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Research Article

Tectona Grandis Leaves: Determination of Total Flavonoid Content, Phenolic Content, Characterization of the Leaves, and Compound Identification in GC-MS

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ABSTRACT

Nowadays, traditional plant study has grown in importance as their use has been increased. *Tectona grandis* (teak) is one of several plants that have been studied for its phytochemical and pharmacological properties. This plant includes a number of secondary metabolites, which may explain its diverse pharmacological properties. Although teak leaf compounds have been examined in the past, there is still little information on the diversity of teak leaf compounds. As a result, we are employing several methodologies to determine the total flavonoids content, phenolic content, water extractable matter, ethanol extractable matter, total ash content, and other component analysis of teak leaves. We found that the average of total flavonoid content in the teak leaves is about 3.93 ± 0.008%w/w, while the average total phenolic concentration in teak leaves is about 4.3±0.15%w/w. The Gas Chromatography-Mass Spectrometry GC-MS study of the methanol extract of *T. grandis* leaves also found twenty-three active chemical compounds (phytochemical components).

Key words: *Tectona grandis*, Teak, Phenolic content, Flavonoids content, Ash content, Water content, Moisture content, GC-MS.

INTRODUCTION

Medicinal plants are becoming more popular as a result of a high number of people seeking health cures with few or no side effects, which is a concern with most chemically created pharmaceuticals.[1] Research on these plants often aims to establish medical properties by evaluating available scientific information and traditional applications of the product. These plants' phytochemicals can be utilized as the basis for further optimization of the lead compounds. According to reports, 25% of medications in developing nations are based on plants and their derivatives.[2,3] One of many plants that have been examined for their phytochemical and pharmacological qualities is *Tectona grandis* L.f (TG) which belongs to the Verbenaceae family and commonly called as teak.[4]

The teak tree is a tropical hardwood that has become the most popular choice for furniture due to its durability and water resistance. This species is indigenous to South and Southeast Asia, specifically India, Sri Lanka, Indonesia, Malaysia, Thailand, Myanmar, and Bangladesh.[5] In terms of the origin, teak is spread from 73◦ E in India to 104◦30' E in Thailand, and from the island of Java at 8◦45' S to the low hills and plains below 800 m in northern Myanmar.[6] This plant contains several secondary metabolites, which may explain its traditional usage as an antiinflammatory, laxative, astringent, and analgesic agent.[7] Teak leaves also demonstrated numerous pharmacological activities, including antibacterial, anticancer, and antioxidant capabilities.[8]

Previous studies have researched Tectona grandis leaves for its phenolic compounds by Reversed-Phase High-Performance Liquid Chromatography method (RP-HPLC);[9] phenyl ethanoid content by Liquid Chromatography Mass Spectrometry (LCMS);[10] flavonoids, phenolic acids, and glucuronides content by LCMS;[11] phenolic acids, flavonoids, and coumarin content by High-Performance Liquid Chromatography method (HPLC);[12] aliphatic ketones, esters & alcohol, anthocyanins content by UV–Visible, Gas Chromatography-Mass Spectrometry (GC-MS), and LCMS;[13] anthraquinones and hemisynthetic derivatives content by column chromatography;[14] also for the preservative and natural dye compounds of teak leaves.[15]

Although teak leaf compounds have been studied before, information on the diversity of teak leaf compounds is still little. As a result, we are trying to determine the total flavonoids content, phenolic content, water extractable matter, ethanol extractable matter, total ash content of teak leaves, other component analysis using various methods.

MATERIALS AND METHODS

Materials

The chemicals and reagent used in this study were ethanol, quercetin, aluminum chloride 10%, sodium acetate 1 M, water, Folin-Ciocalteu reagent, gallic acid, natrium hydroxide 1%, chloroform-saturated water, hydrochloric acid, and helium gas. Meanwhile, the teak leaves were obtained from Jebres, Central Java, Indonesia. The samples were obtained from the forest in the form of fresh and dried flowers. The samples were obtained between May and June 2018

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Procedure for determining of flavonoid content by colorimetric method

Sample preparation: The teak leaf methanol extract sample was precisely weighed at 0.2g before adding ethanol until the solution reached 25 ml. The solution was then stirred for 30 minutes using a magnetic stirrer, and if it was not clear, it was filtered.

Solution of quercetin: After accurately weighing roughly 10 mg of quercetin standard, 25 ml of ethanol was added. The solution was then prepared at a succession of levels for the quercetin standard curve, precisely at 2.4, 5.0, 7.6, and 10 g/ml.

Colorimetric procedure: Pipette separately 0.5 mL of the sample solution and each series of reference solution into a 5 ml volumetric flask, add 1.5 mL ethanol to each flask, 0.1 ml aluminum chloride 10%, 0.1 ml sodium acetate 1 M and 2.8 ml water. Shake and let it idle for 30 minutes at room temperature. Measure the absorbance at a wavelength of 435 nm.

Determination of total phenolic content by Folin-Ciocalteu Reagent

Sample preparation: The teak leaf methanol extract sample was weighed accurately at approximately 0.2 g, then methanol was added until it reached 25 ml. The solution was stirred for 30 minutes with a magnetic stirrer, and if it was not clear, filter the solution.

Solution of galic acid**:** After accurately weighing roughly 10 mg of standard gallic acid, 25 ml of ethanol was added. The solution was then prepared at a succession of levels for the gallic acid standard curve, precisely at 1.62; 3.24; 4.86; 6.48; and 8.10 g/ml.

Procedure: Pipette separately 1 ml of the sample solution and each series of reference solution into the appropriate container, add 5.0 ml Folii-Ciocalteu dilution (7.5% in water). Let it idle for 8 minutes, add 4 ml of natrium hydroxide 1%, and then incubate for 1 hour. Measure the absorbance of each solution at a wavelength of 731.5 nm. Take blank measurements in the same way, without adding any sample solution. Create a calibration curve and calculate the level of sample solution.

Characterization of *Tectona Grandis* Leaves

Procedure determination of water extractable matter: Weigh sample roughly 5 g accurately. Place it in a plugged flask, add 100 ml of chloroform-saturated water, and shake it frequently for the first 6 hours before leaving it for 18 hours. Evaporate 20 ml of the filtrate until it dries in a porcelain cup which has been heated to 105°C and tare weight, heat the remainder at 105°C until it reached a constant weight. Calculate the content in %w/w.

$$\text{Extract content} = \frac{(\text{container weight after drying} - \text{container weight}) x\ 5}{\text{extract weight}} x\ 100\%$$

Procedure for determining ethanol extractable matter: Weigh sample roughly 5 g accurately. Place it in a plugged flask, add 100 mL of ethanol, and shake it often for the first 6 hours before leaving it for 18 hours. Filter immediately to prevent ethanol evaporation, evaporate 20.0 mL of the filtrate until it dries in a porcelain dish heated to 105°C and tare weight, and heat the rest at 105°C until it attained a constant weight. Calculate the content in %w/w.

$$\text{Extract content} = \frac{(\text{container weight after drying} - \text{container weight}) x\ 5}{\text{extract weight}} x\ 100\%$$

Determination of total ash: Accurately weigh 2 to 3 g of the sample material and place it in a silicate crucible that has been heated and tare, heat it to constant weight at 800 ± 25°C slowly until the charcoal runs out, cool and weigh. The total ash content is calculated by weight of the test material, expressed in %w/w.

Determination of acid-insoluble ash: Boil the ash obtained in the determination of total ash content with 25 ml of hydrochloric acid for 5 minutes. Collect the non-acid soluble components, filter through ash-free filter paper, wash with hot water, and heat in a crucible until the weight remains at 800+25°. The acid insoluble ash content is calculated against the weight of the sample material, expressed in %w/w.

Determination of water content by moisture content: The moisture content of the samples was evaluated by weighing three fresh, 10g samples in a weighted, dry, tarred crucible and baking them in a 58°C oven overnight.[16]

GC-MS

On a GC-2010 gas chromatograph-mass spectrometer, the components of *Tectona grandis* leaves were separated and identified. Helium (He) gas was utilized as a carrier gas for analysis using mass spectrometer as a detector. The carrier gas flow rate was set to 1, 20 ml/min. The injector was kept at 250°C.

The injection volume of the solution into the GC-MS was 0.5 L, with a split ratio of 50:1. The column oven temperature was controlled by first holding it at 80°C for 2 minutes, then increasing it by 10°C/min to 130°C, then by 15°C/min to 250°C, and finally by 5 minutes' isothermal at 280°C. Mass spectra were collected at 70 eV with a scan interval of 0.5 second with fragments ranging from 35 to 500 m/z. The overall run time of the GC-MS was 18 minutes. The components were identified by comparing their mass spectra to those in the Wiley Registry of Mass Spectral Data, 7th Edition. ChemStation was used to handle the mass spectra and chromatograms.

RESULTS

Total flavonoid content of methanol extracts of *Tectona grandis* leaves

Using the colorimetric method, a quercetin standard solution was created as a guide to determine the link between quercetin content and absorbance value on a calibration curve and in a calibration equation. A linear regression equation, y=0.07973x-0.0568, is obtained, with a r value of 0.998523. y is the absorbance at 435 nm and x is the sample concentration, resulting in the total flavonoid content being calculated. As shown in table 2, with 3 replications of the sample, the average of total flavonoid content in the teak leaves is about 3.93 ± 0.008%w/w.

Table 1: Absorption of quercetin (µg/mL) standard.

No.	Quercetin solution (µg/ml)	Absorption
1	2.4	0.129
2	5.0	0.351
3	7.6	0.563
4	10.0	0.723

Table 2: Absorption and flavonoid content (%w/w) of methanolic extracts of *Tectona grandis* leaves.

Replication	Absorbance	Initial total flavonoid content (µg/mL)	Average total flavonoid content (%w/w)
1	0.236	363.4	3.93
2	0.235	362.1	3.92
3	0.236	363.4	3.93
		Avg	3.93
		SD	0.008
		RSD (%)	0.002

Table 3: Absorption of gallic acid (µg/mL) standard.

No.	Gallic acid solution (µg/ml)	Absorption
1.	1.62	0.094
2.	3.24	0.221
3.	4.86	0.427
4.	6.48	0.716
5.	8.10	0.927

Table 4: Absorption and total phenolic content (%w/w) of methanolic extracts of *Tectona grandis* leaves.

Replication	Absorbance	Initial total flavonoid content (µg/mL)	Average total flavonoid content (%w/w)
1	0.236	363.4	3.93
2	0.235	362.1	3.92
3	0.236	363.4	3.93
		Avg	3.93
		SD	0.008
		RSD (%)	0.002

Table 5: Results of the water extractable matter.

Container weight (g)	Weight (container + sample) after oven (g)	Deviation (g)	Water extractable matter (%w/w)
46.0465	46.1632	0.1167	11.06
51.3018	51.4170	0.1152	10.92
76.9173	77.0421	0.1248	11.83
		Avg	11.27
		SD	0.005
		RSD (%)	0.043

Table 6: Results of the ethanol extractable matter.

Container weight (g)	Weight (container + sample) after oven (g)	Deviation (g)	Water extractable matter (%w/w)
64.4094	64.6909	0.2815	27.53
69.4133	69.6994	0.2861	27.98
59.6565	59.9434	0.2869	28.06
		Avg	27.86
		SD	0.003
		RSD (%)	0.010

Table 7: Results of the total ash content.

Replication	container weight (mg)	Sample (mg)	Weight (container + sample) after ashing (mg)	Weight (container + sample) after acid addition (mg)	Total ash (%w/w)	Acid-insoluble ash (%w/w)
1	21709.5	3288.3	21899.4	21717.1	5.775	0.231
2	25892.4	3273.3	26089.2	25903.1	6.012	0.327
3	19577.3	3413.1	19767.3	19587.9	5.567	0.311
				Avg	5.8	0.290
				SD	0.2	0.051
				RSD (%)	3.9	17.695

Table 8: Results of the water content by moisture content.

Replication	Moisture content (%w/w)
1	4.28%
2	4.22%
3	5.06%
Avg	4.25
SD	0.47
RSD (%)	11.0

Table 9: Results of the characterization of *Tectona Grandis* leaves.

No.	Parameter	Result (%w/w)
1.	Water extractable matter	11.27 ± 0.005
2.	Ethanol extractable matter	27.86 ± 0.003
3.	Total ash content	5.8 ± 0.2
4.	Acid insoluble ash content	0.290 ± 0.051
5.	Water content by moisture content	4.25 ± 0.47

Table 10: GC-MS analysis of *Tectona grandis* leaves extract.

No.	RT (Minute)	Area	%Area	Name
1	2.157	456697	9.45	Valeraldehyde
2	2.216	332478	6.88	Azetidin-2-One, 3,3-Dimethyl-4-(Ethyl-1-Amino)
3	2.303	209959	4.34	Methyl 7-Octenoate
4	2.359	392674	8.12	1-Methylhexahydroazepin-2-One
5	2.590	347524	7.19	8-Azanonane, 4-Cyano-4-(3,4-Dimethoxyphenyl)
6	2.669	134945	2.79	2-(4,5-Dihydro-3-Methyl-5-Oxo-1-Phenyl-4-Pyrazolyl)-5-Nitrobenzoic Acid
7	2.750	235094	4.86	Propanedioic Acid (CAS), Malonic Acid, Dicarboxymethane
8	2.893	120780	2.50	2-(5-Aminohexyl)Furan
9	3.000	135190	2.80	Hexahydro-3H,6H-2,5a-Methano-1,2-Benzazepine
10	3.179	207423	4.29	N-[2,2,2-Trifluoro-1-(Isopropylamino)-1
11	3.320	177389	3.67	Thiazole, 2-Amino-4,5-Dimethyl-, Hydrobromide
12	3.423	326514	6.75	Linalool
13	3.530	106269	2.20	2-(4,5-Dihydro-3-Methyl-5-Oxo-1-Phenyl-4-Pyrazolyl)-5-Nitrobenzoic Acid
14	3.640	176548	3.65	Peroxide, Bis(Dichlorobenzoyl) (CAS) Dichlorobenzoyl Peroxide
15	3.746	270808	5.60	2-Propen-1-Amine, 2-Bromo-N-Methyl-(CAS) Methylamine, N-(2-Bromo-2-Propenyl)
16	4.050	108485	2.24	3-Butenamide
17	4.110	140037	2.90	Isopropenylacetic Acid
18	4.237	163229	3.38	Quiactin
19	4.360	189649	3.92	Thioxamyl
20	4.631	226184	4.68	Cyclobutanol
21	4.870	103279	2.14	Isovaleraldehyde
22	5.070	140156	2.90	1-Nitrosopiperidine
23	5.312	132793	2.75	3-Methyl-1,1-Cyclobutanedicarboxylic Acid

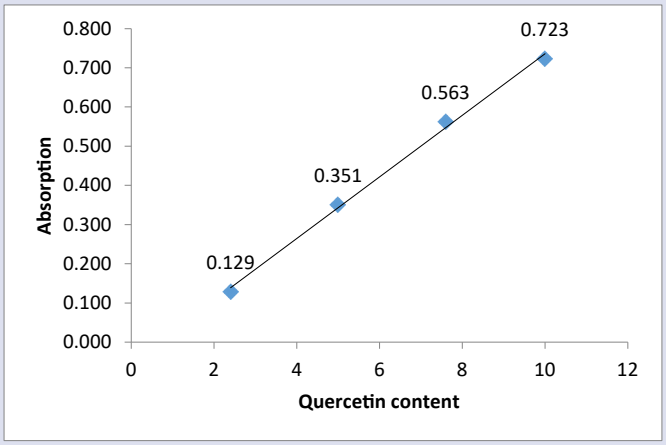

Figure 1: Calibration curve of quercetin standard.

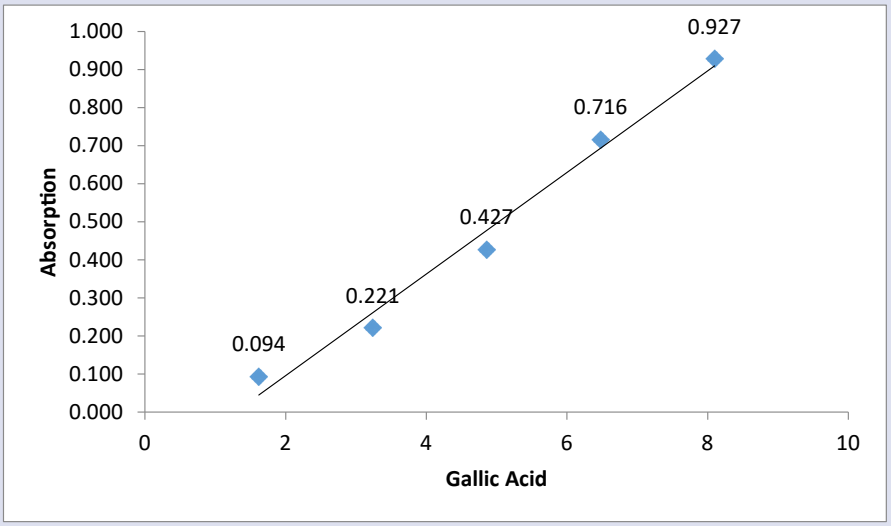

Figure 2: Calibration curve of gallic acid standard.

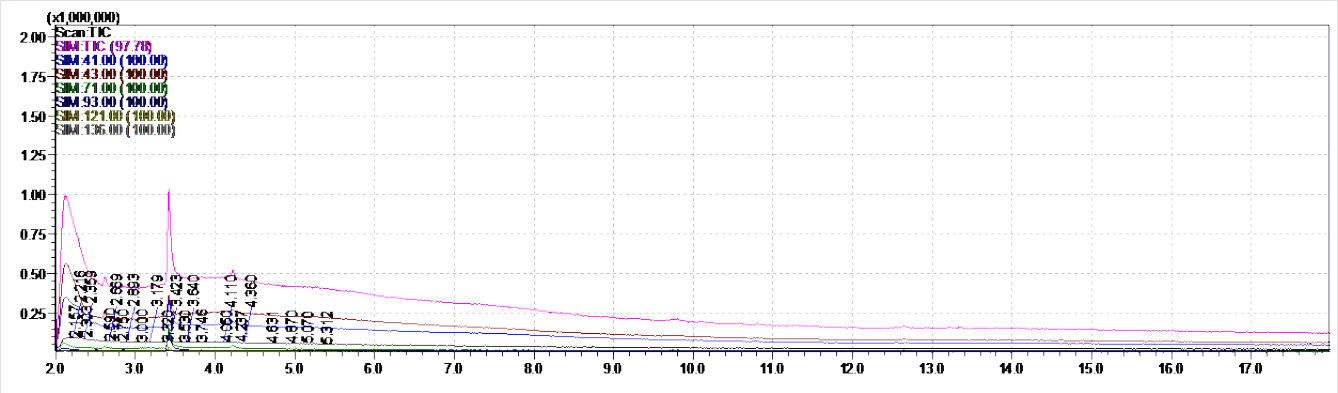

Figure 3: Chromatogram obtained from GC-MS analysis of *Tectona grandis* leaves extract.

Total phenolic content of methanol extracts of *Tectona grandis* leaves

A gallic acid standard solution was made to serve as a reference for determining the relationship between gallic acid concentration and absorbance value on a calibration curve and in a calibration equation. A linear regression equation, y=0.13344x-0.1715, with a r value of 0. 992, is produced. y is the absorbance at 731.5 nm and x is the sample concentration, which will be followed by the calculation of total phenolic content. The average total phenolic concentration in teak leaves is about 4.3±0.15%w/w, as shown in table 2, based on three replications of the sample.

Characterization of *Tectona Grandis* leaves

Water-extractable matter is the soluble fraction of organic matter extracted from the sample under various laboratory conditions.[17] From our result on table 9, the water extractable matter was 11.27 ± 0.005 %w/w and the ethanol extractable matter was 27.86 ± 0.003%w/w. Examination of total ash content is useful to see the mineral content of Tectona grandis.[18] The total ash content was 5.8 ± 0.2%w/w and the acid insoluble ash content was 0.290 ± 0.051%w/w. Water content is one of the physical qualities of a material that indicates how much water it contains.[19] For the water content, we obtained 4.25 ± 0.47%w/w.

DISCUSSION

The study of organic molecules derived from plants and their materials, as well as their composition and activity, has grown in recent years. The GC-MS approach was utilized in this research since it is the most suitable for qualitative and quantitative analysis of bioactive chemicals. The chromatogram of *tectona grandis* leaves was shown in figure 3, while the findings of GC-MS analysis of *tectona grandis* leaves was shown in table 10.

We identified twenty-three active compounds (phytochemical constituents) from the GC-MS analysis of the *Tectona grandis* leaves. Identification of the active compound was confirmed based on retention time, peak area, and molecular formula. The descending order of compounds present in *Tectona grandis* leaves is shown as follows : -Valeraldehyde (9.45%) > 1-Methylhexahydroazepin-2-One (8,12%) > 8-Azanonane, 4-Cyano-4-(3,4-Dimethoxyphenyl) (7,19%) > Azetidin-2-On, 3,3-Dimethyl-4-(Ethyl-1-Amino)(6,88%) > Linalool (6,75%) > 2-Propen-1-Amine, 2-Bromo-N-Methyl- (CAS) Methylamine, N-(2-Bromo-2-Propenyl) (5,60%) > Propanedioic Acid (CAS) Malonic Acid, Dicarboxymethane (4,86%) > Cyclobutanol (4,68%) > Methyl 7-Octenoate (4,34%) > N-[2,2,2-Trifluoro-1-(Isopropylamino)-1 (4,29%) > Thioxamyl (3,92%) > Thiazole, 2-Amino-4,5-Dimethyl-, Hydrobromide (3,67%) > Peroxide, Bis(Dichlorobenzoyl) (CAS) Dichlorobenzoyl Peroxide (3,65%) > Quiactin (3,38%) > Isopropenylacetic

Acid (2,90%) > 1-Nitropiperidine (2,90%) > Hexahydro-3H,6H-2,5a-Methano-1,2-Benzazepine (2,80%) > 2-(4,5-Dihydro-3-Methyl-5-Oxo-1-Phenyl-4-Pyrazolyl)-5-Nitrobenzoic Acid (2,79%) > 3-Methyl-1,1-Cyclobutanedicarboxylic Acid (2,75%) > 2-(5-Aminohexyl)Furan (2,50%) > 3-Butenamide (2,24%) > 2-(4,5-Dihydro-3-Methyl-5-Oxo-1-Phenyl-4-Pyrazolyl)-5-Nitrobenzoic Acid (2,24%) >Isovaleraldehyde (2,14%).

Based on the result, it shows 2-(5-Aminohexyl) Furan with peak area of 2,50%. Previous study was able to identify furanone-based compounds having antimycobacterial activity but low cytotoxicity against a human cell line, and were able to demonstrate potent synergism when combined with the current anti-TB drug rifampicin.[20] Thiazole was found on our GC-MS analysis with peak area of 3.67%. The fact that thiazole nucleus is a key component of penicillin nucleus and some of its derivatives have antibacterial (sulfazole), antiretroviral (ritonavir), antifungal (abafungin), antihistaminic, and antithyroid actions demonstrates its versatility for medicinal usage. Its derivatives might even be used as anticancer (tiazofurin), anthelmintic, vulcanising accelerators (mercaptobenzothiazole), and photographic sensitizers.[21]

Another compound that we found from GC-MS analysis is linalool, with peak area of 6,75%. It was suggested that linalool exerts neuroprotective, anti-inflammatory, and antioxidant effects in the brain.[22] Linalool-rich plant-derived oils also exert antidepressant, anti-anxiety and pro-cognitive effects in rodent models. Linalool may be helpful for social anxiety, as acute administration of linalool (100 mg/kg, i.p.) reduced anxiety-like behaviors in mice and restored social interaction in mice subjected to a social defeat paradigm, when compared to socially stressed mice given saline (controls).[23]

Teak leaf extracts were consisted of numerous types of chemical substances. Previous research found that the amount of volatile chemicals rose with the plant maturity, therefore young teak leaves had a considerably smaller diversity of compounds than mature teak leaves.[24] GC-MS analysis confirms the existence of enumerated nutraceutical components that aid medicinal, nutraceutical, and therapeutic value.

CONCLUSIONS

Based on the research, the following conclusions can be drawn: The total flavonoid content of teak leaves is around 3.93 0.008%w/w. Teak leaves have an average total phenolic content of 4.30.15%w/w. The extractable matter in water was 11.27 0.005%w/w while the extractable matter in ethanol was 27.86 0.003%w/w. The overall ash content was 5.8 0.2%w/w, with an acid insoluble ash value of 0.290 0.051%w/w. We obtained 4.25 0.47%w/w for the water content. Our GC-MS investigation of *T. grandis* leaves yielded twenty-three active chemicals (phytochemical components).

AUTHOR CONTRIBUTIONS

P.B.: drafted the original manuscript. S.S: validation. B.W.: conceptualization. D.M.: review. All authors have read and agreed to the published version of the manuscript.

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Health Research Ethics Committee of the Faculty of Medicine, Universitas Muhammadiyah Surakarta.

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Not Applicable

DATA AVAILABILITY STATEMENT

All data are reported in the manuscript.

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Not Applicable

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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