



E-ISSN: 2321-2187

P-ISSN: 2394-0514

www.florajournal.com

IJHM 2021; 9(4): 37-42

Received: 17-05-2021

Accepted: 19-06-2021

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A phytochemical screening of Bakkala (*Etilingera elatior*) originated from suakbugis, Aceh, Indonesia and its potential in ethnobotany

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Abstract

Etilingera elatior is commonly used as a spices mixed in food and vegetables by Acehnese people. Because these plant species has a strong scent that will make food tasty. However, the use of *Etilingera elatior* as a medicinal plant in Acehnese society is still limited. Hence, we need to reveal the potential of *Etilingera elatior* by phytochemical screening. This study aims to determine the secondary metabolites of *Etilingera elatior* originated from SuakBugis and its potential in ethnobotanical studies. The research was conducted in SuakBugis, Nagan Raya District, Aceh, Indonesia. The laboratory experiment was conducted at Regional Health Laboratory, Jakarta. These research was begun from September to October 2020. The results of phytochemical screening showed that leaves of *Etilingera elatior* detected chemical compositions *i.e.* flavonoids, phenols, tannins, steroids, saponins and alkaloids; *Etilingera elatior* flowers extracts detected the presence of flavonoids, phenols, tannins, terpenoids, saponins and alkaloids; *Etilingera elatior* fruit extract contains chemical substances *i.e.* flavonoids, phenols, tannins, terpenoids and alkaloids. Furthermore, hopefully, based on the chemical compositions of *Etilingera elatior* will bring benefit in ethnobotanical studies, especially traditional medicine and natural cosmetic ingredients.

Keywords: *Etilingera elatior*, Bakkala, Suakbugis, phytochemical screening, Acehnese

1. Introduction

SuakBugis is one of the region, which is located in Darul Makmur Sub-District of Nagan Raya District, Indonesia. The SuakBugis region included in Tripa Peat Swamp Forest, where Tripahas high biodiversity and the largest carbon storage in Aceh [1]. On the other hand, deforestation and forest degradation have occurred over much of Tripain the last one decade. It was probably lead to the loss of biodiversity every years [2-3]. In some areas has converted to palm oil plantations and community garden. However, Tripa still remains plant diversity in primary forests. One of the interesting plants to study in SuakBugis is *Etilingera elatior* Jack. RM Smith. The *Etilingera elatior* is a plant species belongs to Zingiberaceae Familia [4]. In 1980, Rosemary Margaret Smith from the Royal Botanic Gardens Edinburgh introduced these species as *Etilingera elatior*. According to ref [4], *Etilingera elatior* has several synonymous names *i.e.* *Nicolaia elatior*, *Alpinia nutans*, *Phaemeria speciosa*, and *Nicolaia speciosa*. Meanwhile, *Etilingera elatior* also has many local names, such as Kecombrang, Kincung, Honje, Keci-cang, Cekala, Bongkot, Katimbang, Tere, Bungong Kala, *SihalaDairi*, Patikala, Wualae, etc [5-8]. There were many scientific articles reported that the *Etilingera elatior* is native to Malaysia. However, ref. [9] revealed that *Etilingera elatior* has been well known in Indonesia for hundreds of years. The distribution of *Etilingera elatior* (Jack.) in Indonesia including Sumatera, Borneo and Java. Ref. [10] also reported that *Etilingera elatior* is native to Sumatera Island (Indonesia) and Malaysia. In Aceh, these plant is well known as Bakkala. *Etilingera elatior* or Bak Kalais commonly used as a spices mixed in food, such as *guleeplik u*, *guleeungkot*, *guleesieitek*, *sambaibungongkala*, etc. *Etilingera elatior* also has a strong scent that will make food tasty. On the other hand, some literature reported that *Etilingera elatior* is effective as a medicinal plant because of its chemical composition. The previous study revealed that *Etilingera elatior* has potential for the treatment of various diseases such as a heal wounds, breast cancer, diabetes, typhoid fever symptoms, etc [11-13]. In line to ref. [14] kecombrang flower (*Etilingera elatior* (Jack) R.M. Sm.) have been widely used by local people for cancer drugs, tumor and also as a natural cosmetic ingredient. According to [15-16], *Etilingera elatior* contains secondary metabolites *i.e.* glycosides, phenols, terpenoids, tannins, flavonoids, steroids and saponins. In line to [17], the *Etilingera elatior* flower extract contains complex

chemical substances such as flavonoids, tannins, saponins, alkaloids and terpenoids, whereas its leaves extract contain a lot of flavonoids active compounds [18]. Ref. [19] also reported the phytochemical screening results of *Etilingera elatior* flower detected the presence of flavonoids, quinones, tannins, saponins and steroids/triterpenoids. Many literature reveals the potential of *Etilingera elatior* as one of the native plants of Indonesia. However, studies on the phytochemicals of Bak Kala (*Etilingera elatior*) originated from Aceh, especially in SuakBugis relatively limited. Therefore, this study was carried out to determine the secondary metabolites of Bak Kala (*Etilingera elatior*) originated from SuakBugis, Nagan Raya District, Aceh, Indonesia. Furthermore, hopefully, the

secondary metabolites of *Etilingera elatior* will bring benefit in ethnobotanical studies, especially in traditional medicine and natural cosmetic ingredients.

2. Materials and Methods

2.1 Study Area

The research was conducted in SuakBugis, Pulo Kruet Village, Darul Makmur Sub-District of Nagan Raya District, Aceh Province, Indonesia. The laboratory experiment was conducted at the Regional Health Laboratory, Jakarta. These research was begun from September to October 2020. The sampling location of Bak Kala (*Etilingera elatior*) in SuakBugis, Nagan Raya District is presented in Figure 1.



Fig 1: Distribution Map Location of *Etilingera elatior* sampling in Nagan Raya District

3. Procedures

3.1 Sample collection

The research used purposive sampling method, where in those place is considered much overgrown by Bak Kala (*Etilingera elatior*). The specimens were obtained from seven different sampling areas. Data collection in the field is done by exploration and direct collection. Plants samples such as leaves, flowers, fruit and rhizome. The leaves, flowers, fruit and rhizome were taken randomly from each clump of *Etilingera elatior* and cut using a knife. In addition, all samples are stored in plastic bags.

3.2 Extraction of *Etilingera elatior*

3.2.1 Leaves

Samples of *Etilingera elatior* leaves were dried under UV light and then ground into a powder. Maceration was carried out using 75% ethanol as a solvent. The simplicia was poured into the flask and added 75% ethanol and left for 5 days. Shaken at 90 rpm for 20 minutes (twice). After 5 days, as much as 75% ethanol was added to the pulp and shaken to obtain macerate. The macerated was evaporated using a rotary evaporator at a temperature of 60-70°C to obtain a thick extract.

3.2.2 Fruit

Freeze-dried *Etilingera elatior* fruit powder was extracted using 0.1% HCl (v/v) in methanol with a ratio of sample and solvent (1:10). The extraction was carried out 2 times, the first was maceration for 24 hours at room temperature in the dark room, and the second was continued by maceration for 2 hours at room temperature in the dark room. The mixture was

separated using a centrifuge at low temperature (4 °C) at 4200 rpm for 20 minutes. The mixture was then filtered using Whatman filter paper No.1 by vacuum filtration. The filtrate obtained was transferred into an extraction flask and evaporated with a rotary evaporator at a temperature of 50 °C. The extract was kept refrigerated at -21 °C [20].

3.2.3 Flowers

As much as 10 grams of *Etilingera elatior* flowers were dissolved in 10 mL of 96% alcohol. Macerated for 24 hours at room temperature and not exposed to direct sunlight, then filtered. After that, the *Etilingera elatior* flower extract is ready to use [21].

3.3 Phytochemicals screening

The samples used for phytochemical screening were ethanolic extracts of leaves, flowers and fruits of *Etilingera elatior*. For each sample was carried out in Duplo.

3.3.1 Flavonoids Test

A total of 100 mg of extract was dissolved in 10 mL of methanol. Take 1 mL of extract, added 3 mL of methanol, 0.2 mL of 10% AlCl₃, 0.2 mL of potassium acetate, and added distilled water to 10 mL. Kept for 30 minutes in a dark place (room temperature). The absorbance was measured by UV-Vis spectrophotometry at a wavelength of 431 nm. As a standard used quercetin concentration of 10-60 mg/L. Total flavonoid extract was expressed in mg of quercetin equivalent (QE)/gram of extract dry weight [22].

3.1.2 Phenolic Test

The total phenolic content was measured using the Folin-Ciocalteu method. As much as 300 μ L of sample extract was poured into test tube. Mixed with 1.5 mL of Folin-Ciocalteu reagent (10 times dilution) and 1.2 mL of sodium carbonate solution (7.5% w/v). After 30 minutes, the absorbance was measured at a wavelength of 765 nm using a UV Vis spectrophotometer. For the preparation of the standard curve, a standard solution of gallic acid with a concentration of 30–80 ppm (mg/L) was used. Total phenols content is expressed in mg gallic acid equivalent (GAE)/ g dry weight extract [23, 20].

3.1.3 Tannins Test

As much as 1 mL of the test extract solution was reacted with 1% FeCl_3 solution. If a green-brown solution is formed, the tannins are condensed, whereas if it is blue-black then the tannins are hydrolyzed [24].

3.1.4 Steroids and Terpenoid Test

As much as 2 ml of the test solution was evaporated, the residue obtained was evaporated in 0.50 ml of chloroform, then 0.50 ml of anhydrous acetic acid was added. Then the mixture is dripped with 2 ml of concentrated sulfuric acid through the tube wall. If a bluish green colour is formed, it indicates the presence of sterols. If the results is a brownish or violet ring at the boundary of the two solvents it indicates the presence of triterpenoids [25].

3.1.5 Saponins Test

The extract was poured into a test tube, added 10 ml of boiling water and allowing to cool. Then, shaken vigorously vertically for 10 seconds. The presence of saponin is shown by a foam formation lasted more than 10 minutes which has 1-10 cm height. The foam does not disappear by adding 1 drop of 2N HCl [25].

3.1.6 Alkaloids

As much as 2 ml was evaporated on a porcelain cup until it got a residue. The residue was then dissolved with 5 mL of 2N HCL. The solution obtained was divided into 2 test tubes. Three drops of Dragendorff's reagent are added to the first tube. The second tube was added with three drops of Mayer's reagent. The formation of an orange precipitate in the first tube and a white to yellowish precipitate in the second tube showed the presence of alkaloids [25].

4. Results and Discussion

4.1 The Morphology of BakKala (*Etilingera elatior*)

Based on direct observation which took seven different locations in SuakBugis, Darul Makmur Sub-District of Nagan Raya District, Aceh, we found a lot of *Etilingera elatior* species distributed there. Generally, the species of *Etilingera elatior* found in SuakBugis grew as high as 3-4 meters, pseudo-stem, has upright stem with a midrib like a banana, forming rhizomes and green colour. The previous study reported that the height of the *Etilingera elatior* species can reach 2, 5-5 meters with an average of about 3 meters, upright stems and forming rhizomes, as well as has midrib [26-27]. The habitus of *Etilingera elatior* that are found in SuakBugis, Nagan Raya District is presented in Figure 2. Meanwhile, the plant specimens were collected from Suak Bugis are presented in Figure 3.



Fig 2: The plant of *Etilingera elatior* in SuakBugis, Nagan Raya District, Aceh, Indonesia



Fig 3: (a) leaves; (b) fruit; (c) flowers; (d) rhizome of Bak Kala (*Etilingera elatior*)

Based on Fig. 3 shows the leaves, fruit, flowers, and rhizome of *Etilingera elatior* collected from SuakBugis, Nagan Raya District. These plant parts were observed one by one based on its morphology. The morphological characters observed included the colour, shape, number and size of each organ. Generally, the *Etilingera elatior* leaves found in SuakBugis has the same colour as *Etilingera elatior* leaves that found in another place. Meanwhile, the leaves of *Etilingera elatior* originated from SuakBugis are single, leaves are green in colour and lanceolate shape (Figure 3a). The leaves have a length of 20-30 cm, whereas the width of the *Etilingera elatior* leaves of 5-15 cm. These plants have pinnate leaf bones and approximately 15-30 leaves arranged in two alternate rows. The leaves grow in two rows and grow alternately on the pseudo-stem. According to [28] the species of *Etilingera elation* with green leaves on the underside. The outer peduncle reaches 13 cm in length and curving outward when it blooms. In line to [29], *Etilingera elatior* leaves are lanceolate in shape, base and pointed tips of the leaf, as well as leaf buds are light green with yellow edges.

Based on the observation of *Etilingera elatior* fruit, it was found that there was one colour variation of the *Etilingera elatior* fruit, brownish yellow with a reddish tip and this fruit would be green with a dark brown tip when it is still young (Figure 3b). According to [30], *Etilingera elatior* does not have variations in the colour of fruits. The fruit size is small and grows in crowds [19] Reported that the fruit of *Etilingera elatior* looks like a pineapple. Although *Etilingera elatior* fruit found in SuakBugis only in yellow colour, but [28] revealed that the variations of colour in *Etilingera elatior* fruits includes pink, pale pink and white.

The *Etilingera elatior* flower was collected from SuakBugis only has one colour, name lyred (Figure 3c). The colour of Bak Kala flower is caused by anthocyanidins [31]. Anthocyanidins are natural pigments belonging to the flavonoid family. Actually *Etilingera elatior* has many variations of colours on its flowers *i.e.* red, pink, and white [32]. According to [28] the colour of *Etilingera elatior* flowers are pink, pale pink and sometimes white. Meanwhile, the flower of Bak Kala is a tubular compound and the peduncle have a length of 40-80 cm. The Bak Kala flower in flower-shaped collection, with bracts in elliptic-shaped, as well as fleshy. Crown in tubular-shaped, pink, up to 4 cm. The labellum similar to spatula shaped, about 4 cm in length, bright red with white or yellow margins. According to [33] the flower of *Etilingera elatior* in flower-shaped collection or called inflorescences. One inflorescence of Bak Kalacan exist in 3 or 4 months [10]. Reported that the young inflorescences looks like a spear-like head. Flowers stand on stems with a length of 0.8-2.0 meters. If we squeeze the bracts from the inflorescences, they will produce a strong aroma.

In this study, we are not only found leaves, fruit and flowers in SuakBugis but also found of rhizomes in *Etilingera elatior* clumps. Based on our observations to the rhizomes, it showed that rhizome has a diameter of 3-4 cm with a strong aroma. Rhizome was found in SuakBugis close to the soil surface. The rhizome has a reddish colour. According to [4] the rhizome is thick, yellowish white or reddish when young.

4.2 Phytochemical screening

Phytochemical screening was carried out to determine the class of compounds contained in the simplicia leaves, flowers, and fruit of Bak Kala (*Etilingera elatior*). Phytochemical screening were included flavonoids, phenols, tannins, terpenoids, steroids, saponins and alkaloids. The results of the phytochemical analysis of the leaves, flowers, and fruit extracts of Bak Kala (*Etilingera elatior*) are presented in Table 1.

Table 1: Phytochemical Analysis of Bak Kala (*Etilingera elatior*) Originated from SuakBugis, Nagan Raya District, Aceh, Indonesia

Phytochemical components	Test method	Sample Code/ Ethanol Solvent		
		EL	EFL	EFT
Flavonoids		+	+	+
Phenols		+	+	+
Tannins		+	+	+
Terpenoids		-	+	+
Steroids		+	-	-
Saponins		+	+	-
Alkaloids	Mayer	+	+	+
Alkaloids	Wagner	+	+	+
Alkaloids	Dragendorff	+	+	+

Note: + present; - absent

EL: *Etilingera elatior* Leaves

EFL: *Etilingera elatior* Flower

EFT: *Etilingera elatior* Fruit

The analysis of phytochemical in the extract of *Etilingera elatior* leaves showed the presence of the following compounds such as flavonoids, phenols, tannins, steroids, saponins and alkaloids, but no terpenoids (Table 1). Therefore, the undetected terpenoid compounds were represented by the absence of a brownish or purple ring at the boundary of the two solvents. According to [34], the chemical composition found in the pseudo-stems, leaves, flowers and rhizomes of *Etilingera elatior* are saponins and flavonoids. These compounds are mostly found in the leaves [18] also reported that leaf extract on *Etilingera elatior* Jack. RM contain a lot of active flavonoid compounds. Based on phytochemical screening analysis of the Bak Kala (*Etilingera elatior*) flower ethanol extract showed the presence of secondary metabolites such as flavonoids, phenols, tannins, terpenoids, saponins and alkaloids, but no steroids (Table 1). In line to [17] revealed that the *Etilingera elatior* flower extract contains chemical substances such as flavonoids, tannins, saponins, alkaloids and terpenoids [19] also revealed that the phytochemical screening analysis of *Etilingera elatior* flower extract showed that detected compounds such as flavonoids, saponins, tannins, steroids and terpenoids, but no alkaloids were detected. Flavonoids in the ethanol extract of *Etilingera elatior* flowers were indicated by a yellow-orange colour change in the amyl alcohol layer [35]. The preliminary phytochemical screening will help to determine the content of secondary metabolites in plants. The results of phytochemical screening of fruit extracts (Table 1) showed that *Etilingera elatior* fruit extract contains active compounds, *i.e.* flavonoids, phenols, tannins, terpenoids, and alkaloids, but steroids and saponins were not detected. In line to [36], kecombrang fruit extract contains bioactive compounds such as flavonoids, tannins and triterpenoids.

5. Conclusions

Based on the results of morphological observations of the leaves, flowers, fruit, and rhizome showed that the *Etilingera elatior* leaves originated from SuakBugis are single, leaves are green in colour and lanceolate shape; *Etilingera elatior* fruit is brownish yellow with a reddish tip; the *Etilingera elatior* flower is red in colour; and the rhizome has a reddish colour. The results of phytochemical screening showed that the leaves of *Etilingera elatior* detected chemical compositions *i.e.* flavonoids, phenols, tannins, steroids, saponins and alkaloids; *Etilingera elatior* flowers extracts detected the presence of flavonoids, phenols, tannins, terpenoids, saponins and alkaloids; and *Etilingera elatior* fruit extract contains chemical substances *i.e.* flavonoids, phenols, tannins, terpenoids and alkaloids.

6. Acknowledgements

This study has been financially supported by Hibah Penelitian Kerjasama Perguruan Tinggi (PKPT) Kemenristek Dikti No. 089/SP2H/LT/DRPM/2020. The authors are very grateful to WRI (World Resources Institute). Our special thanks to Rayhannisa, S. Si who have helped during in the field.

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