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Phytochemistry and chemical composition by gcms of n-hexane and methanol extract of *Magnolia coco* Flowers

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Abstract
The *Magnolia coco* which is widely used in traditional medicine has been extracted. The extraction used the solvent of methanol and n-hexane. The yield of extraction in methanol and n-hexane were 2.92% and 39.10%. Qualitative analyses of phytochemical constituents in methanol were tannins, phenolic, saponin, and terpenoid. On the other hand, n-hexane has terpenoid compound only. The analysis that used GCMS showed that n-hexane extract have six constituent which were 1,5-Octadiene-3,7-diol; 3,7-dimethyl-(terpenediol I); 2,6-Dimethyl-1,7-octadiene-3,6-diol; 3,5,5-trimethyl-4-(3-oxobutyl)-1-cyclohex-2-enone; 3,7-dimethyl-4-(2-methyl-1-propenyl)-2,4,5,6,7,7a-hexahydro-1H-inden-1-ol,S)-4,5,9,9-Tetramethyl-2,4a,5,6,7,8,9,9a-octahydro-1H-1,5-cyclo-benzocycloheptene and 3-(3-Butynyl)-2-cycloocten-1-valerenol. Meanwhile, methanol extract consist of Methyl 14-Methylpentadecanoate and (1Z)-3-ethenylcyclooctene.

Keywords: GCMS, Magnolia coco, Methanol, n-Hexane

Introduction
The *Magnolia coco* flower which is widely used in traditional medicine has phytochemical compound. Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans more than those attributed to macronutrients and micronutrients [1]. They protect plants from disease and damage and contribute to color, aroma and flavor of plants. Phytochemicals from plants are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans more than those attributed to Macronutrients and micronutrients [2-3]. Secondary constituents are the remaining plant chemicals such as alkaloids, terpenes, flavonoids, lignans, plant steroids, curcumines, saponins, phenolics, flavonoids and glucosides [4]. Aims of the research is to know the composition of the secondary metabolites and chemical composition of n-hexane, ethyl acetate and methanol extract from Magnolia coco by using GCMS.

Material and Methods
General Experimental Procedures
Magnolia coco was harvested from local area in Bogor, West Java. The flower of Magnolia coco has been washed and cutted. Reagents that used were Dragendorff's reagent, Mayer's reagent, methanol, ethyl acetate, *n*-hexane, concentrated sulfuric acid, concentrated HCl, ferric chloride hexahydrate ($FeCl_3.6H_2O$) purchased from Merck. All chemicals used were of analytical grade.

Sample Extraction
Sample preparation was conducted by maceration using several organic solvents. Fresh flower of Magnolia coco ± 25 g were immersed in 100 mL of n-hexane for 3 days, and then filtered. Filtrate was evaporated until dry sample was obtained, and this step resulted raw extract of n-hexane. The residue from first immersion was entirely immersed back in 100 mL ethyl acetate for 3 days to obtain raw extract of ethyl acetate. The maceration process was repeated several times to obtain clear extract containing all of expected chemical species.

Phytochemical Assay
Phytochemical assay of raw extract of n-hexane and ethyl acetate was performed using Ciulei method (1982) [5]. The assay included several test for alkaloid, tannin, saponin, reducing sugar, flavonoid, glucoside, phenolic, glycosidesteroid, and sterol – triterpenoid.

Structural Elucidation of n-hexane And Ethyl acetate Extract by GC-MS

Samples of *n*-hexane, ethyl acetate, and methanol extracts were further analyzed by Gas Chromatography – Mass Spectrometry (GC-MS) to determine the species contained in the samples. The chemical components were identified by matching their mass spectra with those recorded in the mass spectral library. GC-MS analysis was performed by using Agilent 7890A GC system, Agilent 5975C series VL MSD, capillary column type, flow rate of 1 mL/min, carrier gas was helium, constant flow model, injector temperature was 250°C, injection volume was 2 µL, split injection technique, oven temperature was programmed from 40°C for 2 min, with temperature increment rate of 10°C/min, and final temperature of 280°C for 2 min. Total running time was 30 min.

Result and Discussion

Sample Extraction

The results in Table 1 showed that different extracting agent resulted indifferent percentage of yield. From ± 25 g fresh flower of Magnolia coco, it yielded 0.7055 g (2.85%) of greenish yellow solution of raw n-hexane extract, and 9.6607 g (39.10%) of brownish yellow solution of raw methanol extract. The results showed that methanol extract contains yield larger than n-hexane extracts. The percentage of yield of extract indicated the extracting capacity of extracting agent. The yield of methanol extract has extracting capacity for secondary metabolite larger in the *Magnolia coco* flowers compared n-hexane extract. On the other side, the yield of n-hexane extract related to the fact that n-hexane has the lowest extracting capacity. Azmir *et al*. (2013) [6] stated that the efficiencies of extraction methods mostly depend on the understanding the nature of plant matrix and chemistry of bioactive compounds. The possible explanation for this phenomenon was the fact that the secondary metabolites contained in the ethyl acetate extract were semi polar or thus need the extracting agent which has the similar polarity. This explanation must be supported by the further phytochemical assay. The physical appearance of the extract solution also provided supporting information that different kinds of the secondary metabolites were extracted from different solvent.

Table 1: The yields of "kembang telor (*Magnolia coco*)" extracts (per ± 25 g of samples)

Solvent	Wo (g)	W1 (g)	% Yield*
n-hexane	24.7182	0.7055	2.85
Methanol	24.7182	9.6607	39.10

*) % yield is calculated using the following equation (W1/W0) X 100%

Phytochemical Assay

Phytochemical analysis of n-hexane and methanol raw extract revealed that methanol extracts contain too much of tannin, phenols, terpenoids, and litle saponin compound, and the other hand n-hexane extract only terpenoids contain (Table 2).

Table 2: Phytochemical assay of extract of "Kembang telor (*Magnolia coco*)"

No	Phytochemicals	Extracting agent	
		n-hexane	methanol
1	Alkaloids :	-	-
	Meyer	-	-
	Dragendorf	-	-
	Wagner	-	-
2	Flavonoids	-	-
3	Tannins	-	+++
4	Phenols	-	+++
5	Saponins	-	+
6	Terpenoids	++	+++

Table 2 showed that all of the extracts contain terpenoid, but contain no alkaloid and flavonoid. Tannin, phenol and saponin cmpounds was present in methanolic extract. These results showed that the terpenoid may present in all of extracts. Methanol extract showed more completecontents of secondary metabolite, compared to *n*-hexane extracts, thus it can beexpected.

The predicted constituents in the n-hexane extracts were listed in Table 3 and Table 4. A total of 6 compounds were found in *n*-hexane extracts and 2 compounds in methanolic extract. It can be understood that there was larger amount of compounds found in n-hexane compared to that found in methanol extract, since the GC-MS technique only performed well for analyzing volatile compounds, which largely contained in the *n*-hexane extract than in methanolic extract [7].

Table 3: GC-MS analysis of hexane extract of Magnolia coco Flowers

No	Constituents	Retention time (min)	% Area	% Similarity
1	1,5-Octadiene-3,7-diol, 3,7-dimethyl-(terpenediol I)	4.620	1.08	86
2	2,6-Dimethyl-1,7-octadiene-3,6-diol	5.603	2.71	90
3	3,5,5-trimethyl-4-(3-oxobutyl)-1-cyclohex-2-enone	10.062	2.88	91
4	3,7-dimethyl-4-(2-methyl-1-propenyl)-2,4,5,6,7,7a-hexahydro-1H-inden-1-ol	11.843	0.65	89
5	(S)-4,5,9,9-Tetramethyl- 2,4a,5,6,7,8,9,9a-octahydro-1H-1,5-cyclo-benzocycloheptene	13.488	3.79	90
6	that the methanol extract has high bioactivity3-(3-Butynyl)-2-cycloocten-1one valerenol	14.802	1.98	91

Chemical Composition of n-Hexane and Methanol Extract of *Magnolia coco* by GC-MS

Table 3 showed n-hexane extract of Magnolia coco flowers contains 6 terpenoids compound with similarity 86 – 91% and retention time 4.620-14.802 minutes, since phytochemical assay that n-hexane extract consist terpenoids compound only. The six constituent from n-hexane extract were 1,5-Octadiene-3,7-diol, 3,7-dimethyl- (terpenediol I), 2,6-

Dimethyl-1,7-octadiene-3,6-diol, 3,5,5-trimethyl-4-(3-oxobutyl)-1-cyclohex-2-enone, 3,7-dimethyl-4-(2-methyl-1-propenyl)-2,4,5,6,7,7a-hexahydro-1H-inden-1-ol,S)-4,5,9,9-Tetramethyl-2,4a,5,6,7,8,9,9a-octahydro-1H-1,5-cyclo-benzocycloheptene and 3-(3-Butynyl)-2-cycloocten-1one valerenol. The terpenoids compound has healthcare function, such as beta-carotene and the others function.

Table 4: GC-MS analysis of methanol extract of Magnolia coco Flowers

No	Constituents	Retention time (min)	% Area	% Similarity
1	Methyl 14-Methylpentadecanoate	12.698	10.99	92
2	(1Z)-3-ethenylcyclooctene	14.223	10.77	92

Table 4 showed that methanolic extract of Magnolia coco flowers contains 2 chemical compound with similarity 92% and retention time 12.698 and 14.223 minutes. Phytochemical assay showed that methanolic extract contain tannin, phenol and saponin compounds. Methanol extract showed more complete contents of secondary metabolite, but contain chemical compound with high molecule weight and non volatile characteristic.

Conclusion

This study revealed n-hexane extract used GCMS Analysis have six constituent were 1,5-Octadiene-3,7-diol, 3,7-dimethyl- (terpenediol I), 2,6-Dimethyl-1,7-octadiene-3,6-diol, 3,5,5-trimethyl-4-(3-oxobutyl)-1-cyclohex-2-enone, 3,7-dimethyl-4-(2-methyl-1-propenyl)-2,4,5,6,7,7a-hexahydro-1H-inden-1-ol,S)-4,5,9,9-Tetramethyl-2,4a,5,6,7,8,9,9a-octahydro-1H-1,5-cyclo-benzocycloheptene and 3-(3-Butynyl)-2-cycloocten-1one valerenol. On the other hand Methanol extract consist of Methyl 14-Methylpentadecanoate and (1Z)-3-ethenylcyclooctene.

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