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Secondary metabolite compounds of *Moringa oleifera* leaves in two different urban altitude locations, Indonesia

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ABSTRACT

Moringa plants from the Moringa oleifera species are widely cultivated and used by residents in tropical regions, including Indonesia. Indonesians generally in rural areas use this plant as food and traditional medicine. The development of knowledge followed by the results of recent experiments, shows that all moringa organs contain secondary metabolite compounds that are beneficial to humans, especially as a source of good nutritious nutrition, anti-allergic, anti-inflammatory, antihistamine, anticancer, and able to increase the body's immunity. The cultivation of moringa as yard plants in housing is still not popularly carried out by urban residents. In addition to functioning as a source of food, medicine, it is also able to function ecologically for the surrounding environment. The adaptation of growing moringa in an altitude area is quite wide, ranging from 0-1000 m above sea level. This study, conducted in March-April 2022, aimed to analyze the type of diversity and concentration of secondary metabolite compounds in the moringa leaf organs planted at two different altitudes, including the origin of housing in Turangga Village, Bandung City at an altitude of 696 masl and the origin of housing in Kenjeran Village, Surabaya City at an altitude of 4 masl in Indonesia. The analyses of moringa leaf extract through a QP-2010 type chromatography tool identified more phytochemical diversity than those from Bandung, including carbinol, methyl cyclopentane, n-Hexane, neophytadiene, hexa decane, pronalol alaniol, methyl palmitate, and eicosyne. Meanwhile, moringa leaf extract from Surabaya, the dominant type of carbinol was identified, followed by acetic acid, and methyl hydroxylate. The results of the analysis recommend that moringa is effectively used as urban farming to meet food sources, while improving the ecological quality of the surrounding environment. Meanwhile, to improve the quality of phytochemical diversity, it is recommended that the cultivation of moringa should be in moderate rainfall areas with average daily air temperatures ranging from 20 ºC to 30 ºC and high soil moisture.

Keywords: Plant, Altitude, *Moringa oleifera*, Traditional medicine, Extracts, Compounds.
Article type: Research Article.

INTRODUCTION

The moringa plant, *Moringa oleifera* Lam is one of the species of the Moringaceae family that is widely cultivated and used by people in tropical regions, including Indonesia (Santoso 2020). Moringa in Indonesia is generally able to adapt well to almost all types of soil,including marginal soil types, however, will grow more fertile if cultivated on land with a slightly acidic to neutral pH with low to moderate soil moisture, as well as a height of places above sea level up to 1000 m (Kurniasih, 2020; Winarno 2018). Based on regional diversity in Indonesia, moringa has different names, including moringa (in Java, Sundanese, Balinese, Lampung), murong (in Aceh), maronggih (in Madura), moltong (in Flores), keloro (in Bugis), parongge (in Bima), kawona (in Sumbawa), and kelo (in Ternate; Isnan *et al*. 2017; Kurniasih 2020; Plantation Development Research Center 2021). Indonesian people, generally in rural areas, have used several moringa organs as food consumption materials, especially leaf organs, young seeds, flowers, and buds. Leaves, flowers and leaf buds are usually consumed after cooking, but young seed linings are often consumed raw. Meanwhile, Indonesian people in urban areas, still rarely cultivate and utilize moringa organs for these cultivated products, perhaps since they have not been socialized about moringa cultivation techniques on narrow land in urban yards. Based on the results of research by the Plantation Development Research Center (2021), moringa is useful for lowering blood sugar levels, overcoming inflammation, controlling blood pressure, maintaining brain health and function, inhibiting the growth of cancer cells, and increasing endurance. Likewise, the Ministry of Health of the Republic of Indonesia (2021) has recommended that consuming moringa leaves is an effort to prevent attack of the COVID-19 virus. The Covid-19 pandemic that has hit the world since the beginning of 2020 so far, is still an unresolved problem. From time to time more and more human earthlings contracted the coronavirus, leading to death, although many humans recovered and were able to resume their activities. In Indonesia, the latest data at August 22, 2022, the confirmed population of Covid-19 disease is 6,319,506 people, 6,107,680 people recovered, and 153,777 people died (Antaranews 2022). The Corona virus attack will occur if a person's immune system is low while in an environment polluted by the Covid-19 case. Basically, a person's immune system depends on mental health in accepting living conditions so as to avoid stress, consumption patterns of balanced nutritious food, physical activity in maintaining fitness, and a clean and healthy living environment (Winarno 2004; Al-Hadidy & Mostafa 2022). Consuming a variety of foods of high nutritional value with balanced calorie levels for one's activities is a top priority that should be considered, so that one's immune system is always in prime condition. The development of knowledge and experiments about the benefits of moringa, now various types of moringa processed foods are gaining popularity, e.g., mixed for biscuit mixtures, cakes, noodles, salads, vegetable soups, *lodeh* vegetables, refreshing drinks, or other culinary variations (Wickramasinghe *et al*., 2020; Zula *et al*. 2021). As a raw material for traditional medicine, food and refreshing drinks, all moringa organs can be utilized including the roots and bark of the trunk (Karyasari 2005; Wickramasinghe *et al*. 2020). Climatic and soil factors at the site of plant cultivation are external factors that play a role in determining the type of phytochemical contained in a plant. Altitude factors will affect climate factors, especially the average daily temperature, sunlight intensity, rainfall, and soil fertility rate. In addition, the greenhouse effect (global warming) in urban areas plays quite a role in air quality and elements of absorption by plants. Therefore, this study aimed to examine the extent of the difference in altitude at two planting locations in urban areas, including Bandung City (West Java), and Surabaya City (East Java), both are taken from residential hedges, to the phytochemical productivity of moringa leaves. The results of the analysis were obtained in order to add recommendations for the selection of moringa cultivation sites in urban areas (urban farming), and at the same time obtained information on the type of diversity and optimal phytochemical concentration of moringa leaves from two different planting locations altitude. By spreading this information, it is hoped that urban communities will be interested in cultivating moringa as yard forage and at the same time, utilizing moringa leaves as a source of cheap nutritious food, so that they can increase the body's immunity, especially during a pandemic.

MATERIALS AND METHODS

Moringa leaves as samples were picked from two different trees, and came from two different planting locations, namely Turangga Village, Bandung City by a place height of 696 masl and Kenjeran Village, Surabaya City by 4 masl. In each location we took 500 g fresh moringa leaves, then let them to be dried, macerated method (Handayani & Nurcahyanti 2015), evaporated and analyzed with a QP-2010 type GCMS chromatography tool. The two samples were picked in March 2022 (Bandung) and April 2022 (Surabaya) respectively.

Preparation of dried samples of moringa leaves

Adult moringa plants were approximately 4 years old, plant height was approximately 5 m, had sturdy stems, and were leafy lush. The leaves were plucked from secondary branches, bright green in color, while part of the petiole was removed. The leaves were thoroughly washed, dredged for a moment, then put in a special drying oven at a temperature of 50 ºC for 3 × 60 minutes. After the dry leaves were broken, they were smoothed to a powder, then passed through an iron mesh 3 mm in diameter. Preparation of fine dry leaf samples, each carried out in the cities of Bandung and Surabaya in Plant Physiology Laboratory, University of Insan Cendekia Mandiri, Bandung.

Preparing moringa leaf extract with methanol solvent

Distillation to obtain essential oil extracts from moringa leaves was carried out at the Chemical and Service Applications Laboratory, Faculty of Mathematics and Natural Sciences, Padjadjaran University. Samples of moringa leaves were finely weighed as much as 100 g followed by the maceration process with 80% methanol solvent as much as 400 mL or in a ratio of 1: 4, then the macerate was separated from the rest of the pulp until a clear macerateis obtained. Afterward, the macerate was evaporated with the help of a rotary vacuum vaporizer at a temperature of 50 ºC until a viscous extract was obtained. Thereafter, it was re-evaporated with an evaporator at a temperature of less than 40 ºC for 3 × 24 hours until the extract becomes completely dry.

Analysis of the variety and phytochemical levels of moringa leaf

The moringa leaf extract obtained was then examined to identify the type and level of phytochemicals contained in it using the QP-2010 type GCMS phenatographic gas method with pure methanol solvent, and was carried out at the Instrument Chemistry Laboratory of the Faculty of Mathematics and Natural Sciences, Universitas Pendidikan Indonesia, Bandung.

RESULTS AND DISCUSSION

Phytochemical analyses of moringa leaf through the gas chromatography method identified several different chemical compounds, both types and concentrations of moringa leaves grown in the Bandung and Surabaya cities. Table 1 depicts the phytochemical types of moringa leaves from two different planting sites. Based on the chromatograms read on the chromatography tool, there were differences in the types and concentrations of chemical compounds in moringa leaves originating from these two cities. The occurrence of differences is normal, since there are external factors that play an important role in influencing the growth and development of a plant. The difference in the height of the growing place (altitude), affects climate differences, especially temperature, air humidity, soil moisture, and rainfall.

Table 1. Types of phytochemicals identified in moringa leaves at different altitude locations.

No.	ytochemicals identified	Origin: Bandung	ntration(%)	Origin: Surabaya	ntration (%)
1.	Carbinol	√	34.48	√	99.40
2.	Acetic acid			√	0.31
3.	1-Propanol-alaniol	√	1.07	√	0.18
4.	Methyl hydroxylate	√	0.07	√	0.11
5.	2,3 Methyl cyclopentane	√	36.04		
6.	n-Hexane	√	25.58		
7.	Benzene	√	0.44		
8.	Neophytadiene	√	0.70		
9.	Hexadecane	√	1.23		
10.	Methyl palmitate	√	0.09		
11.	Ethyl linoleate	√	0.20		
12.	Eicosyne	√	0.10		

Description: The concentration value is the percentage value of phytochemicals based on the area of the area depicted on thechromatography tool.

Carbinol compound (C_9H_9NO) is the dominant type of phytochemical identified in moringa leaves studied in two different locations. It is an organic compound resulting from secondary metabolites of the long-chain alcohol group, and this type of alcoloid and flavonoid compound is also commonly found in green vegetable plants, including broccoli, mustard greens, kale, and cauliflower (Agustin 2020; The Francis Crick Institute 2022). Carbinol compounds contain complete organic nutrients, including carbon, hydrogen, oxygen, and nitrogen which serve as the main constituent components of complex compounds of carbohydrates, fats, and proteins resulting from the metabolic activities of plants rich in chlorophyll. The difference in the number of atomic elements occurs due to the dehydrogenation process or due to the release of nitrogen elements from carbinol compounds. Some of the derivatives of carbinol compounds include carbinolamine, carbinosamin, propenyl alcohol, allyl alcohol, and iso-propanol. Mandrich & Caputo (2020) conclude that carbinol generally has enzyme activity with a role as an oxidative anti-stress, so that it can play a role in preventing cell damage and other cellular components by active free radicals, so that it can inhibit the growth of cancer cells. All types of carbinol derivatives, generally have the potential for activity as a dehydrolase enzyme in the process of amino acid formation, produce little saturated fat, low triglycerides, high potassium, iron, and vitamin C (Ikrarwati & Anisatun, 2018). Judging from the complexity of nutritional value, carbinol compounds can be ensured to be one of the best sources of nutrition for humans, as well as providing pharmacological effects as antioxidants, antihistamines, anti-inflammatories, antidiabetic, anti-asthmatic, anti-allergic, analgesic, and enhances the body's immunity (Razis *et al*. 2014; Paikra *et al*. 2017). Indole-3-carbinol compound is one of the derivatives of carbinol compounds, often effective for suppressing the growth of abnormal cells in people with cervical cancer, and colon cancer, reducing nausea in pregnant or breast feeding mothers (pregnant and lactating women). The chemical bonds in this compound are stable, play a role in the metabolism of estrogen hormone which is one of the important hormones in supporting women's health (The Francis Crick Institute 2022). Chromatograms of the phytochemical types identified in moringa leaves from Bandung City presented in Figs. 1-4.

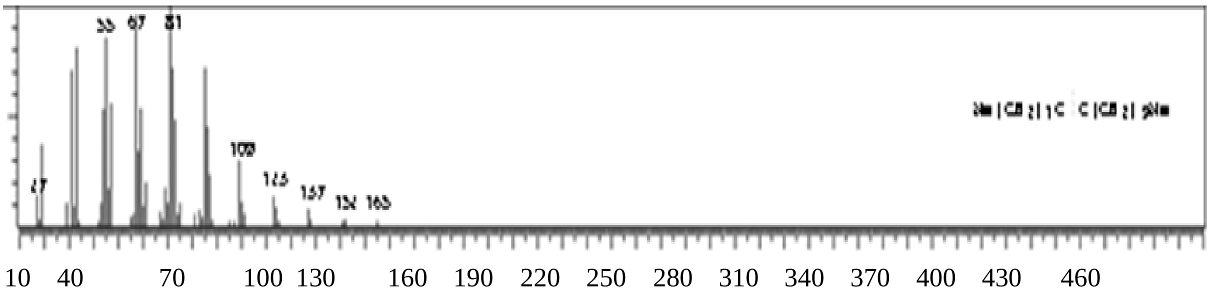

Fig. 1. Chromatograms of the phytochemical types identified in moringa leaves from Bandung City: Methyl cyclopentane compound.

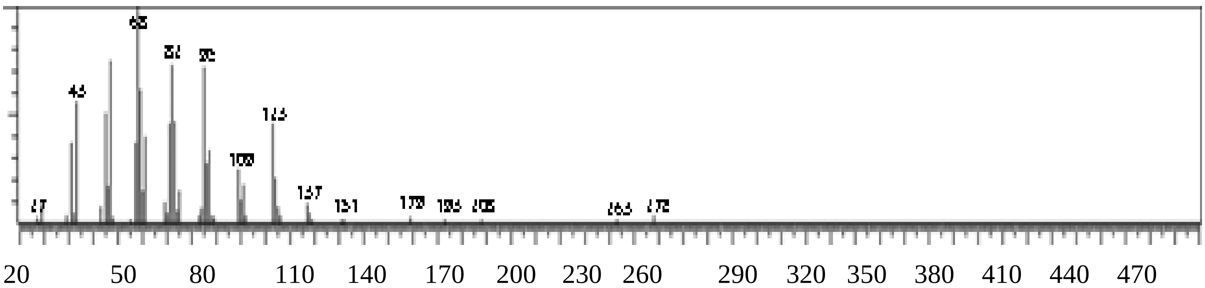

Fig. 2. Chromatograms of the phytochemical types identified in moringa leaves from Bandung City: Neophytadiene compound.

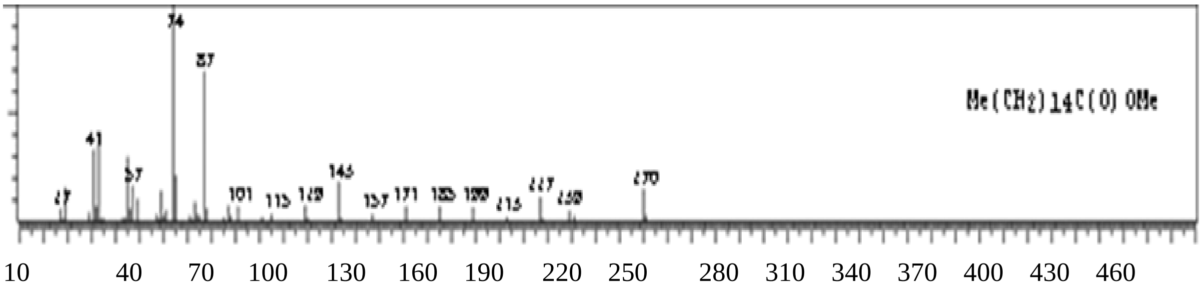

Fig. 3. Chromatograms of the phytochemical types identified in moringa leaves from Bandung City: Methyl palmitate compound.

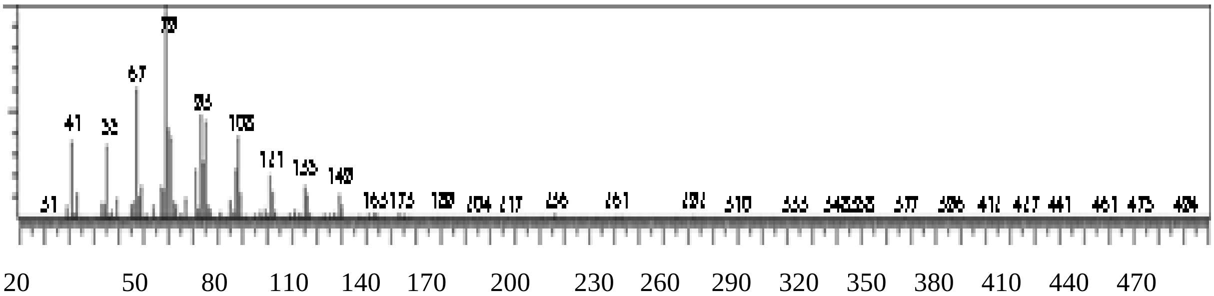

Fig. 4. Chromatograms of the phytochemical types identified in moringa leaves from Bandung City: Ethyl linoleolate compound.

The four types of phytochemical compounds identified in the chromatogram above are the initial compounds that form secondary metabolites from the starch group, proteins, essential elements, vitamins, and the rest of the metabolites in the form of essential minerals. The four types of phytochemicals are identified, which are typical starting compounds that will then react to the role of enzymes as catalysts. So that, the end of the reaction will produce several types of secondary metabolite compounds from the flavonoid, terpenoid, saponin and steroid groups. The results of this analysis support the results of previous studies by experts that moringa leaves, and seeds contain high fibre, protein, minerals such as Zn, Mg, Fe, β carotene, vitamin A, B, C, D, E, and K, active compounds inhibiting LDL cholesterol, as well as antioxidant compounds from the moringinin, quercitine, rhamnetin, and polyphenol groups (Dhakar *et al*. 2011; Melesse *et al*. 2012; Winarno 2018; Saa *et al*. 2019; Kurniasih 2020). The type of arginine protein and polyphenol compounds found to be quite high from moringa leaf extract is the main key that causes moringa leaves to respond to an enhanced immune system. The linoleic acid compound identified in moringa leaf extract from Bandung City, is a type of omega-6 essential fatty acid needed by our body, since the body is not able to synthesize itself, however should receive intake from food sources. Linoleic acid plays an important role in brain development as well as child growth and development. Palmitate acid compounds, also identified in Moringa leaf extract from Bandung City, are one of the plant fatty acids that are often found from the Palmaceae family, such as coconut and oil palm. Palmitic acid is a type of saturated fatty acid, often used as a raw material for the cosmetic industry, especially soap or other types of cleansers, since it has properties as a surfactant that can maintain skin moisture. Chromatograms of the phytochemicals type identified in moringa leaves from Surabaya City were presented in Figs. 5-6.

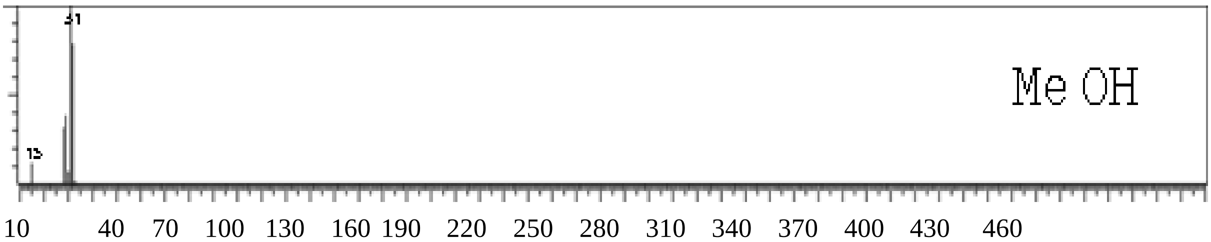

Fig. 5. Chromatograms of the phytochemicals type identified in moringa leaves from Surabaya City: Carbinol compound.

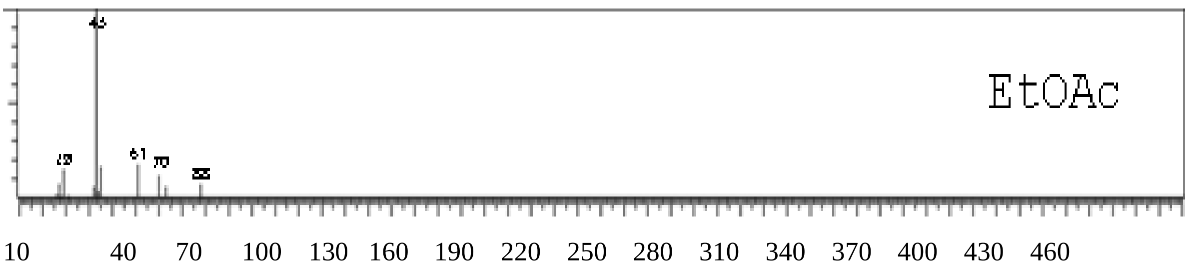

Fig. 6. Chromatograms of the phytochemicals type identified in moringa leaves from Surabaya City: Acetic acid compound.

The type of carbinol compound ranks as the highest percentage of phytochemical diversity was identified in Moringa leaf extract from Surabaya City. Although isomers or derivativesof carbinol compounds are quite a lot, however, in chromatograms not perfectly legible. Nevertheless, it can be ascertained that the long-chain methyl alcohol compounds identified are a type of phytochemical that contains many benefits as a source of food, medicine and cosmetics. Acetic acid compounds are one of the organic compounds from the primary metabolite group. These compounds are acidic and act as a confer of sour taste and aroma in processed foods. As a raw material for the medicinal industry, acetic acid compounds can be used as antibiotics, since they are quite effective in treating fungal and bacterial infections. Secondary metabolites are compounds that plants produce through metabolic pathways in contrast to primary metabolite pathways in metabolism activities. In addition, their presence in small quantities is considered not so important to affect the growth and development of plants. Not all higher plants are capable of producing secondary metabolite compounds, and generally secondary metabolites will be synthesized when plants experience a stressful condition (Kusbiantoro & Purwaningrum 2018). Based on the results of the analysis of the type and phytochemical concentration of moringa leaf extract identified from two different planting locations, i.e., the Bandung and Surabaya cities. It appears that these differences are more influenced by differences in external factors, especially the average daily temperature, air humidity, rainfall intensity and the dominance of urban pollutant compounds. In general, it has been recommended that moringa can be cultivated in tropical or sub-tropical climates with the analtitude of 0-1000 masl. However, the results of the analysis using a QP-2010 type chromatography tool inform that the diversity of identified phytochemical types derived fromthe Bandung City sample is more complex than those from Surabaya City. The average daily temperature of Turangga sub-district, Bandung City when samples were picked ranged from 18ºC to 28 ºC with rainfall of 181-409 mm month^{-1} (March 2022) (BMKG Bandung City 2022). Meanwhile, the average daily temperature of Kenjeran village, Surabaya city when the sample was picked was 26ºC to 34 ºC with rainfall of 151-200 mm month^{-1} (April 2022; BMKG Surabaya City 2022). Thus, the diversity of differences in secondary metabolite compounds of Moringa leaf extract is influenced by external conditions, including altitude which is automatically followed by differences in temperature andrainfall at local planting sites.

CONCLUSION

Based on the results of phytochemical analysis through a QP-2010 type chromatography tool, information on differences in the variety of types and concentrations of secondary metabolite compounds produced from moringa leaf extract was planted in two different locations, including Turangga Village, Bandung City (696 masl) and Kenjeran Village, Surabaya City (4 masl). The altitude of moringa cultivation sites in the Bandung City with relatively lower daily temperatures, monthly rainfall and relatively higher soil moisture compared to those of Surabaya City is quite representative of the recommendations for adaptation to the growth of moringa cultivation on residential vacant land, as an effort to increase sources of high nutritionand urban farming efforts to improve ecological quality.

SUGGESTION

The results of this study obtained are important information for the people of Bandung City, especially to be used as a consideration for moringa cultivation as a greening plant in a residential environment. So that, leaf production can be used as a source of high-nutrition food for efforts to increase body immunity, excellent health for pregnant and lactating women, and support early childhood growth and development. Meanwhile, to improve the quality of phytochemical diversity, it is recommended that the cultivation of moringa plants be in moderate rainfall areas with average daily air temperatures ranging from 20 ºC to 30 ºC and high soil moisture. The delivery of this information will be more advisable through the activities of PKK mothers in Turangga village, Lengkong district, Bandung City.

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