Diversity and phytochemistry analysis in zodia plants organs (Evodia suaveolens)

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Abstract. The Zodia is one of the Indonesian plants from Papua, designated as biophytopharmaca because of its efficacy in preventing mosquito bites in the human body. The variety of types and levels of these organic compounds found in each organ of the plant zodia is the purpose of this study. The results showed that zodia plants dominated by hexane compounds were found in root bark (40.9%), stem bark (81.1%), leaves (88.2%), and seeds (90.2%). Then followed by benzene compounds respectively in the root bark organ (58.1%), stem bark (3.5%), seeds (1.5%), and leaves (1.9%). The total types of phytochemical compounds contained in the root bark organ (43 types), leaves (23 types), stem bark (18 types), and seeds (13 types). The special phytochemical found in root bark is Aristolone, in stem bark is Longiverbanon or Vulgarone B, in leaves is Evodone, and in seeds is Decosane. All types of phytochemicals are detected through GCMS method using GC-2010 and QMS-2010 type GCMS are potential as phytopharmaca if managed appropriately. By knowing the types and levels of phytochemicals found in each organ of zodia, it is expected to continue on drug research opportunity to manage it to be clinically useful for humans.

1. Introduction
The Zodia (Evodia suaveolens) plant is one of the Indonesian flora's riches from West Papua, which has been used by local people as traditional medicine. Classified as medicinal plants commodity by Director General of Horticulture Indonesia in 2016, it also acts as an ornamental plant or floriculture [1], both ornamental plants in pots and yards [2]. Zodia plants emit a distinctive aroma which is a volatile hydrocarbon compound, called volatile oil or essential oil [3]. Essential oils are beneficial for the plant itself, which attracts the attention of insects in the process of pollination, as a food reserve, affects transpiration, has a bitter taste, and smells fragrant. Essential oils from zodia have the potential as vegetable insecticides [4], and the linoil compounds contained there can inhibit the activity of gram-positive and gram-negative bacteria.

It is estimated that there are 13 species of zodia plants scattered in the Madagascar archipelago [4], including Evodia suaveolens, Evodia rutaecarpa, Evodia fattraina, Evodia sp. and so on which are shrub plants from the Rutaceae family, the Magnoliopsida class, and the Sapindales order. Plant height ranges from 50 cm to 200 cm with an average height ranging from 75 cm. Zodia leaves are flat elongated, rather flexible with yellowish green, the length of the leaves is approximately 2 to 20 cm, and can grow well at an altitude of 400 to 2000 M above sea level [1].
Due to the results of a test of the Spice and Medicinal Plant Research Institute (Balittro) by gas chromatography method, oil distilled from plant leaves zodia containing 46% linalool compounds and 13.26% α-pinene, where linalool is the main component of Zodia plant’s essential oil [5]. Maryuni's study found that essential oils from zodia leaves contained evodone or evodiamine compounds (72.32%), menthofuran (7.52%), Limonene (4.73%), curcume (4.28%) and fonenol (1.66%). While Handayani and Nurcahyanti [6] obtained essential oils from zodia leaf extract through maceration methods, including limonene compounds (2.6%), menthofuran (10.2%), benzofuranone (20.1%), and vitamin E (6.7%) [4]. Some literature sources state that the types of chemical compounds mentioned above have the potential to be anti-bacterial, insecticidal, anti-respirant, anti-migraine, anti-pain, reliever fever, itch reliever and bumps caused by mosquito bites, skin diseases, and anti-cancer [3]. The use of essential oils as drugs, insecticides, anti-inflammatory and so on is effective, considering that volatile compounds are natural materials that are easily biodegradable so that they do not pollute the environment and are relatively safe for humans because of their volatile residue [7].

Experiments to obtain essential oil extraction from leaf organs, root bark, stem bark, and seeds of adult zodia were carried out using maceration method, then analyzed by gas chromatography method (GC-MS).

The results of the research obtained will be studied on the diversity and phytochemical levels in each organ of the zodia plant, to obtain information on what organs are effective as phytopena. This research is expected to provide information about the potential of zodia plants as medicinal plants so that they can improve the phytopena industry in Indonesia, while at the same time increasing the variety of biological resources for improving the quality of national health.

2. Materials and Methods

2.1. Preparation of plant samples
The experiment was carried out in September-December 2018. Zodia plant samples were obtained from Turangga village, Lengkong sub-district, Bandung city. Adult zodia plants are approximately 4 (four) years old, the plant height is approximately 100 cm, has complete organs, namely roots, woody stems, leaves, and seeds. The plant was dismantled, separated from the root bark, bark, leaves, and seeds, washed, then dried air under the hot sun for 3-4 days. Each of these organs is then mashed and filtered into powder with an iron sieve with a diameter of 0.5 mm.

2.2 Making of ethanol extract
Distillation of essential oil extract from zodia plants was carried out at the Chemistry Laboratory of the Faculty of Mathematics and Natural Sciences, Padjadjaran University, Jatinangor. Refined plant samples were then weighed as much as 250 g for leaf organs, and each 100 g for root bark, stem bark, and seeds. Each sample was macerated with 80% ethanol as much as 400 mL with a ratio of 1:4 for 3x24 hours, then macerate was separated, and the remaining pulp was macerated again with 80% ethanol to obtain clear macerate. Then each macerate was evaporated with the help of a rotary vacuum evaporator at a temperature of 50 °C until a thick extract was obtained, then dried with a freeze dryer at a temperature of less than 40°C for 24 hours.

2.3 Making suspension of ethanol extract from zodia plant organs
Making suspension of ethanol extract from zodia plant organs, has been carried out at the Chemistry Laboratory of the Faculty of Mathematics and Natural Sciences, Padjadjaran University, Jatinangor. Into the ethanol extract of each zodia organ was given 0.5 g to 1 g of thickener agent CMC type (Carboxy Methyl Cellulose), given warm distilled water with a temperature of 60°C, stirred evenly, closed tightly, and left for 15 minutes until a transparent mass was obtained. After that the extract is added with a little distilled water, stirred quickly to be homogeneous and form a suspension. The suspension placed into a 10 mL vial then tightly closed.
2.4 Analysis of variety and phytochemical levels in each organ of zodia plants
The volatile compound suspension that has been obtained from 4 types of zodia plant organs, then detected by GCMS chromatography gas type GC-2010 and QMS-2010 was carried out at the Chemistry Laboratory of the Faculty of Mathematics and Natural Sciences Universitas Pendidikan Indonesia, Bandung.

3. Results and Discussion

3.1. Extraction component of volatile compound in zodia plant
The results of extracting essential oils from each organ of zodia plants, which are root skin, stem bark, leaves, and seeds with 80% ethanol solvent are presented in Table 1. below. Based on Table 1, it gives a signal that the phytochemical diversity or secondary metabolite compounds in zodia plants, are most commonly found in leaf organs and root bark.

| No. | Type of Zodia plant organ | Mass of sample (g) | Mass of extract (g) | % Result | Colour of zodia organ extract in ethanol solvent |
|-----|--------------------------|--------------------|---------------------|----------|-----------------------------------------------|
| 1   | Root skin                | 100                | 8.82                | 0.09     | Dark yellow                                   |
| 2   | Stem bark                | 100                | 6.06                | 0.06     | Light yellow                                  |
| 3   | Leaves                   | 250                | 22.94               | 0.09     | Black                                         |
| 4   | Seeds                    | 100                | 5.86                | 0.06     | Light brown                                   |

Source: Chemistry Laboratory, Faculty of Mathematics and Natural Sciences Padjadjaran University, Jatinangor, Indonesia. 2018.

3.2. Analysis of secondary metabolites of zodia plant organ in ethanol extract suspension
Each of 0.5 g to 1 g suspension of ethanol extract from zodia plant organ (root bark, stem bark, leaves, seeds) is separated by its metabolite components using GC-MS chromatography type GC-2010 at a wavelength of 400-700, column temperature 60°C, and pressure 80, 20 kPa. Phytochemical fractions were monitored through volatile gas chromatography method approach, and obtained various types of phytochemicals as presented in Table 2. below:

The diversity of phytochemical types as presented in Table 2 shows that all plant organs of zodia (root bark, stem bark, leaves, seeds) were studied, dominated by types of alkaloid compounds. Since the middle of the 20th century, alkaloid compounds found in plants, generally characterized by the presence of Nitrogen atoms forming heterocyclic and alkaline rings, have been known to be effective as anticancer. What types of alkaloid compounds are effective as anti-cancer, are now being studied [8].

3.3. Evodone compound found in leaves organ of zodia plant
Evodone alkaloid compounds or also called Evodiamine presented in Figure 1., have been used by society as traditional anticancer drugs, and or as additional chemotherapy to reduce the side effects of ameliorate compounds and inhibition of glioma proteins causes of mitotic tumor cells or cancer. It is predicted to have the same mechanism with staurosporinone to inhibit Glioma Protein (GLI) [9].

Studies on this alkaloid isolated from Evodia rutaecarpa has also known to show in vitro and in vivo activities to inhibit proliferation, invasion, metastasis, and apoptosis induction in several tumor cells such as breast cancer, prostate cancer, leukemia lymphocyte T cancer, melanoma cancer, backbone cancer, colon and lung cancer [10].

3.4. Aristolone compound found in root skin organ of zodia plant
One of the alkaloid compounds found is quite large in the root skin organ of zodia is aristolone (3 %), besides that aristolone compounds are also found in zodia stem skin organs. Aristolone has been reported
to have the potential as anti oxidant activity and identified to have free radical scavenging activity to superoxide, nitric oxide, and ABTS radical cation analyzed by LC/ MS method [11].

Table 2. Phytochemical diversity in each organ of zodia plant

| No. | Zodia Organ | No. Phytochemical type | Various of Phytochemicals |
|-----|-------------|------------------------|--------------------------|
| 1.  | Root bark   | 43                     | 2-propanone (CAS) Aceton; n-Hexane; Cyclopentane; 2,2-dimetil (CAS) Hexane; 1,2-dimetil Pentanol; 1,3-metil (CAS) Pentanol; Cyclopentanol; Benzene 1,2-dimetil (CAS) o-xylene; Benzene 1,2,3 trimetil (CAS); Benzene 2-etil-1,4-dimetil (CAS); Benzene 1-metil-3-propil (CAS) m-Propiltoluene; Benzene 1-etil-3,5-dimetil m-xylene; Benzene 1-etil-1,4-dimetil (CAS) p-cymene; Benzene 1,3 dietil-5-metil (CAS) 3,5-dietiltoluene; Benzene 1,2,4,5-tetrametil (CAS); Benzene 1-metil-4-1-metilpropil; Benzene dietil-metil (CAS) AR-dietiltoluene; Benzene 1-etiloctadecil (CAS) eicosane, 3-penil; Benzene 1,4-dimetil-2-1-metiletil (CAS) l-isopropil; Benzene 1-etil-2,4,5-trimetil (CAS); 1H-Indene 2,3-dihidrometil (CAS); 1-(2,4-dimetilpenil)-4-metilpentane; Ledene; Octadecane; Azulene; 19-di-torulosol; α-Gurjunene; δ-Gurjunene; Cyclopentane; Octadecane; Zierone; Procerine; 5-Eicosane (CAS); Aristolone; Ledenoxide; 2-Naphtalenol 1,4-chloropenil; 2H-cyclopropa(G)benzofurane; 1-Docosanol; (CAS) behenic alcohol; Isolantolactonoid butenolide A; 7-H-2,4 A-methanonaphtalen; Dehiydroindene. |
| 2.  | Stem bark   | 18                     | Hexane (CAS) n-Hexane; Hexane 2,2-dimetil (CAS) metil cyclopentane; Cyclopentane; Cyclohexane; 3-Dodecanol (CAS); Benzene etil (CAS) EB; Benzene 1,2-dimetil (CAS) o-xylene; Benzene 1,2,3-trimetil (CAS); Benzene 1-metil-3-propil (CAS) m-propiltoluene; Benzene 1-etil-3,5-dimetil (CAS) 4-etil-m-xylene; Benzene 2-etil-1,4-dimetil (CAS); Benzene 1,2,4,5-tetrametil (CAS); Benzene 1,2,3,4-tetrametil (CAS) prehnitol; Octadecane; Nonadecane; Vulgaron B; Aristolone; α–Copaene. |
| 3.  | Leaves      | 23                     | Asam Octan-2-Ynoic; Benzene 1-metil-4 (CAS) p-cymene; Benzene 1,2,4,5 tetrametil; Benzene 1,2,3,4 (CAS) prehnitol; Benzene 1,1-5 dimetil (CAS) ar-Curcune; Benzene 1 butil 4 metoxy (CAS) butane; β Himachalene; Evedone; n-Hexane; δ-Selinine; Furanone; α-Copaene; δ-Cadinene; Mint Furunanone; Naphthaleneamine; Neophytadiene, Cyclopentane metil (CAS); Cyclooctane 1-asam karboxil; metil ester asam Palmitate; 2-benzoifuran asam karboksil; 2-metil-4,2,6 trimetil cyclohexan. |
| 4.  | Seeds       | 13                     | n-Hexane; Hexane 2,2-dimetil (CAS); Cyclopentane; Benzene 1-etil-3-metil (CAS) m-propiltoluene; Benzene 1,2,3-trimetil (CAS); 1,2-Benzene dikarboksilikat; Tetradecane (CAS); 1-Hexadecene (CAS) cetene; Nonadecane (CAS); 1- α Octadecene (CAS); Eicosane (CAS); Decosane (CAS); 1-Eicosanol (CAS). |

Source: Chemistry Laboratory, Faculty of Mathematics and Natural Sciences Indonesia University of Education Bandung (2018)
3.5. Longiverbenon compound found in stem bark organ of zodia plant

The results of chromatographic analysis of alkaloid compounds that only exist in zodia stem bark organs, are longiverbenon compounds or also called vulgaron B. These compounds are derivatives of benzene compounds which are generally also found in other plants, such as *Justicia neesii* [12], *Pimpinella anisum* which has been known as a potential for anti-microbial, anti-protozoa, anti-fungi and anti-pathogens [13, 14]. The structure view of longiverbenon compounds or also called vulgaron B which is detected only on zodia bark is shown in Fig. 3.

3.6. Docosane compound found in zodia seed organs

Docosane is one type of phytochemical which is found quite a lot in the seed organ of zodia plants which also shows an antibacterial activity [15]. The chemical structure of the docosane compound is shown in Fig. 4.
Figure 4. Docosane compound in seeds organ of zodia plants

All zodia plant organs examined in this experiment, namely, root bark, bark, leaves and seeds contain volatile compounds of the type cyclopentane which are known to function as antifungi and anti-bacteria, while octadecane compounds are also found in all organs except leaves, which have the potential to be broader than cyclopentane compounds, namely as antifungal, antibacterial, antitumor, anticancer, antioxidant, and antimicrobial [15].

4. Conclusion
The four root bark organs, stem bark, leaves, and seeds from adult zodia plants were tested by maceration method, then analyzed by GC-MS gas chromatography, having phytochemical diversity of volatile oil groups that differed in type and level, and each of these compounds has the potential to be used as a raw material for the drug and food industry, mainly because of its function as a vegetable insecticide, antibacterial, antifungal, anti-cancer, and anti-phatogen.

It needs to be further investigated regarding the improvement of zodia plant cultivation by utilizing various biological microorganisms as a trigger so that zodia plants can produce large quantities of pharmacically potent metabolites.

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