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Effect of Extraction Time And GC-MS Analysis of Fresh and Dry Frangipani Flower *(Plumeria rubra* L.*)* using Ultrasonic Assisted Extraction

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Abstract: Frangipani flower (Plumeria rubra) is one of the plants that has a role in perfume and aromatherapy technology which have essential compounds that are the main cause of fragrant smelling flowers. Optimization of extract production needs to be done to obtain a high content of active substances. Ultrasonic assisted extraction is a widely used method for extraction of active compounds in certain products due to shorter operating times. This study aims to determine the effect of variations in extraction time 30,45,60 minutes from fresh and dry frangipani flowers using ultrasonic methods on extract yield values and chemical compositions using the GC-MS method. The results showed that the 60 minutes extraction produced the highest yield values, namely 5.11% for fresh yellow frangipani flower extract and 3.43% for dried yellow frangipani flower. The results of the chemical content study using GC-MS showed that the constituent compounds of fresh yellow frangipani flower essential oil consisted of fatty acids (6.59%), alkanoic acids (4.43%), triterpenoids (1.42%) and floral compound groups. Dry frangipani consists of alkanes (7.47%), alcohol (13.31%), fatty acids (1.00%).

Keywords: *Extract, Flower, Frangipani, Hexane, Ultrasonic.*

1. Introduction

People have used plants as a basis for medical remedies since the beginning of human history. The genus Plumeria comprises a large number of species widely distributed throughout the world of which 11 species grow in tropical and subtropical regions of the world. *Plumeria* is a genus of the *Apocynaceae* family, native to the Caribbean, Central America, Mexico and South America and distributed throughout the world. The genus name *plumeria* is taken from the 17th century French botanist Charles Plumier who traveled to America to document plants and animals (Puspita and Nuraini, 2020).

Frangipani is a flowering plant that grows a lot in Indonesia, especially on the island of Java. Frangipani plants do not require special care and are easy to grow in the tropics. Frangipani plant is a succulent plant that can store water in the roots, stems, leaves and flowers (Julianto, 2016). There are so many benefits of frangipani plants, one of the benefits that can be utilized from frangipani plants is the flowers which are fragrant and very long lasting. This flower is often used in traditional and religious events because it emits a distinctive aroma and beautiful

color (Kumari *et al*., 2012). The colors of frangipani flower plants are very diverse, ranging from white, pink, red and yellow (Dyah *et al*., 2020).

Ethnomedical uses of different parts of Plumeria rubra have been cited as beneficial in various ailments. Flowers, leaves and bark of *Plumeria rubra* contain many bioactive compounds with anticancer, anti-inflammatory and antimicrobial activities (Lim, 2014). The literature study shows that in Frangipani there are also several volatile compounds, which are the main cause of sweet-smelling flowers that are widely used as a mixture of perfumes, cosmetics and aromatherapy (Dwi Saputra and Megawati, 2012). The benefits contained in frangipani flower decoction are traditionally used by Mexicans to treat diabetes mellitus. The flowers and sap are used to treat toothaches, and also to clean the eyes. A decoction of the bark and roots is used in the treatment of tamarind, relieves constipation, promotes menstruation, reduces fever. In terms of the phytochemical constituents, various compounds have been isolated and identified from Plumeria rubra including iridoids, triterpenoids, flavonoids, glycosides, phenolics, alkaloids, carbohydrates, amino acids, fatty acid esters, sphingolipids, lignin, monoglycerides, coumarins, and several other organic compounds (Bihani, 2021).

Extraction is a method of separating the content of simplex compounds using a suitable solvent. The choice of this method depends on the type, the nature of the compound, the solvent used and the physical properties (Hanani,2015). Percolation, soxhletation, maceration, reflux, distillation, infusion, and decoction are the most common methods used in extraction. Ultrasonic Assisted Extraction (UAE) is the most innovative modern extraction method, where this extraction technique uses organic solvents assisted by ultrasound. Ultrasound is a sound wave with a frequency (>20 KHz) higher than human hearing, which increases the permeability of the cell wall and allows the contents of the cell to escape (Hanani, 2015). Some of the advantages of the ultrasonic extraction process are that there is no need to add chemicals and other additives, the processing time is shorter, and it doesn't cost a lot, and there is no significant change in the chemical structure and compounds of the materials used during the processing (Sekarsari *et al*., 2019).

2. Materials and Methods

2.1.Materials

The tools used for the production of frangipani flower extract consisted of an ultrasonic bath extraction tool (Branson), an analytical balance (Ohaus), a G60 F254 silica gel plate (Merck), a chamber, a vacuum rotary evaporator (IKA/Heidolph), karl fischer (AQV-300), UV lamp 254 nm and 366 nm (Camag), capillary tube, oven (Memmert Un 110), Erlenmeyer (Iwaki), porcelain cup, water bath (Satria medika H-WBE-8L), sieve mesh no. 40, 500 ml beaker glass (Pyrex), 100 ml measuring cup (Iwaki), dropper pipettes, test tubes (Iwaki), and glassware commonly used in laboratories. The tool used to test the identification of compound components uses a Gas Chromatography-Mass Spectrometry (GC-MS). The material used are fresh yellow frangipani flowers (*Plumeria rubra*), dry powder of yellow frangipani flowers (Plumeria rubra), n-hexane solvent, distilled water, HCl 2N, HCl (Merck), concentrated H2SO4 (Merck), concentrated anhydrous acetic acid (Merck). , Mayer (Merck), Bouchardat (Merck), Dragendorf (Merck), 1% $FeCl_3$ (Merck), Quercetin (Sigma), Magnesium powder (Merck), filter paper, and chemicals for analysis.

2.2.*Method*

Fresh yellow frangipani flowers (*Plumeria rubra*) were obtained from the Research Institute for Spices and Aromatic Medicinal Plants (BALITTRO) Bogor. To ensure the correctness of the simplicia to be used in research, plant determination was carried out at the National Research and Innovation Agency (BRIN), Cibinong, Bogor-West Java.

Fresh frangipani flowers, flowers that have bloomed and are old enough to be harvested are collected, then sorted to reduce impurities that are still present in the material collection process. Frangipani flowers are then washed dried at room temperature, spaced and not stacked to facilitate the drying process and prevent moisture from causing the flowers to rot. After the dry raw materials are mashed using a blender to form a powder. The obtained powder is sieved, to obtain a fine degree of powder, it is sieved using a sieve with a size of 40 mesh, weighed and the results are recorded.

The extraction was carried out using the ultrasonic method, the frangipani flower powder and fresh flowers which had been weighed 100 grams were mixed together with 765 ml of n-hexane solvent with a ratio (1: 5) in a beaker glass which had been divided into 3 beaker glasses and covered with aluminum foil, then inserted into the ultrasonic device. After the ultrasonic tool that has been filled with aquadest with a temperature of 50ºC is ready, then the extraction time is set. Extraction parameter was carried out by looking at the yield at 30, 45, and 60 minutes (Budiastra et al., 2020). After the extraction process is complete, the filtering process is carried out using filter paper. Then the solvent was evaporated using a rotary vacuum evaporator, and followed by a water bath to obtain a thick extract.

The characterization of extract was determine using organoleptic examination, yield calculation and water content of the extract using Karl Fischer titration. The viscous extract of the yellow frangipani flower that has been obtained was then subjected to a phytochemical screening test to determine the content of secondary metabolites. In this study the identified secondary metabolites included saponins, glycosides, alkaloids, flavonoids, tannins, and triterpenoids/steroids using chemical test and TLC test.

Analysis of the composition of the frangipani flower extract compounds was carried out using GC-MS (Gas Chromathography-Mass Spectrometry), which was equipped with a Capillary Column Model Number: Agilent 19091S433 HP 5 MS 5% Phenyl Methyl Siloxane (250 μm inner diameter, 30 m long, and film thickness of 0.25 μm) and the detector used is FID. The GC condition with an initial temperature of 60°C was increased to 250°C (4°C/min) then maintained at 250°C for 20 minutes, Helium carrier gas with a flow rate of 20 mL/min. Compounds were identified by comparing the retention index and comparing the mass spectra with those in the Wiley library database and the NIST library (Baihaqi *et al.*, 2018)

3. Results and Discussion

The extraction process is intended to extract the chemical compounds contained in the simplicia. The principle of the extraction process is based on the nature of like dissolves like, the mass transfer of the solute components into the appropriate solvent or has the same polarity level. The ultrasonic extraction method used has several advantages, it can speed up the extraction process in a short time, compared to conventional extraction methods. This method is safer, energy efficient and can increase the amount of yield with n-hexane solvent. The principle of this tool is to reduce the pressure of the round flask and rotate the flask so that the solvent can evaporate below its boiling point more quickly. Then do the thickening in the waterbath tool. The results obtained by the n-hexane extract can be seen in Table 1.

Tabel 1. Yield and Water content of the frangipani extract

Parameter	Fresh yellow frangipani flower extract			Dried yellow frangipani flower extract		
	30 min	45 min	60 min	30 min	45 min	60 min
Yield	1.8%	3.44%	5.11%	2.11%	2.74%	3.43%
Water content	8.78%	14.97%	16.65%	4.19%	2.61%	4.19%

From the results obtained with 2 different variations, namely fresh ingredients and dry powder ingredients, it was found that the fresh ingredients had a higher percentage than the dry powder ingredients, although they were not much different. According to (Rifkowaty & Martanto, 2016) in the wet extraction method, the fineness and crushing rate of raw materials are higher than dry extraction in powder form. The finer the material, the faster the material cells are destroyed and broken, making it easier for the solvent to penetrate the material cells. In addition, the wet method does not go through the drying stage so that very little material is evaporated. It has been emphasized that the simpler the molecular structure, the greater the porosity or pores of the material which makes it easier for the solvent to diffuse into the cells of the extracted material. However, for the yield results with this variation, there is no significant difference.

In the results of the time variation it is known that the longer the extraction process, the higher the yield value. From the variations of the two materials with variations of 3 times, namely 30 minutes, 45 minutes, and 60 minutes, it was found that the 60 minute time had a higher yield. This is because materials that are in contact with the solvent for a longer period of time and high temperature can increase the kinetic energy of the solution and thereby increase the diffusion of the solvent into the tissue cells. Based on the statistical test results, it shows that the ultrasonic extraction time has an effect on the yield of fresh yellow frangipani flowers. on the yield value produced by the influence of 3 variations of ultrasonic assisted extraction time. In the statistical test results for dried frangipani flowers, the results were obtained with a sig value of 0.019 where <0.05, the results contained in this sample showed no significant difference in time and yield. This can be influenced by the lack of extraction time so that the contact time between the material and the solvent is not optimal. It could also be due to factors in the simplicia processing process where sifting is not carried out with a larger mesh size because the finer the material used, the wider the contact area between the material and the solvent until the compound limit is extracted out in the material (Syamsul *et al*., 2020).

Based on previous studies, the temperature of 70ºC and 20 minutes can affect the value of the extraction yield in the ultrasonic method Moringa leaves (Rifkia, 2020). In the results of previous studies, the longer the extraction, the yields obtained are increasing (Yuswi N.C.R., 2017). From these results it can be seen that the ultrasonic method can give very different results compared to conventional methods, where a short treatment time can produce high yield values compared to conventional methods which take several days.

Determination of the water content provides a limit or range of the minimum amount of material contained in the extract, the higher the water content the easier it is for fungi and mold to grow so that during storage it can reduce the biological activity of the extract (Najib *et al*., 2018). The long drying process affects the water content, the drier the simplicia, the lower the water content. The results of this determination of levels can be seen in Table 1.

Phytochemical screening is a method that provides information regarding the presence of secondary metabolites in plants (Harborne,1987). Plants generally contain active compounds in the form of plants often contain active compounds in the form of flavonoids, triterpenoids, tannins, alkaloids, steroids, saponins and others. This test helps provide information about the types of compounds found in plants that can be used in the field of pharmacology such as antioxidants, blood anticoagulants, inhibition of carcinogenesis and besides that they can also be used as anticancer, antibiotics and anti-pest control agents (Ergina, 2014). Based on the results of the phytochemical screening identification test of n-hexane extract of fresh yellow frangipani flowers, it showed the presence of chemical compounds such as flavonoids, saponins, phenols, tannins and terpenoids. The results of the identification test for dry yellow frangipani flower extract showed the presence of flavonoids, saponins, triterpenoids, and tannins. The results can be seen in table 3.

Tabel 3. Phytochemical screening of frangipani flower extract

Secondary metabolite	Test	Fresh flower			Dried flower		
		30 min	45 min	60 min	30 min	45 min	60 min
Alkaloid	Dragendorff	-	-	-	-	-	-
	Mayer	-	-	-	-	-	-
	Bouchardat	-	-	-	-	-	-
Flavonoid	Mg + HCL	+	+	+	+	+	+
	TLC test using quercetin as a standard	+	+	+	+	+	+
Phenolic	$FeCl_3$	+	+	+	-	-	-
Saponin	Hot water + HCl	+	+	+	+	+	+
Tannin	Gelatin 10%	+	+	+	+	+	+
Terpenoid	Lieberman bouchart	+	+	+	+	+	+
Steroid	Lieberman bouchart	-	-	-	-	-	-

Notes : (+) = present, (-) = not present

The purpose of the analysis using the GC-MS method was to determine the compounds contained in the n-hexane extract of dried and fresh yellow frangipani flowers with an extraction time of 30 minutes using the ultrasonic method. This method is often used to identify compounds, both single components and mixtures. Mass spectrophotometers are used to identify fragments and molecules and identify components present in small quantities. Retention time is the time required for a compound to pass through the column to the detector. Retention time measurement is based on the time the sample is injected until the sample reaches the maximum peak height (Suryowati *et al*., 2015).

The results of the GC-MS analysis of frangipani flower extract with n-hexane solvent and fresh flower samples yielded 33 peaks where of the 33 peaks in the chromatogram, 17 compounds had the highest similarity quality to the computer database. The results of the chromatogram of compounds isolated from yellow frangipani flowers with n-hexane solvent can be seen in Figure 3. Retention time, % content, structural formula, molecular weight and name of the resulting compound can be seen in Table 6. GC-MS analysis of extracts isolated from frangipani flowers with n-hexane solvent and dried flower samples produced 32 peaks where of

the 32 peaks in the chromatogram, 21 compounds were found to have the highest quality of similarity to the computer database. The chromatogram results of the compounds isolated from frangipani flowers with n-hexane solvent can be seen in Figure 1 and 2.

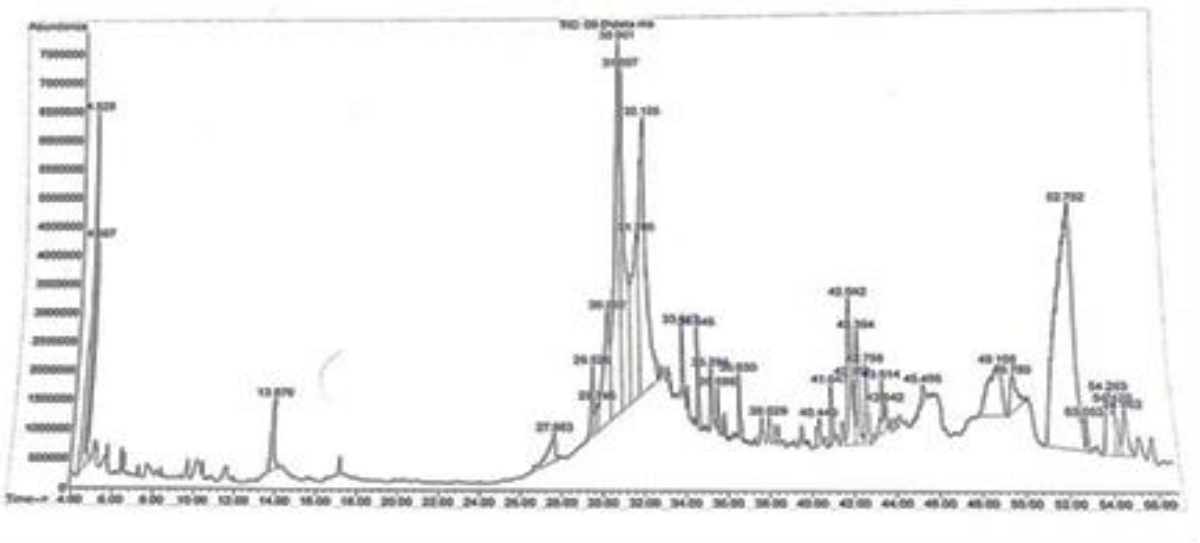

Figure 1. Chromatogram of Fresh Yellow Frangipani Flower Extract

The results of the GC-MS analysis showed that each sample of fresh and dry yellow frangipani flower extract had the same chemical compound components and some were different. From the results of the GC-MS analysis which has been compared with the database, it shows that there are 17 compounds in the extraction of fresh yellow frangipani flowers. The most dominant peak (highest abundance) is A'-Neogammacer-22(29)-EN-3 OL,.BETA.,21.BETA.) at the peak with a retention time of 52.754 which has the largest content of 24.84%. From the results of the GC-MS analysis in the table above, there are also other ingredients found with relatively large abundances of other ingredients, namely Hexadecenoic Acid (11.59%) (palmitic acid), (9E)-9-Octadecenoic Acid (11.51%) (elaidic acid), Acetic acid (4.43%) (alkanoic acid), A-NOR-DINOSTEROL (4.11%) each of which has a retention time of 30.903; 32.123; 4.528; 49.106, while the other components are ≤ 4%.

The database shows that the compound with the most abundance from extraction is the Hexadecenoic Acid compound with the chemical formula C16H32O2 and has the structural formula shown in Figure 5. Hexadecenoic acid was chosen as one of the bioactive compounds suspected of being produced from the extraction of fresh yellow frangipani flowers because these compounds have similar qualities of 99 and detected peaks at half-lives 30,289, 30,903, 31,054, 31,785 with a percentage content of 4.35% - 11.59%. From the data it can be seen that the compound Hexadecenoic acid or palmitic acid has different retention times. In this case, for several variations in different retention times, only a short distance is normal. This can be due to errors in every change in the column including temperature changes and residual effects from previous rounds, pressure and flow that are not properly regulated, matrix effects or analyte interaction effects etc. Hexadecenoic acid or palmitic acid (Figure 3) is the most common fatty acid, which is a saturated fatty acid found in animals, plants and microorganisms with antibacterial properties that can damage the structure of cell walls and membranes. Palmitic acid is also useful in stimulating the development of insulin which plays a role in the treatment of diabetes (Hidayati and Syahnandiaratri, 2018).

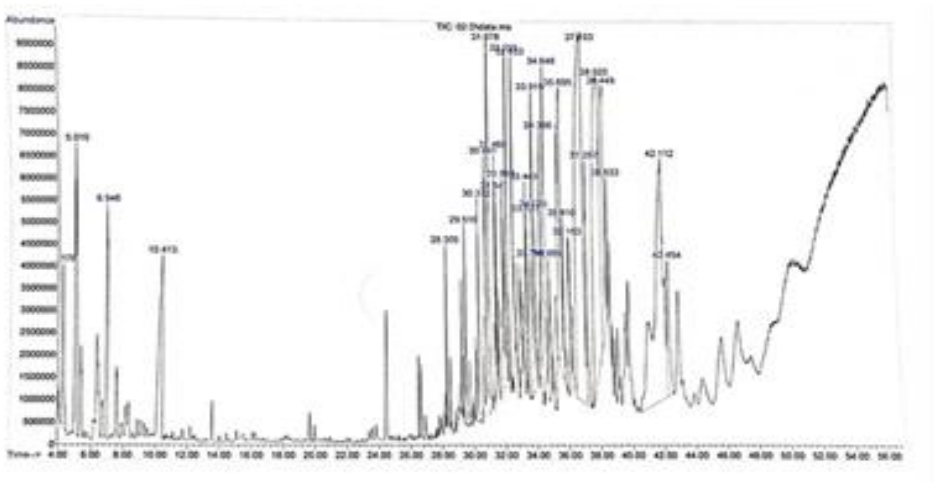

Figure 2. Chromatogram of Dried Yellow Frangipani Flower Extract

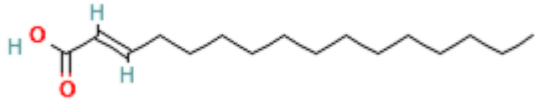

Figure 3. Chemical structure of Palmitic acid *(Pubchem)*

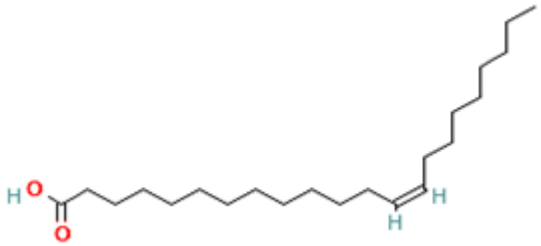

Figure 3. Chemical structure of Eruric acid *(Pubchem)*

The results of the extraction of dried yellow frangipani flowers had 21 compounds with 8 compounds having the most dominant peaks selected, namely the peaks with retention times respectively 37.102, 42.114, 38.019, 35.695, 38.446, 10.410, 5.018, 34.647. From the results of the identification of compounds that have the highest % content, it is suspected that Erucic acid, Pentacosane, Nonadecyl trifluoroacetate, Heptacosane, Octadecane, 2-cyclohexen-1-one, 3,5,5-trimethyl, Benzene, 1,3-dimethyl-, (9E)-9-Octadecenoic Acid. From the database for dried yellow frangipani flowers it shows that the compound with high abundance from extraction is the Erucic acid compound with the chemical formula C22H42O2 and has the structural formula shown in Figure 6. As suspected one of the bioactive compounds because it has a similarity of 98 and is detected at a peak of 37.102 with percentage content of 16.97%. Erucic acid is a very long single-chain unsaturated fatty acid with a 22-carbon backbone and a single double bond originating from the 9th position from the methyl end, with the double bond in the cis configuration. Erucic acid (Figure 4) is a docosanoic acid having a cis- double bond at C-13, found mainly in brassica. Eruric acid is a major component of mustard and rapeseed oils and is produced by broccoli, Brussels sprouts, kale, and wallflowers.

From the results of the research test above, dried and fresh yellow frangipani flowers are suspected to have compounds contained in the essential oils of frangipani flowers, where the two samples contain essential oils of different groups, namely the presence of alkane group compounds such as Heptacosane (5.79%), Octadecane (5.73%), Hexadecanal (2.67%). The alkene class compound is 1-Octadecene (2.50%). Fatty acids such as -1,2-Benzenedirboxylic acid, mono (2-ethylheyl) ester (phatalyc acid) (1,28%), 9,12-Octadecadienoic acid (Z,Z)-,methyl ester (methyl linoleate) (1 ,00%). Alcohol group compounds such as Heptacosanol (13.31%) in dry yellow frangipani flower extract. The presence of fatty acid compounds such as Hexadecenoic acid (4.35-11.59%), Tetradecanoic acid (1.28%), Steroids, namely Stigmastan-3,5-diene (1.63%), alkanoic acids such as acetic acid (4.43%), and Triterpenoid beta amyrin (1.42%) which is found in fresh yellow frangipani flowers.

4. Conclusion

The highest yield results from the effect of time on fresh and dry yellow frangipani flowers were ultrasonic extraction in 60 minutes with yield percents of 5.11% and 3.44% respectively. The results of the phytochemical screening test using color reagents found that there were secondary metabolites of flavonoids, saponins, tannins, triterpenoids, and the presence of phenolic compounds in fresh flowers. The results of the chemical content study using GC-MS showed that the constituent compounds of fresh yellow frangipani flower essential oil consisted of fatty acids (6.59%), alkanoic acids (4.43%), triterpenoids (1.42%) and floral compound groups. dry frangipani consists of alkanes (7.47%), alcohol (13.31%), fatty acids (1.00%).

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Conflict of Interest

All Authors declare no conflict of interest and agree with the content of the manuscript.

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