Fruit and seed variation of wild nutmeg (*Myristica schefferi* Warb.) in South Aceh, Indonesia

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Abstract. Several species of the genus *Myristica* are found in Indonesia, one of which is Aceh wild nutmeg (*M. schefferi* Warb). This study aims to identify fruit and seed variations and chemical compounds that might be potential for further use. The research was carried out at a hilly area in Lubuk Arang Village, South Aceh in the year 2017. There is a high variation of the 42 trees observed in yield, fruit, seed, and mace weight, meanwhile low variation in flesh thickness. Plant size, leaves, fruits, and seeds are bigger than other types of nutmeg. In general, the fruit shape was round to ovate with greenish when young and brownish-green in ripe fruit. The seed shape was rounded to oval with dark brown color when mature. Mace net covered the seed with an orange color when mature. Flesh fruit, mace, and seeds were not aromatic. The main chemical compound of seed ethanol extract analyzed using GCMS was *Otobaine*, Heptaleno (1,2-C) Furan 1-3H)-one-3,3-dimethoxy6,7,9,11-tetramethyl-, Palmitic acid, and Austrobailignan-6, while in mace were Dihydrojasmone, *Otobaine*, Palmitic acid, cis- Vasenic acid, and Austrobailignan. Cluster analysis of 42 trees based on fruit and seed characters, separated into three groups with a similarity of around 50%.

1. Introduction

Nutmeg known used as a spice since pre-historic times. Several species belong to the genus *Myristica* found in Indonesia [1] i.e. *Myristica argentea* Warb. *M. fatua* Houtt, *M. schefferi* Warb., *M. speciosa* Warb., *M. succedanea* Bl., *M. littoralis* and *M. teysmannii*. Distribution of *Myristica argentea* Warb. in the West Papua, while *M. schefferi* Warb is in the Moluccas and Sumatra. Identification of nutmeg population at Tidore and Patani, Central Halmahera, North Moluccas [2], consists of *Myristica fragrans*, *Myristica fatua*, *Myristica argentea*, *Myristica succedanea*, and several unknown nutmeg species [2].

The genus *Myristica* is perennial trees, with monopodial branching, single leaf alternates, with a slightly rough underside of the leaves, pointed leaf tip, glandular or fluffy. The flower is axillary, consisting of 2-4 flowers. The seeds have a hard seed coat, covered with aril containing aromatic oil or fat [3].

In Western Ghats India, there are five wild nutmeg populations, i.e. *M. malabarica* Lam, *M. fatua* Houtt, *M. beddomei* King, *Gynmacranthera farquhariana* Warb, and *Knema attenuate* Warb. [4]. The populations are fragmented, and the size has become smaller due to over-exploitation for seed arils used in commercial and medicinal applications.

Aceh wild/forest nutmeg (*M. schefferi* Warb) is a perennial tree with a plant height of up to 20 meters, even more. This plant is usually grown in the same area as common nutmeg (*M. fragrans*) on a sloping...
edge of land and is not easy to reach. It even can grow well on rocks. Its grow spotted and separated, there are not many crops that are in the same location area. In general, the species is underutilized.

Traditional people at South Aceh usually used tree trunks for building wood. Seed and mace mix with common nutmeg, which should not be allowed because it may reduce the quality of nutmeg seeds. After all, the constituents may be different. Recently, people also used forest nutmeg seeds as a rootstock on nutmeg grafting to overcome root diseases.

This study aims to identify fruit and seed variation among Aceh wild nutmeg trees based on morphological characters and identify seeds and mace extract compounds related to the potential used as medicine.

2. Material and methods

Plant observation was conducted at the hilly area of Lubuk Arang Village, Tapaktuan, South Aceh, in 2017. As many as 42 plants were collected and recorded for the tree height, ages, canopy width, trunk diameter, and fruit yield. Location of wild nutmeg usually in the rocky slope and hard to be reached. It makes harvesting fruit very difficult. Only ten fruit tree-1 harvested then sorted for five fruit which already ripe for measurement. Bulk seed and bulk mace then sun dry and ethanol extracted for looking of the chemical compound using GCMS.

2.1. Plant morphological observation

Detail observations of Morphological characters were carried out by looking at fruit, seed, and leaves characters. The observed variable refers to the tropical fruit descriptor [5] in general. The observation on quantitative characters was conducted on fruit (weight, length, width, and thickness), seed characters (weight, length, width, and mace weight), and leaves characters (length, width, and petiole length). Data of 42 trees calculated for the means and standard deviation, and coefficient of variation using excel. The relationship among the selected trees based on fruit and seed was analyzed using the Statistical Tool for Agricultural Research (STAR) software to generate a dendrogram. Genetic variation among wild nutmeg trees calculated using Euclidean distance coefficient (Agglomeration method: Ward's method).

2.2. Chemical constituent of seed and mace identification

The seeds and mace ethanol extracts were analyzed using the GCMS (Gas Chromatography-Mass Spectrophotometry) (Shimadzu) to determine the chemical constituents. The GC conditions used are as follows: using a capillary column DB-5ms, inner column diameter 0.25 mm, film layer thickness 0.25 um, the gas carrier was helium at a pressure 100 kPa, injection volume 2 ul using with split injection technique. Identification of chemical components was by comparing fractions detected with library compounds by NIST (National Institute of Standard and Technology) based on the value of LRI (Linear Retention Indices).

3. Result and discussion

3.1. Plant and fruit characteristic

Aceh wild nutmeg is a tree plant. The plant's growth was varied because of the differences in ages and growing places. Plants observation results in Lubuk Arang, Tapaktuan, South Aceh showed that tree ages range from 17 – 80 years old, with a trunk diameter 40-200 cm and fruit yield per plant per year was 1000-7500 fruit (Table 1). Nutmeg tree which produced 3000 fruits have stability in bearing, possessing high mace and nut weight is considered as high yielder [6]. Among the 42 trees of Aceh wild nutmeg, there are 23 trees having yield 3000 fruit tree-1 or more, and only view plants yield more than 5000 fruit tree-1. Yield variation might be due to differences in plants' ages and genetics. Plants developed from many trees and seeds which are not homogenous yet.

Fruit shape is rounded to slightly oval with skin color greenish when young and getting tanned when ripe to greenish-brown color. Fruit skin is a bit downy, not as smooth as common nutmeg fruit skin. Fruits are grown in the axils of the leaves. An inflorescence consists of many flowers, but those develop into big fruit generally only 1 - 3 flowers inflorescence-1. The cut fruit releases rather sticky sap. Seeds are covered with aril, with creamy yellowish color when young and bright orange when ripe. The mace is very dense, almost covers the whole seed. Seed shape rounded to oval with light brown seed coat
color when young and tent to dark brown to black when ripe. Plants performance, fruit, seeds, mace, and leaves can be seen in Figure 1. The flesh fruit is thin, and the seed size is big.

| Characters                  | Range    | Average | Deviation | CV (%) |
|-----------------------------|----------|---------|-----------|--------|
| Plant ages (years)          | 17-80    | 43.21   | 15.05     | 34.83  |
| Plant height (m)            | 15-40    | 23.55   | 6.53      | 27.72  |
| Trunk diameter (cm)         | 40-200   | 112.1   | 42.33     | 37.36  |
| Yield per plant (fruit)     | 1000-7500| 3185    | 1437.3    | 45.13  |

Table 1. Plant and yield characteristic of Aceh wild nutmeg (*M. schefferi*).

Figure 1. *M schefferi* plant, flower, fruit, seeds, and leaves.

Measurement results of the fruit and seeds showed a high coefficient of variation in fruit, seed, and leaves characters with the value of CV > 10 (Table 2.). The Higher variation is in the weight of fruit, seeds dan mace. Fruit weight of Aceh wild nutmeg range from 116 – 206 gram per fruit, the fruit length 6.84 – 8.92 cm, and the width 4.76 – 6.78 cm. Tree-to-tree variation was recorded in fruit traits (fruit weight, pulp thickness, fruit diameter) within the population of *Trichoscypha acuminate* [7]. The fruit size of Aceh wild nutmeg is quite large. Common nutmeg (*M. fragrans*) in Aceh usually have fruit characters such as Banda variety. According to the description, the Banda variety has a fruit size of 59.50 ± 7.83 (SK number 4059/Kpts/SR.120/12/2009). Meanwhile, the size fruit of the Tiangau Agribun variety is 64.32 ± 8.70 g (SK number 48/Kpts/KB.010/3/2020). Another genus myristica that has a big fruit size is *M. argentea* Warb or Papua nutmeg. The fruit weights of the selected mother trees of Papua nutmeg vary from 93 -105 g with a fruit length of 6.5- 7.9 cm and fruit width 4.3-6.2 cm [8].
Table 2. Fruit, seed, and leaves characteristic of Aceh wild nutmeg (M. schefferi).

| Characters               | Range     | Average | Deviation | CV (%) |
|--------------------------|-----------|---------|-----------|--------|
| Fruit weight (g)         | 116 – 206 | 157.7   | 22.7      | 14.4   |
| Fruit length (cm)        | 6.84 - 8.92 | 7.91   | 0.62      | 7.84   |
| Fruit width (cm)         | 4.76 - 6.78 | 5.85   | 0.46      | 7.79   |
| Ratio L/W                | 1.10 – 1.71 | 1.36   | 0.16      | 11.46  |
| Fruit petiole (cm)       | 1.00 – 2.04 | 1.46   | 0.38      | 26.27  |
| Flesh thickness (cm)     | 1.20– 1.40 | 1.29   | 0.06      | 4.89   |
| Seed included mace weight (g) | 32.69 – 74.20 | 53.42 | 10.86     | 20.34  |
| Seed length (cm)         | 4.70 – 7.60 | 6.17   | 0.75      | 12.18  |
| Seed width (cm)          | 3.28 – 4.30 | 3.85   | 0.3       | 7.91   |
| Ratio L/W                | 1.26 – 1.90 | 1.57   | 0.19      | 12.07  |
| Mace weight (g)          | 7.90 – 23.61 | 14.48  | 4.98      | 34.46  |
| Leaves length (cm)       | 19.80 – 35.00 | 27.15  | 3.99      | 14.7   |
| Leaves width (cm)        | 7.04 – 13.00 | 9.91   | 1.76      | 17.81  |
| Ratio L/W                | 2.20 – 3.56  | 2.77   | 0.31      | 11.32  |
| Petiole length           | 1.30 – 3.12  | 2.29   | 0.55      | 24.19  |

The seed weight of wild nutmeg, including mace, is varied, ranging from 32.69 – 74.20 g. The larger size is almost similar to the weight of fruit of common nutmeg. The mace weighs 7.69 – 23.61 g. This weight is similar or even bigger to the weight of common nutmeg (M. fragrans) seed which is an average weight of around 10 g.

3.2. Cluster analysis

A dendrogram of 42 Aceh wild nutmeg generated based on fruit and seed characters demonstrated a high genetic similarity between HLM1 and HLM3 tree, RUD3, and RUD5 tree, and KSM5 and KSM7 with a similarity level of almost 100% (Figure 1). Nutmeg trees with many similarities in both characters mean having a close relationship. While genotypes that have little or no similarity, mean having a distant relationship [9]. A dendrogram showed that at the similarity of about 50%, Aceh wild nutmeg trees were divided into three groups. Group I consisted of 13 trees, i.e. BHT, MSR2, AZM4, MSR1, AZM1, JPN1, AZM2, ARM2, JPN3, JPN2, ARM1, AZM3, and RHM tree. Group II consisted of six trees i.e. JML, KSM1, HLM1, HLM3, HLM2, and JSM tree. While group III consisted of 23 trees i.e. KSM4, RJD3, RJD5, RJD4, RJD1, RJD2, JSM1, ABD, JSM2, ZND, RSL1, PPT, KSM3, SRY, WSN2, KSM6, WSN1, BKH1, BKH2, KSM2, RSL2, KSM5, and KSM7 tree. Overall, Aceh wild nutmeg trees from the same field and farmer have a close relationship and were in the same group except for KSM. KSM1 has a distant relationship among other KSM's nutmeg trees. KSM1 at group II but KSM2-KSM7 at group III. Fruit and seed variation between KSM1 and other KSM's nutmeg trees reached 60%. Nutmeg is usually developed by seeds. Aceh wild nutmeg plants belonging to the same farmer tend to be closely related probably because of the seeds that grow come from the broodstock around the land.
3.3 Seed and mace chemical compound

Fruit, seed, and mace of wild nutmeg *M. schefferi* have no aroma. Both the seed and mace are slightly hard tough and sticky when squeezed. In contrast to the common nutmeg (*M. fragrans*), which have an aromatic flavor and are not sticky when squeezed. Based on GCMS analysis, there are 23 compounds identified in the ethanol seed extract, meanwhile on mace extract were identified 21 compounds. The major compounds in the seed extracts were otobaine, Heptaleno (1,2-C) Furan 1-3H)-one, 3,3-dimethoxy 6,7,9,11-tetramethyl, palmitic acid, and austrobailignan-6 (Table 3.). The only compound found in the wild nutmeg and the common nutmeg is myristicin, but low concentration.

The majority of compounds found in wild nutmeg is different from the constituent found in the common nutmeg (*M. fragrans*). The main constituent of methanol extract of nutmeg seeds (*M. fragrans*) is apigenin, caffeic acid, catechin, ferulic acid, isoeugenol, eugenol, isorhamnetin, kaempferol, methyl eugenol, myristic acid, myristicin, oleanolic, ursolic acid, protocatechuic acid, quercetin, trimyristin [10][11]. Seeds methanol extract of nutmeg identified 10 major compounds i.e. difluorodi azene, methyl eugenol, benzene, 1,2-dimethoxy-4-(1-propanyl), myristicin, elemicin, methoxyeugenol, isoelminic, methyl tetradecanoate, tetradecanoic acid, and corynan-10,18,19-dihydro-10-methoxy [12].

The major constituent of common nutmeg of Banda nutmeg seed oils is alpha-pinene, beta-pinene, beta-phellandrene, terpineol [13]. Moreover, [14], major chemical compound identified in nutmeg germplasm vary, i.e. myristicin 1.50-17.0%, alpha-pinene 6.38-19.0%, sabinen 10-40%, terpin-4-ol 1.39-14% dan safrol 0.36-19.6%.

Otobaine could become a marker compound of the Myristicaceae family. It has been isolated from several species of the Myristicaceae, including the seeds of *Brochoneura acuminata* (Lam.) Warb. [15], *Iryanthera juruensis*. Its also detected in *Myristica cagayanensis* MERR, besides trimyristin, malabaricone, otobanone, cagayanin, and cagayanone [16]. There is no information yet in South Aceh regarding the use of wild nutmeg traditionally. In the tropical Andean region (Panama, Colombia, Peru, and Ecuador), otoba-wax a mixture of Iryanthera, Osteophloeum, Otoha, and Virola which have otobain compound is used as an exotic product for hair treatment [17]. Another Myristicaceae, *B. acuminata* reported traditionally use as wound healing and the methanol extract of seeds showed a potent scavenging activity [17].
Table 3. The seed chemical constituents of wild nutmeg *M schefferi* using GCMS analysis.

| No. | Chemical constituent                                      | RT  | Area (%) | Quality (%) |
|-----|----------------------------------------------------------|-----|----------|-------------|
| 1   | Tetradecanoic acid                                       | 26.397 | 0.52    | 99          |
| 2   | Palmitic acid                                            | 28.300 | 4.15    | 99          |
| 3   | Grape seed oil                                           | 29.341 | 1.94    | 97          |
| 4   | Octadecanoic acid                                        | 29.431 | 1.28    | 95          |
| 5   | Olealdehyde                                              | 30.189 | 0.26    | 94          |
| 6   | cis-13-Eicosenoic acid                                   | 30.279 | 1.24    | 95          |
| 7   | 2-Hexadecanoyl glycerol                                  | 31.023 | 0.63    | 91          |
| 8   | Otobaine                                                 | 31.161 | 16.69   | 96          |
| 9   | Heptaleno (1,2-C) Furan 1-3H)-one-, 3,3-dimethoxy6,7,9,11-tetramethyl- | 31.423 | 17.14   | 87          |
| 10  | Austrobailignan-6                                        | 31.610 | 4.92    | 64          |
| 11  | Otobaine                                                 | 31.768 | 36.02   | 58          |
| 12  | Austrobailignan-6                                        | 31.830 | 1.94    | 80          |
| 13  | Homovanilic acid methyl ester                            | 31.947 | 1.24    | 70          |
| 14  | Alizarin1,2-O-(phenylboranediyl)                         | 32.078 | 2.28    | 38          |
| 15  | Carbofuran-3-hydroxy-7-phenol                            | 32.209 | 0.5     | 80          |
| 16  | 2-methyl-3-acetyl-4,5,6,7-tetrahy-drobenzo (B) furan     | 32.375 | 1.67    | 38          |
| 17  | Piperonylamine                                            | 32.575 | 1.95    | 49          |
| 18  | Myristicin                                                | 32.644 | 2.47    | 55          |
| 19  | Galbacin                                                 | 32.837 | 1.01    | 93          |
| 20  | 10H-phenoxaphosphine,8-fluoro-10-hydroxy-2,4-dimethyl-10-oxide | 33.030 | 0.61    | 62          |
| 21  | 1,2-Di-4-Anisoyl-1,2-diazetidin-3-one                    | 33.368 | 0.31    | 25          |
| 22  | Stigmastan-3,5-diene                                     | 34.588 | 0.31    | 64          |
| 23  | gamma-sitosterol                                          | 37.360 | 0.93    | 99          |

The major constituent in mace extract are Dihydrojasmone (24.21%), Otobaine 18.79, Palmitic acid 16.59, cis- Vasenic acid (9.26%) and Austrobailignan (8.57%) (Table 3). Dihydrojasmone is not found in the seed of Aceh wild nutmeg. This compound can be found in *Polygonum hydropiper* [18], *Satureja hortensis* [19]. Jasmon has biological activity and can be used to decrease aphid growth and reproduction [20]. Meanwhile, austrobailignan has the potential for anticancer [21]. In Thai herbal medicine, Leard-Ngam remedy (LG), which is used for curing primary dysmenorrhea, leucorrhoea, and balancing women's health reported having eugenol, austrobailignan, aceto-eugenol, and piperine [22]. Based on the information available in the references on the use of several phytochemical compounds, the compounds found in Aceh wild nutmeg may be utilized for medicinal uses.

Table 4. The mace chemical constituents of wild nutmeg *M schefferi* using GCMS analysis.

| No. | Chemical constituent | RT  | Area (%) | Quality (%) |
|-----|----------------------|-----|----------|-------------|
| 1   | Tetradecanoic acid   | 26.403 | 0.44    | 99          |
| 2   | Ethyl palmitate      | 28.182 | 0.46    | 99          |
| 3   | Palmitic acid        | 28.444 | 16.59   | 99          |
| 4   | Hexadecanoic acid    | 28.734 | 0.05    | 99          |
Aceh wild nutmeg has high variation in yield, fruit, seed, and mace weight but is low in flesh thickness. Plant, leaves, fruits, and seeds size was larger category. In general, the fruit shape was globular to oval with fruit color was greenish in young fruit and brownish-green when ripe. The seed shape was rounded to oval with dark brown color when mature. Mace net fully covered the seed with an orange color when mature. Flesh fruit, mace, and seeds have no aroma.

The major chemical compound of seed ethanol extract detected using GCMS were Otobaine, and Austrobailignan-6, while in mace were Dihydrojasmone, Otobaine, and Austrobailignan. Based on the information available in the references on the use of several phytochemical compounds, the compounds found in Aceh wild nutmeg may be utilized for medicinal purposes. However, further studies are needed before such compounds are recommended.

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