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ORIGINAL ARTICLE

LC-MS/MS based metabolite profiling and lipase enzyme inhibitory activity of *Kaempferia angustifolia* Rosc. with different extracting solvents

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Abstract *Kaempferia angustifolia* also known as kunci pepet in Indonesia, has been widely used as a traditional medicine to treat cold, cough, stomachache, diarrhea, fever, and dysentery, also used as a slimming agent. The level of biological activity depends on the composition and concentration of bioactive compounds present in the plants. In addition, extraction solvents affects the composition and concentration of bioactive compounds. Therefore, this study aimed at identifying the metabolite profile of *K. angustifolia* and to evaluate the inhibitory potential of their various solvent extracts towards lipase enzyme. Extracts were prepared using water and different concentration of ethanol (30–99%) and then analyzed their metabolite profile using LC-MS/MS. Lipase inhibitory activity was assessed using *in vitro* enzymatic inhibition assay. In this study, profile of *K. angustifolia* was shown to be rich in terpenoids (monoterpenoids, sesquiterpenoids, diterpenoids), and phenolics (carboxylic acid and phenolic acid). Most of the identified compounds were detected in ethanol extract of *K. angustifolia*. The ethanol extract at 100 μg/mL displayed 59.82% inhibitory activity

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towards lipase and was found to have the highest inhibitory activity compared to the other extracts. A partial least square-discriminant analysis (PLS-DA) was performed for clustering the extracts based on the peak area of 53 putatively identified compounds. Based on the result obtained, 50% ethanol extract is the best extract that gives the highest inhibition results and 15 metabolites were identified, mainly from the carboxylic acid and terpenoid groups.

1. Introduction

Kaempferia angustifolia is a medicinal plant cultivated in Indonesia and other parts of the Southeast Asia region. This species is known by the local name of *kunci pepet* or white turmeric and belongs the the Zingiberaceae family. *K. angustifolia* has been widely used as a folk remedy to treat cold, cough, stomachache, diarrhea, fever, and dysentery (Yeap et al., 2017). This plant also has several biological activities, such as antioxidant, antimicrobial, antiobesity, and antiallergic activity (Yeap et al., 2017; Tang et al., 2014; Hanif et al., 2021; Madaka and Tewtrakul, 2011). A previous study showed the presence of antioxidant compounds in *K. angustifolia* such as boesenboxide, crotepoxide, 2′-hydroxy-4,4′, 6′trimethoxychalcone, kaempfolienol, and zeylenol (Yeap et al., 2017). In addition 2′-hydroxy-4,4′,6′-trimethoxychalcone and 25-dien-3-ol showed the strongest cytotoxic activity against cell lines HL-60 (human promyelocytic leukemia) and MCF-7 (human breast cancer) (Tang et al., 2014).

The metabolites in the Kaempferia genus are mainly terpenoids (monoterpenoid, sesquiterpenoid, and diterpenoid), phenolic acid, and flavonoids such as flavanone (Elshamy et al., 2019). *K. angustifolia* has compounds from the cyclohexane diepoxide derivative, terpenoids (monoterpenoid, sesquiterpenoid, diterpenoid; steroids, and saponins), phenylpropanoid, flavonoid, chalcone, etc. (Woerdenbag et al., 2004; Tang et al., 2011, 2014; Hanif et al., 2021). The composition and content of the metabolites in a plant may differ depending on the site of growth, growing season, harvest time, the part of the plant used, and the type and composition of the extracting solvents (Rafi et al., 2020). Differences in the composition and content of these metabolites will affect their level of biological activity.

Changes in the profile of plant metabolites can be evaluated by metabolomics analysis either with metabolite fingerprinting or profiling under various circumstances (Sajak et al., 2016). LC-MS/MS is a sophisticated technique that can be used to profile metabolites in a sample due to its high sensitivity and selectivity. Also, this technique can identify and measure the metabolites by reducing the complexity of samples and allowing the separation of metabolites before they are detected (Xiao et al., 2012). As we mentioned above, different extracting solvent is one factor the composition and concentration of metabolite extracted will be different. Thus, the level of biological activity will also differ. So, we took this opportunity to profile the metabolites and determine the percentage inhibition of lipase enzyme activity of *K. angustifolia* because there has been no reported paper about this. As previously reported, *K. angustifolia* has antiobesity activity, so we also evaluated the inhibition of lipase enzyme activity as one mechanism to prevent obesity. This study provides the composition of metabolites and level of inhibition lipase enzyme activity of *K. angustifolia* extract based on the types and the solvent compositions.

2. Material and methods

2.1. Chemicals and reagents

The materials used were methanol, acetone, water grade LC-MS, dipotassium hydrogen phosphate, potassium dihydrogen phosphate, sodium chloride, sodium hydroxide, acetonitrile, lipase from porcine pancreas, and *p*-nitrophenyl butyrate (*p*NPB) were purchased from Merck (Darmstadt, Germany). The UHPLC-Q Exactive Plus Orbitrap HRMS (Thermo Fisher, Waltham, USA) was used for metabolite profiling and the microplate reader (Epoch BioTek, Winooski, USA) was used for *in vitro* enzymatic activity measurement.

2.2. Sample preparation and extraction

Kaempferia angustifolia was obtained from the Tropical Biopharmaca Research Center (TropBRC), IPB University, Bogor, West Java, Indonesia. The specimen has been identified with a voucher specimen (BMK0433012018) by Mr. Taufik Ridwan, a botanist from TropBRC. Before being used for extraction, we dried and pulverized the rhizomes of *K. angustofolia*. The dried sample was macerated for about 12 h with a ratio of 1:15 in ethanol p.a., 70% ethanol, 50% ethanol, 30% ethanol, and water. The filtrate was concentrated using a rotary evaporator to produce the extract. Then each sample extract was analyzed for the LC-MS/MS analysis and inhibition of lipase enzyme activity.

2.3. LC-MS/MS analysis

The metabolite profiles were analyzed using UHPLC-Q Orbitrap HRMS. The column used was Accucore C_{18} (100 × 2.1 m m), with a particle size of 1.5 µm. The column was eluted using 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B), with gradient conditions of 0–2 min 5% B, 2–3 min 5–25% B, 3–14 min 25–100% B; 14–19 min 100% B, and 19–23 min 5% B with a flow rate of 0.2 mL/min. The injection volume was 5 µL. The ionization source is ESI with positive and negative ionization modes. The mass scan range was set to m/z 100–1500. The mass spectrometry was conditioned: Capillary temperature 320 °C, spray voltage 3.2 kV, S lens RF level 50, sheath gas and aux gas flow rates 12 and 3, respectively. The power resolution was set to 70,000 FWHM. Accurately weighed 10 mg extract was dissolved in 5 mL methanol and sonicated for 30 min. The solution was filtered using 0.2 µm PTFE membrane and analyzed by UHPLC-Q Orbitrap HRMS.

2.4. In vitro lipase inhibition assay

Enzymatic inhibition assay was performed according to Chedda et al. (2016). Briefly, *p*NPB was used as a substrate in the enzymatic reaction. The test tube contained 100 µL of phosphate buffer (pH 7.2), 50 µL of pancreatic lipase, 25 µL of *p*NPB, and 25 µL of *K. angustifolia* extract (100 µg/mL) in total volume of 200 µL. The blank tube for 100% activity was also prepared by replacing the 25 µL of *K. angustifolia* extract with 100 µL of phosphate buffer (pH 7.2). All tubes

were incubated at 37 °C for 30 min. Orlistat was used as an inhibitor for positive control. *para*-nitrophenol (*p*NP) produced from the enzymatic reaction was measured spectrophotometrically by measuring the absorbances at 400 nm using a microplate reader. The percentage of inhibitory activity was calculated using the following formula:

$$\frac{(\text{Absorbance of blank} - \text{Absorbance of sample})}{\text{Absorbance of blank}} \times 100\%$$

2.5. Data analysis

The metabolite profiles were processed using the Compounds Discoverer 3.2 (Thermo Fisher, Waltham, USA) with in-house databases of metabolites collected from the Kaempferia genus. The steps in identifying the metabolites began with selecting the spectra, aligning the retention times, detecting the unknown compounds, grouping the unknown compounds, predicting the compositions, searching the mass list, filling the gaps, normalizing the areas and marking the background compounds. The MS2 was confirmed to identify the metabolites.

PLS-DA was performed to determine the pattern of extract grouping based on the extracting solvents using area data of the successfully identified compounds or metabolites. The PLS-DA analysis used The Unscrambler X software version 10.1 (Camo, Oslo, Norway). The success of the extract grouping was seen in the number of key components involved, the total variation represented, and the score plot's visualization.

Percentage of inhibition lipase enzyme activity was presented as mean ± standard error for at least three independent experiments. Statistical comparison for inhibition lipase enzyme activity was carried out using one-way analysis of variance (ANOVA) followed by the Tukey test. We used a significant difference at the 95% confidence level ($p < 0.05$) was used.

3. Results and discussion

Forty-two compounds were successfully identified in *K. angustifolia* extract through UHPLC-Q Orbitrap HRMS analysis (Table 1 and Fig. S1). After being confirmed using full mass spectra and MS2 fragmentation patterns, the identified compounds were compared to some relevant published data or MS2 references such as MassBank. Most identified compounds are terpenoids (monoterpenoids, sesquiterpenoids, diterpenoids), and phenolics (carboxylic acid and phenolic acid). Other compounds also successfully identified are flavonoids (flavanone), aldehydes, ketones, fatty acids, furan, anthraquinone, coumarin, lactone, chalconic acid, vinylogous acid, and steroids. Base peak chromatogram patterns with positive (Fig. 1) and negative ionization modes (Fig. 2) off all extracts produce different patterns in each solvent composition. It indicates a difference in the number of metabolites per extract. The total metabolites identified are 17 compounds in water extract, 18 compounds in 30% EtOH extract, 17 compounds in 50% EtOH extract, 29 compounds in 70% EtOH extract, and 43 compounds in 96% EtOH extract (Table 2).

3.1. Carboxylic acids

There are eight carboxylic acids identified in the *K. angustifolia* extract, namely citric acid (1), *trans*-aconitic acid (2), 2-isopropylmalic acid (4), phenyllactate (6), azelaic acid (9), 4-hydroxybenzoic acid (11), benzoic acid (17), gemfibrozil (23). Fragmentation of the carboxylic acids mostly by releasing one or two H_2O molecules characterized by the ion fragments $[M - H - 18]^-$ and CO_2, at *m/z* 129 $[M - H - CO_2]^-$ and 111 $[M - H - CO_2 - H_2O]^-$ for *trans*-aconitic acid, *m/z* 147 $[M - H - H_2O]^-$ and 103 $[M - H - H_2O - CO_2]^-$ for phenyllactate. Compounds with *m/z* 176 identified as 2-isopropylmalic acid provide fragments at *m/z* 157 $[M - H - H_2O]^-$, 131 $[M - H - H_2O - 26]^-$, and 113 $[M - H - 2H_2O - 26]^-$.

Azelaic acid with $[M - H]^-$ at *m/z* 187 is fragmented by releasing one CO_2 molecule at $[M - H - 44]^-$, one H_2O molecule at $[M - H - 44–18]^-$, and one CO molecule at $[M - H - 44–18 - 28]^-$. The compound (1) is citric acid, as indicated by fragments at *m/z* 173, 147, 129, 111, and 87. The compound (11) at *m/z* 137 is identified as a 4-hydroxybenzoic acid for fragments at *m/z* 108, 93, and 65. Compound (17) at *m/z* 123 is known as benzoic acid, and compound (23) is identified as gemfibrozil-produced fragments at *m/z* 233, 191, 105, and 71.

3.2. Terpenoids

Terpenoids are predominant in the extract (Elshamy et al., 2019), and are divided into three types, namely monoterpenoids (2 compounds), diterpenoids (3 compounds), and sesquiterpenoids (12 compounds). The monoterpenoids are camphor (37) at *m/z* 153 which produces fragments at *m/z* 135 $[M + H - H_2O]^+$, 109 $[M + H - H_2O - C_2H_2]^+$, 95 $[M + H - H_2O - C_2H_2 - CH_2]^+$ and camphene (42) at *m/z* 137 with fragments at *m/z* 95 $[M + H - 42]^+$ and $[M + H - 42 - CH_2]^+$ (Turek and Stinting, 2011). The three diterpenoids are retinoic acid (14), traversianal (27), and retinal (40). Retinoic acid with $[M + H]^+$ at *m/z* 301 fragmented at *m/z* 283 $[M + H - H_2O]^+$, 255 $[M + H - H_2O - CO]^+$, 227 $[M + H - H_2O - CO - 2CH_2]^+$, 213 $[M + H - H_2O - CO - 3CH_2]^+$, 185 $[M + H - H_2O - CO - 5CH_2]^+$, 157 $[M + H - H_2O - CO - 7CH_2]^+$, and 143 $[M + H - H_2O - CO - 8CH_2]^+$. Compounds (27) at *m/z* 317 are recognized as traversianal with fragments at *m/z* 299 $[M + H - H_2O]^+$, 271 $[M + H - H_2 - CO]^+$, and 253 $[M + H - 2H_2O - CO]^+$. Retinal with $[M + H]^+$ at *m/z* 285 produces fragments at *m/z* 285, 215, 201, 159, 143, and 83.

In addition, 12 sesquiterpenoids are identified in the extract. Furanogermenone (12) at *m/z* 233 $[M + H]^+$ is identified at retention times of 7.71 min with fragmentation at *m/z* 175, 147, 119, 105, and 91. The compound (13) is identified as curzeone at *m/z* 229 $[M + H]^+$ with fragments at *m/z* 211 $[M + H - H_2O]^+$. The compound (16) at *m/z* 253 $[M + H]^+$ is known as zedoarondiol with fragments at *m/z* 235 $[M + H - H_2O]^+$, 175 $[M + H - H_2O - 60]^+$ showing the opening of cyclopentane and loss of H_2O molecules, and 147 $[M + H - H_2O - 60 - 2CH_2]^+$. Compound (20) at *m/z* 237 $[M + H]^+$ is identified as neocurdione with a fragment releasing one H_2O molecule at *m/z* 219 $[M + H - 18]^+$. The compound (21) at *m/z* 239 $[M + H]^+$ is recognized as a culmorin with fragments at *m/z* 221, 203, 177, 163, 161, 149, 147, 137, 133, 121, 107, 95, and 81 (Hao et al., 2019).

The compound (22) is known as zederone at *m/z* 247 $[M + H]^+$ fragmented at *m/z* 229 $[M + H - H_2O]^+$ and

Table 1 Metabolites in *K. rotunda* extracts as identified by UHPLC-Q Orbitrap HRMS.

No	RT [min]	Molecular Weight	Ion Mode	MS^2 (*m*/*z*)	Formula	Compound	Group
1	1.16	192.0264	$[M-H]^-$	173, 147, 129, 111, 87	$C_6H_8O_7$	Citric acid	Carboxylic acids
2	1.56	174.0157	$[M-H]^-$	173, 129, 111, 85	$C_6H_6O_6$	*trans*-Aconitic acid	Carboxylic acids
3	1.64	164.0472	$[M+H]^+$	165, 147, 123, 95	$C_9H_8O_3$	*o*-Coumaric acid	Phenolic acids
4	5.27	176.0679	$[M-H]^-$	175, 157, 131, 115, 113, 85	$C_7H_{12}O_5$	2-Isopropylmalic acid	Carboxylic acids
5	5.99	196.0735	$[M+H]^+$	123, 105, 95, 79	$C_{10}H_{12}O_4$	2,4,5-Trimethoxybenzaldehyde	Phenolics
6	6.38	166.0623	$[M-H]^-$	165, 147, 119, 103	$C_9H_{10}O_3$	Phenyllactate	Carboxylic acids
7	6.47	166.0994	$[M+H]^+$	167, 149, 121, 107, 93, 81, 79, 67	$C_{10}H_{14}O_2$	4-*tert*-Butylcatechol	Phenolics
8	6.69	264.1359	$[M+H]^+$	265, 247, 229, 105	$C_{15}H_{20}O_4$	Zedoarofuran	Furans
9	6.90	188.1044	$[M-H]^-$	187, 143, 125, 97, 57	$C_9H_{16}O_4$	Azelaic acid	Carboxylic acids
10	7.19	282.1470	$[M-H]^-$	281, 237, 139, 125	$C_{15}H_{22}O_5$	Octyl gallate	Phenolic acids
11	7.26	138.0311	$[M-H]^-$	137, 108, 93, 65	$C_7H_6O_3$	4-Hydroxybenzoic acid	Carboxylic acids
12	7.71	232.1461	$[M+H]^+$	233, 175, 147, 119, 105, 91	$C_{15}H_{20}O_2$	Furanogermenone	Germacrane sesquiterpenoids
13	7.93	228.1150	$[M+H]^+$	229, 211	$C_{15}H_{16}O_2$	Curzeone	Sesquiterpenoids
14	7.95	300.2083	$[M+H]^+$	301, 283, 255, 227, 213, 185, 157, 143	$C_{20}H_{28}O_2$	Retinoic acid	Diterpenoids
15	8.03	264.1360	$[M+H]^+$	265, 247, 219, 177, 145	$C_{15}H_{20}O_4$	Hulupinic acid	Vinylogous acids
16	8.49	252.1722	$[M+H]^+$	253, 235, 175, 147	$C_{15}H_{24}O_3$	Zedoarondiol	Sesquiterpenoids
17	8.69	122.0367	$[M+H]^+$	123, 81	$C_7H_6O_2$	Benzoic acid	Carboxylic acids
18	8.79	248.1410	$[M+H]^+$	249, 203, 143, 105	$C_{15}H_{20}O_3$	Curcumenolactone A	Lactones
19	9.38	272.1045	$[M+H]^+$	273, 255, 199, 185	$C_{16}H_{16}O_4$	Eleutherin	Anthraquinones
20	9.42	236.1772	$[M+H]^+$	237, 219	$C_{15}H_{24}O_2$	Neocurdione	Germacrane sesquiterpenoids
21	9.68	238.1931	$[M+H]^+$	239, 221, 203, 177, 163, 161, 149, 147, 137, 133, 121, 107, 95, 81	$C_{15}H_{26}O_2$	Culmorin	Sesquiterpenoids
22	9.87	246.1255	$[M+H]^+$	247, 229, 183	$C_{15}H_{18}O_3$	Zederone	Germacrane sesquiterpenoids
23	9.89	250.1566	$[M+H]^+$	233, 191, 105, 71	$C_{15}H_{22}O_3$	Gemfibrozil	Carboxylic acids
24	10.05	220.1825	$[M+H]^+$	221, 175, 161, 147, 95	$C_{15}H_{24}O$	Caryophyllene oxide	Sesquiterpenoids
25	10.16	256.0738	$[M-H]^-$	255, 213, 211, 151, 145	$C_{15}H_{12}O_4$	Pinocembrine	Flavanones
26	10.50	234.1617	$[M+H]^+$	235, 217	$C_{15}H_{22}O_2$	Curcumenol	Guaiane sesquiterpenoids
27	10.71	316.2037	$[M+H]^+$	317, 299, 271, 253	$C_{20}H_{28}O_3$	Traversianal	Diterpenoids
28	10.76	216.1513	$[M+H]^+$	217, 119, 157	$C_{15}H_{20}O$	Furanodiene	Germacrane sesquiterpenoids
29	10.86	166.1358	$[M+H]^+$	167, 123, 111, 107, 97, 93, 81	$C_{11}H_{18}O$	Dihydrojasmone	Cyclic ketones
30	11.10	148.0525	$[M+H]^+$	149, 121, 107	$C_9H_8O_2$	3,4-Dihydrocoumarin	Coumarins
31	11.10	106.0421	$[M+H]^+$	107, 106, 79	C_7H_6O	Benzaldehyde	Aldehydes
32	11.18	302.2243	$[M+H]^+$	303, 285, 267, 229, 163, 109	$C_{20}H_{30}O_2$	Eicosapentaenoic acid	Fatty acids
33	11.21	270.0888	$[M+H]^+$	271, 253, 229, 167, 131	$C_{16}H_{14}O_4$	Alpinetin	Flavanones
34	11.43	298.1929	$[M+H]^+$	299, 281, 241, 229, 199, 185, 171, 83, 55	$C_{20}H_{26}O_2$	Norethindrone	Steroids
35	11.62	228.0785	$[M+H]^+$	229, 151, 105, 95, 77, 53	$C_{14}H_{12}O_3$	Benzyl salicylate	Vinylogous acids
36	11.87	222.1982	$[M+H]^+$	223, 73	$C_{15}H_{26}O$	*cis*-Nerolidol	Sesquiterpenoids
37	12.55	152.1201	$[M+H]^+$	153, 135, 109, 95	$C_{10}H_{16}O$	Camphor	Monoterpenoids
38	13.05	314.1147	$[M+H]^+$	315, 273, 191, 190, 151	$C_{18}H_{18}O_5$	2′-Hydroxy-2,4,4′-trimethoxychalcone	Chalcones
39	13.41	218.1667	$[M+H]^+$	219, 163	$C_{15}H_{22}O$	α-Cyperone	Cycloeudesmane sesquiterpenoids
40	14.73	284.2138	$[M+H]^+$	285, 215, 201, 159, 143, 83	$C_{20}H_{28}O$	Retinal	Diterpenoids
41	15.03	212.1201	$[M+H]^+$	213, 198, 128	$C_{15}H_{16}O$	Pyrocurzerenone	Sesquiterpenoids
42	15.45	136.1252	$[M+H]^+$	137, 95, 81	$C_{10}H_{16}$	Camphene	Monoterpenoids
43	10.424	442.1261	$[M+H]^+$	443, 105, 77	$C_{23}H_{22}O_9$	2-acetylrotepoxide A	Polyoxygenated cyclohexane
44	10.312	426.1312	$[M+H]^+$	427, 105, 77	$C_{23}H_{22}O_8$	(-) 6-acetylzeylenol	Polyoxygenated cyclohexane
45	14.006	240.0784	$[M+H]^+$	241, 105	$C_{15}H_{12}O_3$	5,7-Dihydroxyflavanone	Flavanoid
46	10.196	384.1204	$[M+H]^+$	385, 263, 123, 105	$C_{21}H_{20}O_7$	Zeylenol	Polyoxygenated cyclohexane
47	16.388	281.2714	$[M+H]^+$	282, 265	$C_{18}H_{35}NO$	9-octadecenamide	Sesquiterpenoids
48	13.593	212.0839	$[M+H]^+$	213, 105	$C_{14}H_{12}O_2$	Benzylbenzoat	Benzoic acid
49	9.93	108.0578	$[M+H]^+$	109, 91	C_7H_8O	Benzylalcohol	Benzylalcohol

Table 1 (*continued*)

No	RT [min]	Molecular Weight	Ion Mode	MS^2 (*m/z*)	Formula	Compound	Group
50	18.55	390.27645	$[M + H]^+$	391, 167, 149, 71	$C_{24}H_{38}O_4$	Bis (2-ethylHexyl) phthalate	Phthalates
51	12.435	180.0788	$[M + H]^+$	181	$C_{10}H_{12}O_3$	Propylparaben	Benzoic acid
52	18.422	272.2502	$[M + H]^+$	273	$C_{20}H_{32}$	Sandaracopimaradiene	Diterpenoid
53	21.428	412.3702	$[M + H]^+$	413, 107, 81	$C_{29}H_{48}O$	Stigmasterol	Steroid

Remarks: RT = retention time.

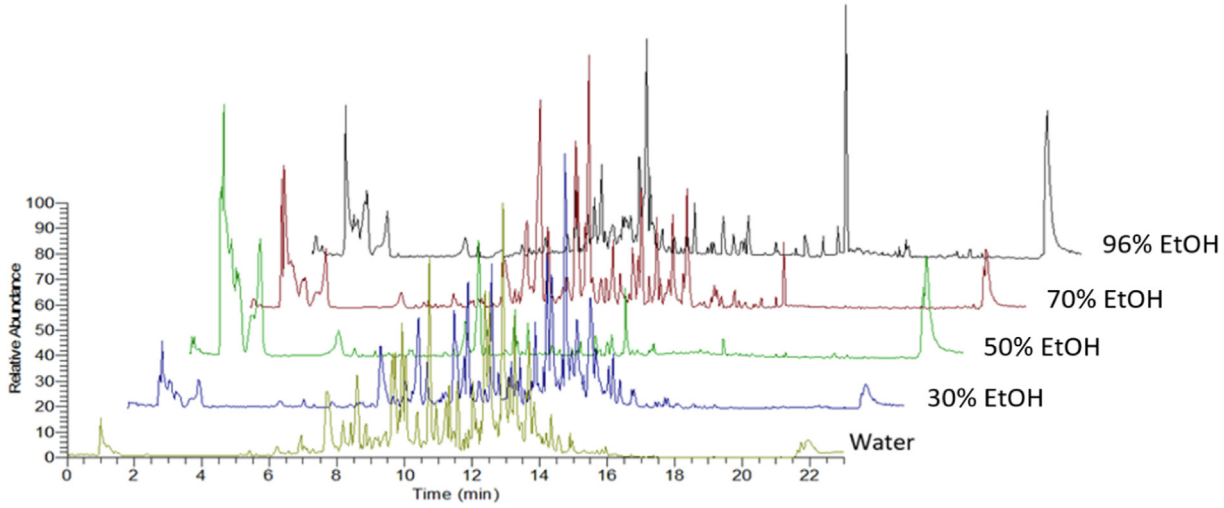

Fig. 1 Base peak chromatograms of positive ionization mode of the five *K. rotunda* extracts.

183 $[M + H - H_2O - C_2H_6O]^+$. Compounds (24) is caryophyllene oxide at *m/z* 221 $[M + H]^+$ produces fragments at *m/z* 175, 161, 147, and 95 (Turek et al., 2011). The compound (26) is curcumenol at *m/z* 235 $[M + H]^+$ and the fragments at *m/z* 217 $[M + H - H_2O]^+$. Compound (28), furanodiene at *m/z* 217 $[M + H]^+$ has fragments at *m/z* 157 and 119. From the compound (36) *cis*-nerolidol at *m/z* 223 $[M + H]^+$ there are fragments at *m/z* 73 $[M + H - 150]^+$ and compound (39) α-cyperone at *m/z* 219 $[M + H]^+$ has fragments at *m/z* 163. The compound (41) at *m/z* 213 $[M + H]^+$ is pyrocurzerenone as indicated by fragments at *m/z* 198 $[M + H - CH_3]^+$ and 128 $[M + H - 85]^+$ (Hao et al., 2019).

3.3. Phenolic acids

A total of 2 phenolic acids were identified in *K. angustifolia* extract and 2 simple phenolic compounds. The first compound identified as phenolic acid is *o*-coumaric acid (3) at *m/z* 165 $[M + H]^+$ with fragments at *m/z* 147 $[M + H - H_2O]^+$, 123 $[M + H - C_3H_6]^+$, 95 $[M + H - C_3H_6 - CO]^+$. The second compound (10) is octyl gallate at *m/z* 281 $[M - H]^-$ fragmented at *m/z* 237 $[M - H - CO_2]^-$, 139 $[M - H - CO_2 - 98]^-$, and 125 $[M - H - CO_2 - 139]$. The simple phenolic compounds identified are 2,4,5-trimethoxybenzaldehyde (*m/z* 197 $[M + H]^+$) and 4-*tert*-butylcatechol (*m/z* 167 $[M + H]^+$).

3.4. Flavonoids

There are two flavonoid compounds identified and belong to the flavanones. Pinocembrine (25) at *m/z* 255 $[M - H]^-$ is identified at a retention time of 10.16 min with fragmentation at *m/z* 213 $[M + H - C_3H_6]^+$, 211 $[M + H - C_2H_4O]^+$, 151 $[M + H - C_8H_8]^+$, and 145 $[M + H - C_7H_{10}O]^+$. Alpinetin (33) at *m/z* 271 $[M + H]^+$ has fragments at *m/z* 253 $[M + H - H_2O]^+$, 229 $[M + H - C_3H_6]^+$, 167 $[M + H - H_2O - C_5H_{11}O]^+$, and 131 $[M + H - H_2O - C_5H_{14}O_3]^+$.

3.5. Other groups of compound

Some other groups of compounds in the extract are aldehyde (benzaldehyde), ketone (dihydrojasmone), fatty acid (eicosapentaenoic acid), furan (zedoarofuran), anthraquinone (eleutherin), coumarin (3,4-dihydrocoumarin), lactone (curcumenolactone A), chalcone (2′-hydroxy-2,4,4′-trimethoxychal cone), vinilogous acid (hulupinic acid and benzyl salicylate), and steroid (norethindrone). Compound (31) is benzaldehyde at *m/z* 107 $[M + H]^+$. The dihydrojasmone (29) is identified at *m/z* 167 $[M + H]^+$ with fragments at *m/z* 123, 111, 107, 97, 93, and 81. The compound (32) at *m/z* 303 $[M + H]^+$ is recognized as eicosapentaenoic acid and has fragments at *m/z* 285, 267, 229, 163, and 109.

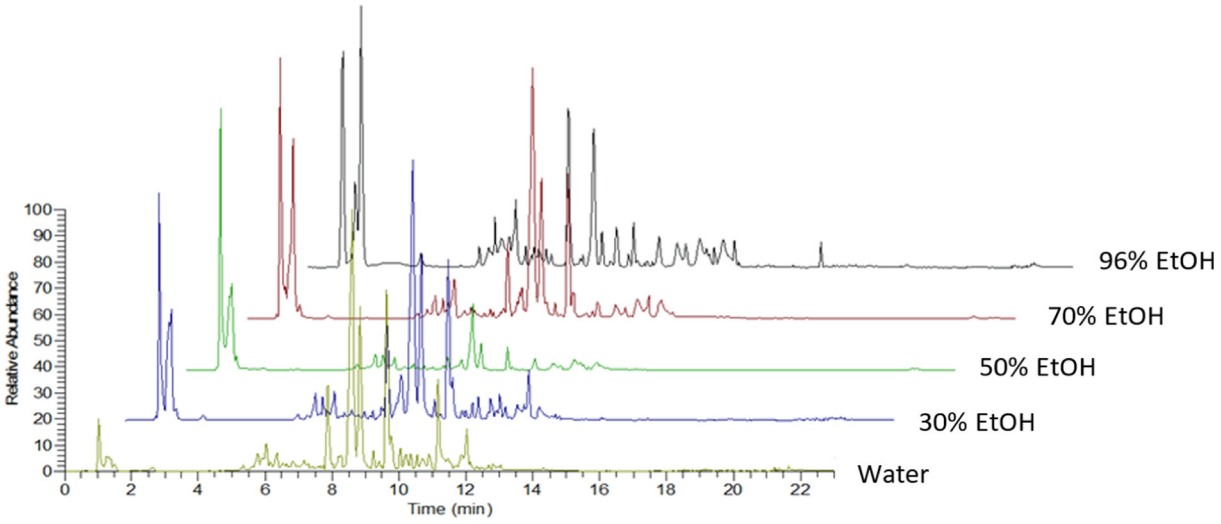

Fig. 2 Base peak chromatograms of negative ionization mode of the five *K. rotunda* extracts.

Zedoarofuran (8) is identified at *m/z* 265 $[M + H]^+$ with fragments at *m/z* 247 $[M + H - H_2O]^+$, 229 $[M + H - 2H_2O]^+$, and 105 $[M + H - 2H_2O - C_8H_{12}O]^+$ (Hao et al., 2019). The compound (19) at *m/z* 273 $[M + H]^+$ is known as eleutherin with its fragments at *m/z* 255, 199, and 185. Compound (30) at *m/z* 149 $[M + H]^+$ is 3,4-dihydrocoumarin with fragmentation releasing CO $[M + H - 28]^+$ and CH_2 $[M + H - CO - 14]^+$. Curcumenolactone A (18) is identified at *m/z* 249 $[M + H]^+$ producing fragments at *m/z* 203, 143, and 105 (Hao et al., 2019). The compound (38) 2′-hydroxy-2,4,4′-trimethoxychalcone is at *m/z* 315 $[M + H]^+$ giving fragments at *m/z* 273, 191, 190, and 151.

Hulupunic acid (15) at *m/z* 265 $[M + H]^+$ produces fragments at *m/z* 247 $[M + H - H_2O]^+$, 219 $[M + H - H_2O - CO]^+$, 177 $[M + H - H_2O - CO - C_3H_6]^+$, and 145 $[M + H - H_2O - CO - C_4H_{10}O]^+$. The compound (35) at *m/z* 229 $[M + H]^+$ is identified as benzyl salicylate with its fragments at *m/z* 151, 105, 95, 77, and 53. The compound (34) norethindrone in at *m/z* 299 $[M + H]^+$ with fragments at *m/z* 281, 241, 229, 199, 185, 171, 83, and 55.

2-acetylrotepoxide A (43) pada *m/z* 427 $[M + H]^+$ produce a fragmentation at *m/z* 105 $[M + H - C_{16}H_{16}O_8]^+$, follow by releasing CO molecule at *m/z* 77 $[M + H - C_{16}H_{16}O_8 - CO]^+$. (-) 6-acetylzeylenol (44) at *m/z* 427 $[M + H]^+$ fragmented at *m/z* 105 $[M + H - C_{16}H_{17}O_7]^+$ and follow with the releasing CO molecule at *m/z* 77 $[M + H - C_{16}H_{17}O_7 - CO]^+$. 5,7-dihydroxyflavanone (45), a compound of the flavonoid group, fragmented at *m/z* 105 after retro diels alder reaction (RDA). Zeylenol (46) was identified with *m/z* 385 and fragmented at *m/z* 263, 123, and 105.

9-octadecenamide (47) at *m/z* 282 was fragmented with releasing NH_2 at *m/z* 265 $[M + H - NH_2]^+$. Benzylbenzoat (48) was identified at *m/z* 213 and gave fragmentation by releasing the C_7H_7O molecule at *m/z* 105 $[M + H - C_7H_7O]^+$. Benzylalcohol (49) at *m/z* 109 produces a fragment at *m/z* 91 by releasing H_2O $[M + H - H_2O]^+$. Bis (2-ethylhexyl) phthalate (50) at *m/z* 391 produces a fragment at *m/z* 391, 167, 149, dan 71. Propylparaben (51) was identified at a retention time of 12.435 min at *m/z* 181. Sandaracopimaradiene (52) was identified in 18.422 min at *m/z* 273 $[M + H]^+$. The stigmasterol (53) was identified with *m/z* 413, 107, dan 81.

3.6. Classification of Kaempferia angustifolia extracts

K. angustifolia extracts with different extracting solvents are classified based on the area of the identified compound. The variables used in this PLS-DA analysis were the area of 53 identified compounds. Each point on the score plot represents a single sample, and the samples indicating similarities were grouped (Liu et al., 2016). Fig. 2 shows the plot score, explaining that 99% of the total variation (PC1 = 97%, and PC2 = 2%). Samples with the same label or color are grouped in adjacent positions (96% ethanol and water extracts). Some single samples do not represent a good grouping (30%, 50%, and 70% EtOH extracts).

The resulting score plot (Fig. 1) explains that the extracts from the solvent composition can be differentiated into three groups: Group 1 (EtOH), Group 2 (50% EtOH), and group 3 (30, 70% EtOH, and water). The 96% and 50% EtOH extracts can be classified well, while water, 30%, and 70% EtOH do not group well. It indicates that the 96% and 50 ethanol extracts have different metabolite compositions, while those of water, 30%, and 70% EtOH extracts have similar metabolite content as the compositions are close together.

The PCA biplots can explain the compounds that play a role in or influence the sample grouping patterns (Fig. 3). Each point on the PLS-DA loading plot is a component variable that contributes significantly to the differences between groups, which is the furthest component of the main group (Taha et al., 2020). Several variables or compounds affect the samples' grouping pattern differentiation based on the combined plot between the score plot and loading (biplot). The biplots (Fig. 2) show that some compounds can be used as markers in grouping *K. angustifolia* extracts based on extracting solvents. The compounds that play a role in the grouping pattern are α-cyperone, neocurdione, 2′-hydroxy-2,4,4′-trimethoxychalcone (Fig. 4).

Table 2 Metabolites in water, 30% ethanol, 50% ethanol, 70% ethanol, and 96% ethanol extracts.

No	Compound	Solvent				
		Water	30% EtOH	50% EtOH	70% EtOH	96% EtOH
1	Citric acid				√	
2	*trans*-Aconitic acid	√				
3	*o*-Coumaric acid	√	√	√	√	
4	2-Isopropylmalic acid	√	√	√	√	
5	2,4,5-Trimethoxybenzaldehyde				√	√
6	Phenyllactate	√				
7	4-*tert*-Butylcatechol					√
8	Zedoarofuran					√
9	Azelaic acid	√	√	√	√	√
10	Octyl gallate					√
11	4-Hydroxybenzoic acid		√			
12	Furanogermenone	√	√	√	√	√
13	Curzeone					√
14	Retinoic acid	√	√	√	√	√
15	Hulupinic acid					√
16	Zedoarondiol		√	√	√	√
17	Benzoic acid	√	√	√	√	√
18	Curcumenolactone A	√	√		√	√
19	Eleutherin		√	√	√	√
20	Neocurdione	√	√	√	√	√
21	Culmorin					√
22	Zederone				√	√
23	Gemfibrozil					√
24	Caryophyllene oxide	√	√	√	√	√
25	Pinocembrine					√
26	Curcumenol					√
27	Traversianal					√
28	Furanodiene					√
29	Dihydrojasmone					√
30	3,4-Dihydrocoumarin					√
31	Benzaldehyde					√
32	Eicosapentaenoic acid		√	√	√	√
33	Alpinetin					√
34	Norethindrone					√
35	Benzyl salicylate				√	√
36	*cis*-Nerolidol					√
37	Camphor	√				
38	2′-Hydroxy-2,4,4′-trimethoxychalcone				√	
39	α − Χψπερονε				√	√
40	Retinal					√
41	Pyrocurzerenone					√
42	Camphene				√	√
43	2-acetylrotepoxide A	√	√	√	√	√
44	(-) 6-acetylzeylenol	√	√	√	√	√
45	5,7-Dihydroxyflavanone					√
46	Zeylenol	√	√	√	√	√
47	9-octadecenamide				√	√
48	Benzylbenzoat				√	
49	Benzylalcohol	√	√	√	√	√
50	Bis (2-ethylHexyl) phthalate	√	√	√	√	√
51	Propylparaben				√	
52	Sandaracopimaradiene				√	√
53	Stigmasterol			√	√	√

3.7. In vitro pancreatic lipase inhibition activity

Inhibition of water extract and ethanol extract of *K. angustifolia* on pancreatic lipase enzyme activity was measured *in vitro* and the results were compared with Orlistat as a positive control. As shown in Fig. 5, Orlistat produced a high percentage inhibition value of about 72.54%, indicating strong inhibitory activity towards pancreatic lipase. For the sample extracts with a 100 μg/mL concentration, 50% ethanol extract of *K. angustifolia* gave the highest inhibition, about 59.82%, compared to

the other extracts. These results show that *K. angustifolia* extracted with 50% ethanol contains chemical compounds that have the potency of pancreatic lipase inhibitors.

Several compounds identified in the water and EtOH extracts of *K. angustifolia*, namely citric acid, 4-hydroxybenzoic acid, benzoic acid, pinocembrine, propylparaben, and stigmasterol, have been reported to have inhibitory activity against lipase enzymes (Ekinci et al., 2015, Ong et al., 2016, Liu et al., 2020, Sari et al., 2021). Other compounds such as 2,4,5-trimethoxybenzaldehyde, azelaic acid, octyl gallate, retinoic acid, and benzyl benzoate are also reported to have potency as antiobesity agents but through other mechanisms other than lipase inhibitors (Ha et al., 2004, Taha et al., 2008, Neels 2013, Wang & Kuo 2014, Khairudin et al., 2018, Lee et al., 2021).

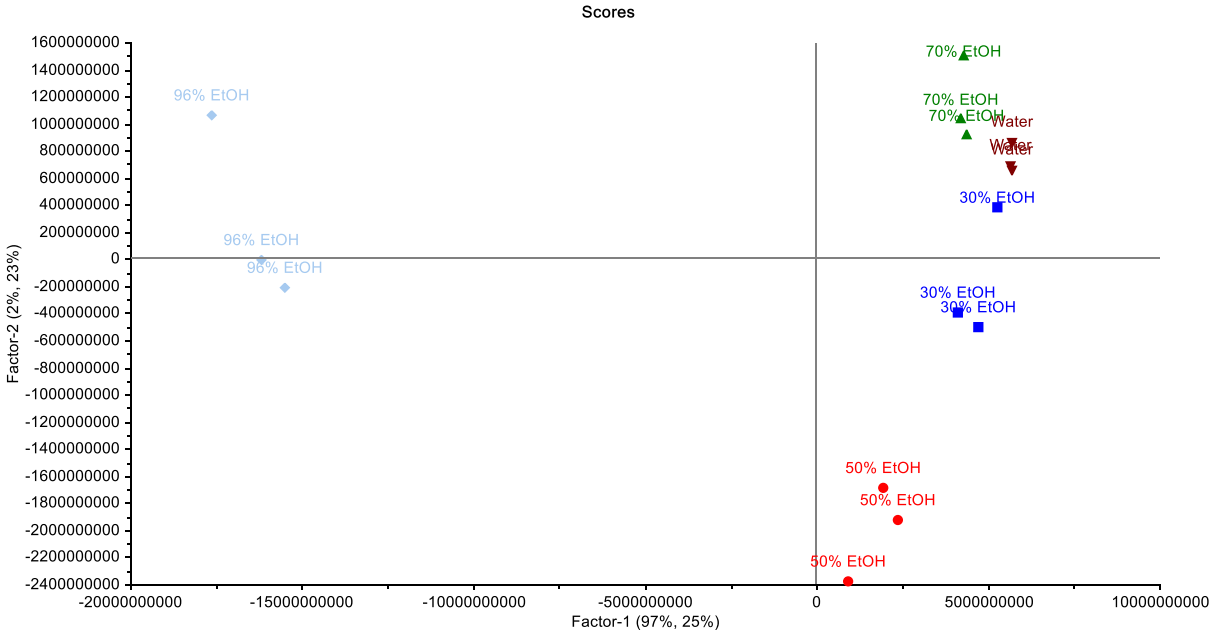

Fig. 3 Plot score of PLS-DA of the five *K. rotunda* extracts.

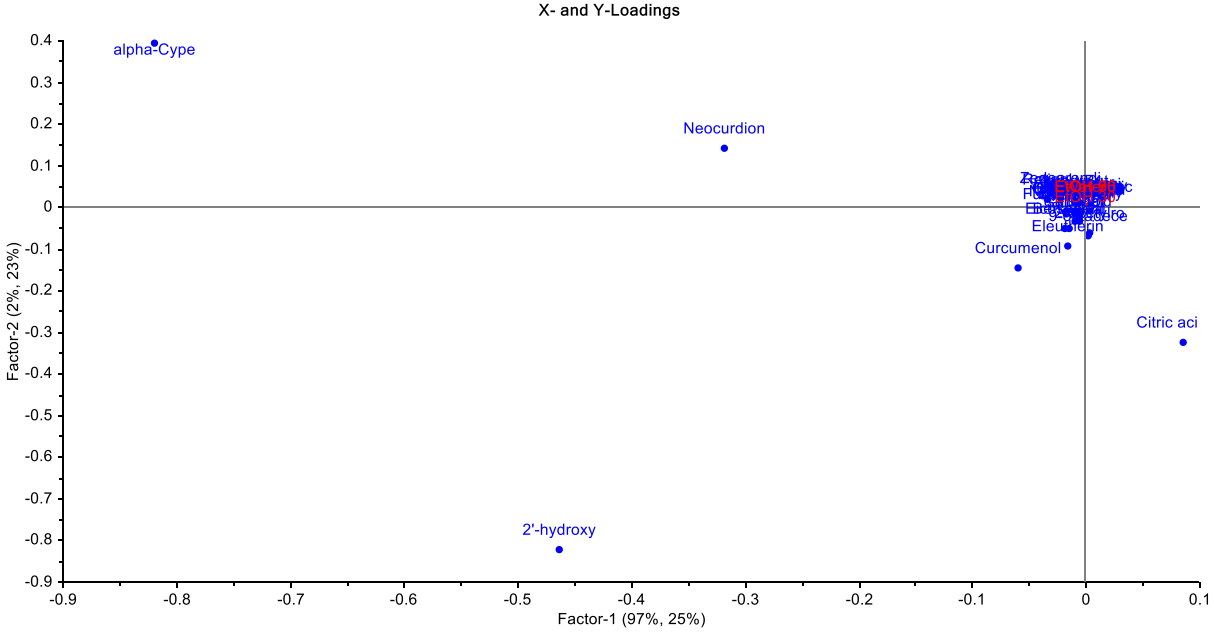

Fig. 4 Biplot of PLS-DA of the five *K. rotunda* extracts.

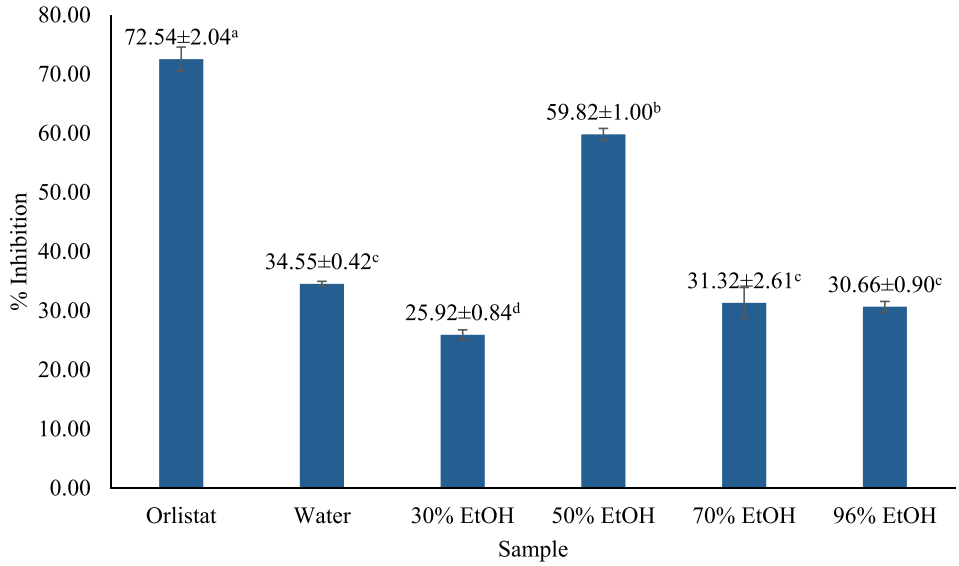

Fig. 5 Pancreatic lipase inhibition activity of *K. angustifolia* extracts. Numbers followed by different letters show a significant difference ($p < 0.05$).

4. Conclusions

Putative identification of metabolite in *K. angustifolia* extracts using water, ethanol, and their mix as extracting solvent using LC-MS/MS resulted in 53 metabolites identified. The predominant metabolites in this plant extract are 19 compounds that belong to terpenoids. We found 50% ethanol extract of *K. angustifolia* gave the highest inhibition, about 59.82%, compared to the other extracts. The PLS-DA can classify the extracts based on different types of solvent composition by utilizing the peak intensity of the identified compounds. Thus, the selection of this solvent proved to be easy and efficient in producing *K. angustifolia* extract, which has the potential to inhibit lipase enzyme activity. However, it is necessary to pay attention to the stability related to the safety of extracts produced from solvents to guarantee the quality of the extracts.

CRediT authorship contribution statement

Mohamad Rafi: Conceptualization, Methodology, Validation, Writing – review & editing. **Alfi Hudatul Karomah:** Investigation, Formal analysis, Visualization, Writing – original draft. **Dewi Anggraini Septaningsih:** Investigation, Formal analysis, Visualization, Writing – original draft. **Trivadila:** Investigation, Formal analysis, Writing – original draft, Project administration. **Min Rahminiwati:** Conceptualization, Methodology, Writing – review & editing. **Sastia Prama Putri:** Conceptualization, Methodology, Writing – review & editing. **Dyah Iswantini:** Conceptualization, Methodology, Investigation, Funding acquisition, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.arabjc.2022.104232.

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