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GCMS ANALYSIS OF BIOACTIVE COMPOUNDS OF CHINESE BEAT LEAVES (*Peperromia pellucida*) WITH EXTRACTION APPLICATION USING SOXHLET ATION METHOD

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ABSTRACT

Chinese betel leaf (*Perperomia pellucida*) is generally widely used as a medicinal plant. Many people use Chinese betel leaf as a traditional medicine based on natural ingredients which are believed to be able to treat various diseases with fewer side effects than synthetic drugs. In general, China betel leaf has several secondary metabolites, generally flavonoids, steroids, alkaloids and terpenoids. However, in testing for secondary metabolites, there is still no clear description of the plant parts taken. So this study aims to determine the secondary metabolites in the young leaves of Chinese betel (Peperromia pellucida) by gas chromatography. The results obtained based on the GCMS analysis contained 18 secondary metabolites. Phytol, 2,3-BIS (4-(CA RBOXYMETHYL) PHENYL) BENZO [B] FURAN, and 6-ALLYL-4,5 - DIMETHOXY- 1,3-BENZOD IOXOLE are the 3 highest bioactive com pounds based on the percentage of content.

Introduction

Indonesia have a tropical climate with a stable frequency of seasons, this is what makes Indonesia one of the countries that has abundant plant diversity. So that many people use plants as traditional medicine empirically. Indonesia is known as one of the countries that has abundant plant diversity so that not a few people use plants as traditional medicine until now. Gita and Januji, 2021 stated the results of their study that in Pamekasan district there are 13 types of medicinal plants that are used empirically by the community. In addition to being used as medicine, people can also use plants as materials that have economic value, food and also a driver of progress, plant diversity has also begun to be applied to the abundance of other living things such as aquatic biota which has antibacterial benefits (Restuati and Endang, 2019).

The increasing use of plants as an alternative in treatment is directly

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proportional to the increase in cases of metabolic syndrome disease that are starting to get out of control with synthetic drugs and also because synthetic drugs have a lot of side effects in their use, so it is necessary to carry out an in-depth study using herbs to suppress side effects. Pane *et al.*, 2021). Mapping of plants in several regions of America has been carried out from 2010 for the American region and studies were carried out by several researchers from the University of Lahore by grouping these plants based on chemical content that is beneficial to health (Nisar et al., 2018).

The case report data released by WHO states that Asian countries have a habit of high attraction to treatment with plant-based traditional medicines, the People's Republic of China reaches 90% with the application of herbal consumption, followed by Japan with a percentage of 60% - 70% of things and the remaining around 50% are in other Asian regions including the ASEAN region, namely Indonesia is in the range of 40% (WH0, 2019).

The study of drug discovery by applying herbs turned out to be very intensively carried out during the COVID-19 pandemic. A study conducted by (Tejada *et al.*, 2021) with a sample collection time of 1 month obtained 17 plants that have potential as drugs that can inhibit the activity of SARS -COV-2 with a P-Value index value <0.05 and only one that is > 0.05.

A follow-up study on molecular studies was also carried out by (Nugraha *et al.*, 2020) stating that several types of herbs such as *Curcuma Xanthorriza* have effects that cause modification of the molecular pathway of Renin Angiotensin (RAA) so that it occupies the active site of the SARS-CoV 2 virus, and can also release inflammatory mediators such as cytokines.

Mapping of typical Indonesian plants has also been carried out by (Kholilah, 2016) based on their secondary metabolites with large yields and potential as modern medicines from the previous ones as traditional medicines with pharma ceutical studies conducted.

However, there are still very many in-depth studies carried out on each plant found and by applying complete methods and descriptions to carry out the identification method. The review study conducted by (Altemimi *et al.*, 2017) identified several articles that were correct with the method parameters, results and objectives. The results obtained from several articles show that there are some who still use identification with the wrong extraction. One of the plants that is widely used is Ketumpang water/china betel (*Peperomia pellucida*). Chinese betel (*Peperomia pellucida*) is a plant that grows wild in humid areas, however, this plant has not been used optimally to be used as a potential plant in agriculture, health and beauty. This is because there are still few who conduct in-depth studies on the bioactive compounds contained in it.

Chinese betel (*Peperomia pelluci da*) contains main group compounds such as alkaloids, cardenolids, saponins and tannins (Egwuche and erukainure 2011). Extracts from betel china given to experimental animals did not have a negative effect with the study of organ damage parameters by induction of betel china with experimental animals giving the results in the form of a histological picture shown at each dose (500 mg/KgBW, 1000mg/KgBW, 2000 mg /KgBW and 4000 mg/KgBW) comparisons of liver, heart, kidney with control experimental animals did not make a difference in the level of organ damage, so it is necessary to conduct an in-depth study to identify the metabolite compounds found in Chinese betel with the right method (Waty *et al*., 2017).

Materials and Methods

Place and time of research

This research was conducted at the Indonesian Nanotechnology Laboratory (NRE) with herbal and phytochemical studies in September 2021.

Population and sample

The population in this study was the Chinese betel plant (*Peperomia pellucida*) obtained from Tanjung Morawa with details

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in the form of young leaves used leaves derived from strands 1-3 from leaf shoots.

Tools and materials

Tools used in the research: Beaker glass (500 ml), Erlenmeyer (1000 ml), measuring cup (1000 ml) blender, scales, filter paper, knife, stirring rod, watch glass, clamps, stative and stative coasters, bath, pump aquarium, hoses, TLC plate vials and a set of GC-MS tools

The material used in this study was the china betel plant (Peperomia pellucida) using young leaves, 98% ethanol, aquades, tissue, aquades, silica gel, and cotton.

Manufacture of methanol extract of young Chinese betel leaf (*Peperomia pellucida)*Puree the china betel leaf with a blender then weigh as much as 127 grams, wrap it with filter paper with the provisions of the top and bottom of the filter paper tied with wool thread and put into the klongsong, after that add 98% ethanol solvent and put ethanol into a round bottom flask, connect the flask to the cladding and condenser as well as to the water cooler, heat the flask with a water bath, and extract for 3 hours, i.e.

51 times filtering so that the oil extracted from soxhletation is obtained, then weigh the empty evaporation flask and enter the oil extraction into the empty flask and evaporate with solvent by evaporation on the evaporator until it is obtained in the form of a thick extract of 4.99 grams (Lutfia and atun, 2018).

Identification with GC-MS

The sample was filtered using a filter and injected as much as 20 l in the stationary phase silica C18 and while in the mobile phase using a mixture of ethanol: water (9:1) by means of gradient elution and a flow rate of 1.0 ml/minute with a duration of 10 minutes and wavelength of 368 nm and operate the tool to detect pure compounds, which are shown by the formation of peaks with a certain duration of time, and provide identity for these compounds in order to obtain results in the form of evaluating the level of content.

Results and Discussion

The leaves used as fresh leaves as much as 6 kg were then subjected to a drying process for 3-5 days and blended until smooth before being added to the GCMS apparatus. Extraction was carried out first using the *soxhletation* method to separate the unwanted components from the expected bioactive compounds.

(Egwuche and erukainure 2011) stated that china betel has a high ash content and water content, so identification methods and appropriate extraction methods must be carried out to obtain optimal results to remove pollutant levels. The dry yield obtained was 127 grams, then with the viscous extraction after passing through 51 cycles for 3 hours, a thick extract was obtained as much as 4.99 grams which was then used as an ingredient for identification with GC-MS.

The bioactive compounds found in young Chinese betel leaves (Peperomia pellucida) were found as many as 16 bioactive compounds as follows:

Table 1. The results of the identification of the bioactive compound of Chinese betel (*Peperomia pellucida*) with GCMS

No	Sample Code	RT	Qty	Compound	Content
	Ekstrak etanol dirih china (*Peperomia pell ucida)*	13.606	99	Beta-elemene	2,88
		14.509	99	Caryophyllene	4,34
		17.267	99	Lepidosene	1,54

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Table 1. *Cont…*

No	Sample Code	RT	Qty	Compound	Content
		20.487	93	Sphatulenol	2,47
		22.390	98	6-allyl-4,5-dimethoxy- 1,3-benzod ioxole	9,42
		27.120	99	Neophytadiene	4,30
		27.382	89	Neophytadiene	2,10
		27.562	95	Neophytadiene	3,06
		28.830	91	1,6,10,14-Hexadecaterraen-3-ol, 3,7,11,15- tetramethyl-	1,20
		29.389	90	Phytol	18.99
	Ekstrak etanol dirih china (*Peperomia pell ucida)*	30.154	62	Hexadecanoic acid	4,30
		30.251	95	Hexadecanoic acid	3,40
		30.699	95	Tetracosane	7,31
		31.133	97	Pentacosane	5,82
		32.940	64	2,3-bis (4-(carboxymethyl) phenyl) benzo [b] furan	11,34
		33.368	90	3,6-Dimethoxy-2-ethylbenzaldehyde	1,80
		34.733	83	3,5-cis(p- dimethylaminostryl) 2,2-dimethyl-2H-Pyrrole 1-Oxide	1,26
		34.955	95	Nonadecane	1,08
		35.740	96	Vitamin E	1,91
		37.126	99	Tadalafil	1,81
		37.567	99	Tadalafil	2,65

phytol, 2,3-bis (4-(carboxymethyl) phenyl) benzo [b] furan, and 6-allyl-4,5-dimethoxy- 1,3-benzod ioxole are the 3 highest bioactive compounds based on the percentage of content

From Table 1, it can be seen that from the 16 secondary metabolites produced, it can be categorized that based on the literature review, phytol (Figure 1) is a compound that plays an important role as an anti-inflammatory which plays an important role in wound healing by stimulating the action of histamine to close the wound (Silva et al. , 2013).

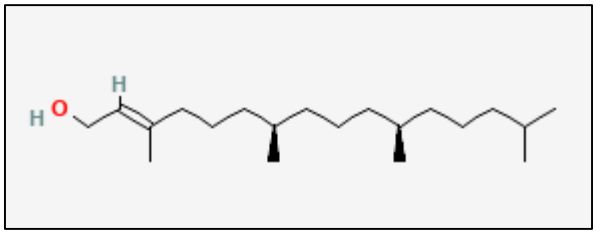

Figure 1. Chemical structure of phytol
Ring bonds are directly related to Fibroblasts
(Pubchem, NCBI) which plays a role in wound closure

The second highest bioactive compound is Benzofuran, benzofuran is composed of a bromine and alkyl ring (Figure 2) which plays an important role as a bond between anti-cancer derivatives (Napiorkowska et al., 2019)

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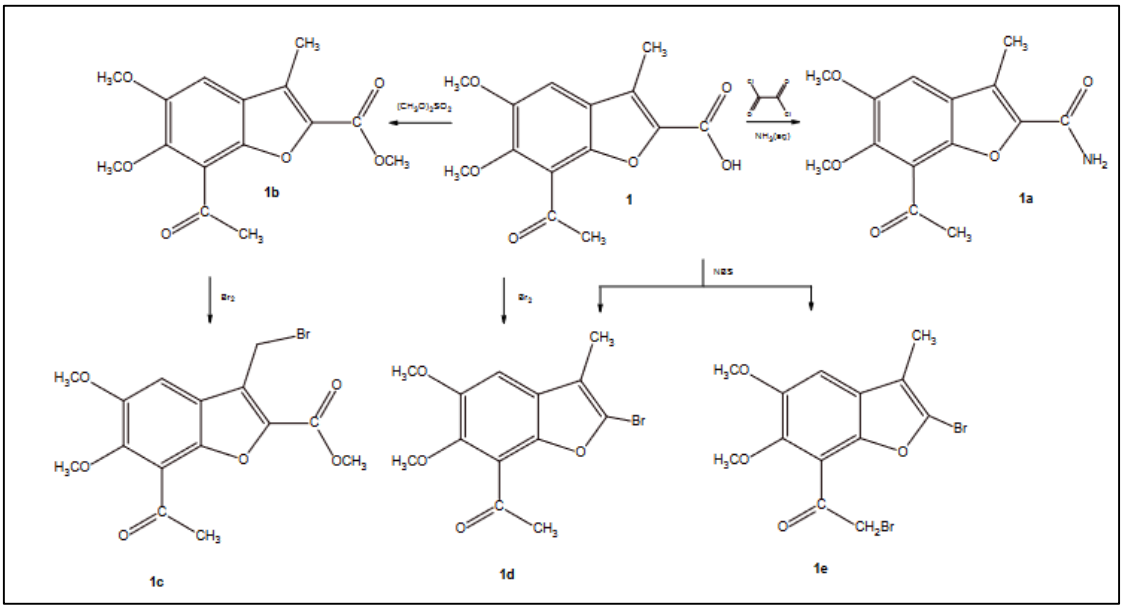

Figure 2. Chemical structure and ring making up of Benzofuran
Benzofuran is composed of a bromine and alkyl ring

The second highest bioactive compound is Benzofuran, benzofuran is composed of a bromine and alkyl ring (Figure 3) which plays an important role as a bond between anti-cancer derivatives (Goodarzi et al., 2016).

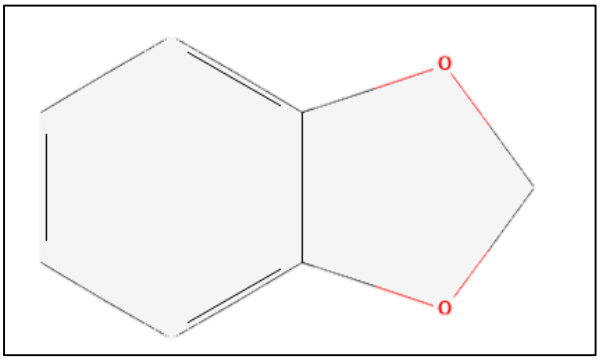

Figure 3. Chemical Structure of Benzodioxole
The ring bond is directly related to the Jaks pathway of cancer (Pubchem, NCBI) which plays a role in wound closure

Conclusion

Based on identification with GCMS, 16 bioactive compounds have specific roles, each of which is the largest 3, namely Phytol, Benzofuran and Benzodioxole and based on studies with literature on anti-inflammatory and anticancer roles.

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