/scent, savory/tasty, and no colour changing compared to both MSG added and control. A unique odour/scent in soup with an additional of bekai leaves described fresh scent and very tempting to taste it. Our sense of smell guards us safe by serving us to choose fresh food and avoid rotten/bad food. Various readings have considered the appeal of scents arising from an object, or a service associated with it being perceived as pleasant or unpleasant,

Table 3. Comparison of organoleptic tests presence in soup with addition of bekai leaf, MSG, and without both additional (control)

Soup Variant	Class	Odour	Flavour	Colour	Remarks
Soup with bekai leaves	1	0	0	0	A unique smell/scent, savoury/tasty, and no colour changing
	2	0	0	0	
	3	2	0	3	
	4	7	4	2	
	5	11	16	15	
Soup with MSG	1	0	0	0	As usual/tasty, and more colouring
	2	0	0	0	
	3	4	0	7	
	4	6	7	2	
	5	10	13	11	
Soup without bekai and MSG (control)	1	0	0	0	As usual/commonly tasty, and no colour changing
	2	0	0	0	
	3	3	3	7	
	4	15	14	11	
	5	2	3	2	

Remarks : - 20 children in average 10-12 years old ; - Hedonic scale with 5 classifications (very dislike=1, dislike=2, neutral=3, like=4, and very like=5)

and also establishes a positive perception of a particular commercial environment (Bone & Ellen, 1999; Chebat & Michon, 2003).

The flavour or taste sense is the most important and most developed of all senses (Randhir et al., 2016). Organoleptic tests showed that the soup with the an addition of bekai leaves had a fresher, savory, and slightly sweet taste, when viewed from the children's perceptions as panelists who really like and have experience tasting in various types of soup. Furthermore, Randhir et al. (2016) stated that very few of our taste preferences are biologically preset. Much rather they are linked with some sort of experience. Once a flavour or food is accepted, this can also influence the preference for and acceptance of new flavours or foods. Another example of natural substance that could improve organoleptic test results is *Mentha arvensis*. The use of *Mentha arvensis* extract on whet-based pineapple mint beverages could improve the colour, taste, appearance and acceptability of the respondents (Kumar et al., 2017).

#### IV. CONCLUSION

A recent study showed that the qualitative screening of phytochemical compounds in bekai leaves ethanolic extracts revealed the presence of alkaloids, flavonoids, tannins and steroids. Antioxidants of bekai leaves using DPPH method showed high activity 68.1% in 100 ppm, 80.1% in 50 ppm dilution, and 83.2% in 25 ppm dilution, respectively. Ethanol leaf extract of bekai contained five major compounds, i.e. Oxirane dodecyl, gamma sitosterol, vitamin E ( $\alpha$  tokoferol), 9.12-Octadecadienoic acid (*Z, Z*)-(natural linoleic acid), and 3-Tetradecanynoic acid (myristic acid). Bekai leaves were considered to be one of the powerful antioxidants which can be used as a flavouring agent in cuisine and commonly used in some food industries.

Results of organoleptic (hedonic) tests presence in soup with bekai leaves added has been provided a unique smell, tasty without changing in original colour of soup compared with additional MSG and or control. It can be proved that soup with bekai leaves has been

a preference for children's taste perceptions, even though the current generation is very comfortable and accustomed to adding MSG to their consumed food. Thus, this study provided an innovative product of herbal medicine served in cuisine (soup) that it would be predicted to attract consumers to the new market opportunities, and to promote general positive image for early generation consumers to prefer more healthy food.

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