

## KORESPONDENSI ARTIKEL

Judul Artikel : Chemical composition, antioxidant activities, and total phenolic content of combination of mangosteen (*garcinia mangostana* L.) Peel kodavan (*Centella asiatica* L. Urban) active fractions

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4 Januari 2023	Editor and reviewer comments
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## Fw: Fw: Manuscript submission to Tropical Journal of Natural Product Research

1 message

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**Ryan Munandar** <ryanmunandar0496@alumni.undip.ac.id>  
To: Khairul ANAM <k.anam@live.undip.ac.id>

Wed, Jun 12, 2024 at 8:58 AM

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**From:** Editor-in-Chief Tjnpr <editor.tjnpr@gmail.com>  
**Sent:** Saturday, December 17, 2022 2:44 AM  
**To:** Ryan Munandar <ryanmunandar0496@students.undip.ac.id>  
**Subject:** Re: Fw: Manuscript submission to Tropical Journal of Natural Product Research

Dear Ryan,  
Thank you for submitting your original manuscript to the Tropical Journal of Natural Product Research ([www.tjnpr](http://www.tjnpr)) <https://www.scopus.com/sourceid/21100933230> SCOPUS [..published by the University of Benin and Natural Product Research Group.](#)

The peer-review process will commence immediately, as the manuscript will be passed to an editor for initial assessment as soon as possible.

Title: Chemical composition, antioxidant activities, and total phenolic content of combination of mangosteen (*garcinia mangostana* l.) Peel-kodavan (*centella asiatica* l. Urban) active fractions

Best regards

Abiodun

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### Professor Abiodun Falodun, PhD

Editor-in-Chief:  
Tropical Journal of Natural Product Research (TJNPR)  
Head, Natural Product Research Group, University of Benin  
Email: [editor.tjnpr@uniben.edu](mailto:editor.tjnpr@uniben.edu); [editor.tjnpr@gmail.com](mailto:editor.tjnpr@gmail.com)  
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Professor of Pharmaceutical Chemistry  
Fellow, Fulbright (USA)  
Deputy Vice-Chancellor (Academic) 2014-2016  
Faculty of Pharmacy  
University of Benin  
Phone: +234-807-318-4488;  
email: [faloabi@uniben.edu](mailto:faloabi@uniben.edu); [abiodun.falodun@fulbrightmail.org](mailto:abiodun.falodun@fulbrightmail.org)  
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<https://orcid.org/0000-0003-2929-3305authorId=12794326500#top>



On Fri, 16 Dec 2022 at 09:34, Ryan Munandar <[ryanmunandar0496@students.undip.ac.id](mailto:ryanmunandar0496@students.undip.ac.id)> wrote:

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**From:** Ryan Munandar  
**Sent:** Tuesday, December 13, 2022 8:46 PM  
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Dear Professor A. Falodun,

Good morning, My Name is Ryan Munandar, I am a magister student of chemistry from Universitas Diponegoro, Semarang City of Indonesia.

I am honoured to submit our manuscript entitled " Chemical composition, antioxidant activities, and total phenolic content of combination of mangosteen (*garcinia mangostana* L.) Peel-kodavan (*centella asiatica* L. Urban) active fractions " by Khairul Anam, Ryan Munandar\*, Octavia Nur Wulandari, Aninda Bibit Lestari, Rivany Eshamia Farada, Dwi Hudyanti, Agustina Lulustyaningati Nurul Aminin to be considered for publication as research article in the Tropical Journal of Natural Product Research.

Dear professor, about 12 days ago (4-12-2022), I submitted this manuscript with the email address of [ryanmunandar0496@gmail.com](mailto:ryanmunandar0496@gmail.com). That was followed by your email reply that we have to complete the documents since I only attached the manuscript and cover letter.

Please refer to this resubmission of manuscript and the email address for the following correspondence ([ryanmunandar0496@students.undip.ac.id](mailto:ryanmunandar0496@students.undip.ac.id))

Here I attached some of documents requested, namely:

1. The full paper of manuscript
2. Cover letter
3. List of potential reviewers
4. List of the authors and contribution
5. Copyright agreement


Thank you very much professor, I hope this manuscript can be published here since Tropical Journal of Natural Products Research is one the best and suitable to our topic of research.

## revised manuscript, proofreading, and turnitin

Ryan Munandar <ryanmunandar0496@students.undip.ac.id>

Tue 17/01/2023 09:21

To:Khairul Anam <k.anam@lecturer.undip.ac.id>

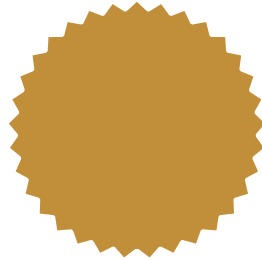
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Chemical composition, antioxidant activities, and total phenolic content of combination of mangosteen (garcinia mangostana l.) Peel-kodavan (centella asiatica l. Urban) active fractions.pdf; (ORIGINAL TITLE) CERTIFICATE FULL PAPER GOODLINGUA.pdf; responses to reviewer's comments.docx; Revised Manuscript of TJNPRNOV155ARN.docx; Manuscript\_Ryan Munandar\_Chemical\_TRACKING.docx;

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## Manuscript Title

Chemical Composition, Antioxidant Activities, and Total Phenolic Content of Combination of Mangosteen (*garcinia mangostana* l.) Peel-kodavan (*centella asiatica* l. Urban) Active Fractions

## Author(s)

Khairul Anam, Ryan Munandar, Octavia Nur Wulandari, Aninda Bibit Lestari, Rivany Eshamia Farada, Dwi Hudiyaniti, Agustina Lulustyaningati Nurul Aminin

## Date Issued

January 16, 2023



PT. Internasional Translasi Edukasi, Jakarta

No.	Reviewer's comments	Correction/responses	Description
1.	<p><i>Better condense it to be one sentences:</i></p> <p>The use of herbs is being reconsidered at the moment. Herbs is believed to be safer. Herbs are combination of several natural ingredients that contain various active chemicals</p>	Herbs, known for their naturally active chemicals, are currently being reconsidered as a safer alternative.	See line 20-21
2.	<p><i>What it is mean?:</i></p> <p>Each crude ethanolic extract</p>	The GM and CA crude ethanolic extract (CEE)	See line 102
3.	<p><i>Make this statement more simple:</i></p> <p>About 10 mg of each extract and fraction of GM, and about 20 mg of each extract and fraction of CA were dissolved in 10 mL ethanol, respectively.</p>	10 and 20 mg of GM and CA extracts and their respective fractions are dissolved in 10 mL ethanol.	See line 110-111
4.	<p><i>Please authors explain how TLC could know exactly the groups of secondary metabolites? Authors used HPTLC??:</i></p> <p>The CEE and its fraction of CA contain secondary metabolites, such as flavonoids, terpenoids, and phenolic compounds</p>	The CEE and its fraction of CA displayed a spot of a different color. Further analysis was conducted using specific spray reagents as preliminary identification of the chemical compound groups, such as flavonoids, terpenoids, and phenolic compounds	See line 166-168
5.	<p><i>Authors used the change of color under UV lamp to justified the group of secondary</i></p>	The obtained spot was identified using specific spray dyeing reagents and examined under a UV	See line 113-118

	<p><i>metabolites?</i> <i>Authors should prove this approach :</i></p> <p>Identification of phytochemical compounds in this research is based on the change of color obtain from the TLC plate when it identify under UV lamp at 365 nm. In CA extract and fractions, the presence of flavonols are denoted by the change of color into orange. The presence of flavons are denoted by the change of color into blue, yellow, and green under UV lamp at 365 nm</p>	<p>lamp at 254 nm and 365 nm. Flavanoids were identified using AlCl<sub>3</sub> 1%, and their presence is denoted by the change of color to blue, yellow, green, and orange. Phenolic compounds were detected using FeCl<sub>3</sub> 5%, and their presence is denoted by the change of color to black. Additionally, terpenoids were identified by the lieberman-burchad reagent, and their presence is denoted by the change of color to pink</p>	
6.	<p><i>Please check some reference that have red mark:</i></p> <p>Nur Khusnawati N, Pramono S, Sasmito E. <b>EFFECT OF 50% ETHANOLIC EXTRACT OF PEGAGAN HERB (Centella asiatica (L.) Urban) ON CELL PROLIFERATION OF LYMPHOCYTES IN Balb/c MALE MICE INDUCED BY HEPATITIS B VACCINE</b> PENGARUH EKSTRAK ETANOLIK 50% HERBA PEGAGAN (Centella asiatica</p>	<p>Nur Khusnawati N, Pramono S, Sasmito E. Effect of 50% Ethanolic Extract of Pegagan Herb (<i>Centella asiatica</i> (L.) Urban) on Cell Proliferation of Lymphocytes in Balb/C Male Mice Induced by Hepatitis B Vaccine. Trad Med J. 2015; 20(3):164-169.</p>	See line 325-327

	(L.) Urban) TERHADAP PENINGKATAN PROLIFERASI SEL LIMFOSIT MENCIT JANTAN GALUR Balb/c YANG DIINDUKSI VAKSIN HEPATITIS B. Tradit Med J. 2015;20(3):2015.		
7.	<i>Please make clear result of GM extract and GM Fractions, separately</i>	CEE: crude ethanolic extract; EAF: ethyl acetate fraction; EF: ethanolic fraction.	See line 361
8.	<i>Please authors put IC50 of positive control also:</i>	Positive control : Quersetin IC50: 4.63	See Table 3
9.	<i>Which active fraction that authors mean?</i>  The Antioxidant activities of GM-CA active fraction combinations	The Antioxidant activities of GM-CA ethyl acetate fraction combination	See Table 4



1 Chemical composition, antioxidant activities, and total phenolic content of combination of  
2 mangosteen (*garcinia mangostana* L.) Peel-kodavan (*centella asiatica* L. Urban) active  
3 fractions

4 KHAIRUL ANAM<sup>1,2,a</sup>, RYAN MUNANDAR<sup>1,b\*</sup>, OCTAVIA N. WULANDARI<sup>1,c</sup>,  
5 ANINDA B. LESTARI<sup>1,d</sup>, RIVANY E. FARADA<sup>1,e</sup>, DWI HUDIYANTI<sup>1,f</sup>, AGUSTINA  
6 L.N. AMININ<sup>1,g</sup>.

7 <sup>1</sup>Department of Chemistry, Faculty of Science and Mathematics, Universitas Diponegoro. Jl.  
8 Prof. Jacob Rais, Tembalang Semarang-50275, Central Java, Indonesia. Tel. (024)7474754,

9 <sup>2</sup>Department of Pharmacy, Faculty of Medicine, Universitas Diponegoro, Jl. Prof Soedharto,  
10 SH.Tembalang Semarang, Central Java, Indonesia. Tel/Fax. 024 76928010/024 76928011

11 <sup>a</sup>email: [k.anam@live.undip.ac.id](mailto:k.anam@live.undip.ac.id)

12 <sup>\*b</sup>email: [ryanmunandar0496@students.undip.ac.id](mailto:ryanmunandar0496@students.undip.ac.id) HP. +62895365074432

13 <sup>c</sup>email: [octavianurwulandari@students.undip.ac.id](mailto:octavianurwulandari@students.undip.ac.id)

14 <sup>d</sup>email: [anindabibitlestari@students.undip.ac.id](mailto:anindabibitlestari@students.undip.ac.id)

15 <sup>e</sup>email: [rivanyeshamiafarada@students.undip.ac.id](mailto:rivanyeshamiafarada@students.undip.ac.id)

16 <sup>f</sup>email: [dwi.hudiyanti@live.undip.ac.id](mailto:dwi.hudiyanti@live.undip.ac.id)

17 <sup>g</sup>email: [agustina.aminin@live.undip.ac.id](mailto:agustina.aminin@live.undip.ac.id)

18

19 **Abstract**

20 Herbs, known for their naturally active chemicals, are currently being reconsidered as a safer  
21 alternative. The natural substances that are known to have lots of bioactivities, such as  
22 antioxidant properties, are mangosteen (*Garcinia mangosata* L.) (GM) and kodavan  
23 (*Centella asiatica* L. Urban) (CA). However, the antioxidant activities of the GM-CA  
24 combination have not been previously reported. It is essential to investigate the properties of  
25 the active fractions to determine which fraction possesses the best antioxidant activities.  
26 Therefore, this research aimed to determine the chemical composition and evaluate the  
27 antioxidant activities (IC<sub>50</sub>) and total phenolic content (TPC) of GM, CA, and their  
28 combination. The combination is expected to exhibit a synergistic effect and an increase in  
29 antioxidant activities. GM and CA were percolated using ethanol and successively partitioned  
30 by n-hexane and ethyl acetate. Antioxidant activities and TPC were evaluated using DPPH and  
31 Folin Ciocalteu methods, respectively. Chemical components were determined through LC-  
32 MS/MS analysis and phytochemical screening. The combination of ethyl acetate fractions  
33 (EAF) of GM-CA in a 1:3 ratio indicates synergistic interaction, strong antioxidant activities  
34 IC<sub>50</sub> = 62.00 ppm, and 132.38 mgGAE/g of TPC. Phytochemical screening showed the  
35 presence of flavonoids, terpenoids, and polyphenols. LC-MS/MS identified several compounds  
36 in GM, such as (+/-) gomisin M2, archangelicin, biodinin A, α-mangosteen, sarcandin acetate,  
37 and achilin. In CA, 5,7,2',5'-tetrahydroxy-flavon,5-hydroxy-6,4'-dimethoxy-flavone-7-O-β-  
38 D-glucopyranose, asiaticoside, kaempferol-3,7-diglucoside, madecassoside, 3β,6β,23-  
39 trihydroxy-urs-12-en-28-oic acid, kaempferol-3-O-rutinoside, and Mahuanin, were  
40 discovered.

41 **Keywords:** Antioxidant, *Centella asiatica* L. Urban, *Garcinia mangostana* L.,  
42 Phytochemistry, Total phenolic content.

43 **INTRODUCTION**

44 The use of herbs as traditional medicine is increasing as they are being reconsidered by  
45 people worldwide. Their advantages include easy accessibility, inexpensiveness, and safer due  
46 to the natural source. Herbs are composed of various mixtures of ingredients that possess  
47 different chemical compositions. Combining two natural ingredients with different chemical  
48 content is expected to produce a better therapeutic effect through the synergistic interaction of  
49 the various active components.

50 Mangosteen (*Garcinia mangostana* L.) (GM) and Kodavan (*Centella asiatica* L. Urban)  
51 (CA) are widely spread in Indonesia, India, and many other Southeast Asia countries. These  
52 herbs have been a natural medicine due to their bioactivities. GM and CA have been reported  
53 to possess several bioactive properties, such as anti-cancer,<sup>1</sup> anti-proliferation,<sup>2</sup> and  
54 antioxidant.<sup>3,4</sup>

55 Antioxidants protect the body from free radicals caused by unhealthy lifestyles and air  
56 pollution. An excess of free radicals in the body can lead to damage to the cells and tissues.  
57 Andri reported that GM's acetone and ethyl acetate extracts have strong and moderate  
58 antioxidant activities, respectively.<sup>5</sup> Meanwhile, for CA, it is reported as weak to moderate.<sup>6</sup>  
59 However, the antioxidant activities from the combination of GM and CA (GM-CA) active  
60 fractions have not been evaluated previously.

61 Evaluating the antioxidant activities and total phenolic content (TPC) from the  
62 combination of GM-CA active fraction is of great interest. The combination could enhance its  
63 antioxidant activities due to the synergism of its secondary metabolites. Furthermore, it is also  
64 expected to have anti-bacterial and immunomodulatory activities.

65 GM is reported to contain secondary metabolites, such as phenolic compounds,<sup>7</sup> while  
66 CA consists of phenolic and terpenoid compounds.<sup>8</sup> The main active component in GM is  $\alpha$ -  
67 mangosteen, which belongs to the xanthone group, a class of polyphenols, with a chemical  
68 structure consisting of a C6-C1-C6 backbone. It is discovered in large amounts in GM's

69 pericarp and possesses many biological activities.<sup>9</sup> Asiaticoside, a pentacyclic triterpene of the  
70 ursane class, is one of the main components identified in CA. It is a triterpene glycoside with  
71 glucose attached to its C-28 (ring E) and possesses many biological activities.<sup>10</sup> These  
72 secondary metabolites are expected to exhibit synergistic effects and the strongest antioxidant  
73 activities.

74 Previous studies have investigated the antioxidant activities of ethanolic extracts. In this  
75 research, GM and CA were examined from the different polarity of the solvents, such as crude  
76 ethanolic extract (CEE), ethyl acetate fraction (EAF), and an ethanolic fraction (EF), to  
77 determine which fraction has the highest level. GM-CA combination will be made in several  
78 compositions of ratios to obtain the best ratio with the strongest antioxidant activities as a result  
79 of synergism interaction.

80 The primary aim of this research was to investigate the hypothesis that the CEE, EAF, EF,  
81 and the combination of GM-CA active fractions exhibit different antioxidant activities, which  
82 were positively correlated with the ratio of their chemical compositions.

## 83 **MATERIALS AND METHODS**

### 84 **Procedures**

#### 85 *Materials and chemicals*

86 The materials used in this research include GM peel powder (Java Plant, Indonesia), CA  
87 powder (Java Plant, Indonesia), Ethanol 96% (Happy Lab, Indonesia), ethyl acetate  
88 (Bratachem, Indonesia), n-hexane (Bratachem, Indonesia), phytochemistry and TLC dyeing  
89 reagents (Merck, Germany), ethanol (Merck, Germany), Na<sub>2</sub>SO<sub>4</sub>, benzene (Merck, Germany),  
90 ethyl acetate (Merck, Germany), chloroform (Merck, Germany), butanol (Merck, Germany),  
91 dichloromethane (Merck, Germany), sulfuric acid (Merck, Germany), gallic acid (Sigma  
92 Aldrich, USA), Quercetin (Sigma Aldrich, Japan), DPPH (Sigma Aldrich, USA) Folin-  
93 Ciocalteu (Merck, Germany), Sodium Carbonate (Merck, Germany), Distilled water, TLC

94 Silica gel G<sub>60</sub> F<sub>254</sub> (Merck, Germany), UV-Vis Spectrophotometer (Genesys 10S), Analytical  
95 Balance (Ohaus, model PA323), UV Quartz cuvette, and rotary evaporator (Scilogex RE-100  
96 pro).

#### 97 *Extraction and fractionation methods*

98 The GM and CA dried powdered plant material (5kg) were each percolated with Ethanol  
99 96% at a ratio of 1:4 (w/v) continuously for 7 days at room temperature. Following this,  
100 filtration was performed, and the solvent was removed using a rotary evaporator to obtain the  
101 crude ethanolic extract (CEE)

102 The GM and CA crude ethanolic extract (CEE) were each dissolved in ethanol 1:4 (w/v),  
103 partitioned by n-hexane 1:1 (v/v), and separated. Distilled water was then added to the ethanolic  
104 phase 1:1 (v/v) and successively partitioned by ethyl acetate 1:1 (v/v) to obtain ethyl acetate  
105 fraction (EAF). The residue of the last partition is known as the ethanolic fraction (EF). Finally,  
106 all the fractions are filtered, and their solvent is removed using a rotary evaporator.

#### 107 *Phytochemicals screening analysis method*

108 Qualitative phytochemical screening analyses were performed using standard methods.<sup>11</sup>  
109 Silica gel G<sub>60</sub> F<sub>254</sub> was used as a stationary phase and activated by heating at 100 °C for 10  
110 minutes. Furthermore, 10 and 20 mg of GM and CA extracts and their respective fractions are  
111 dissolved in 10 mL ethanol. The solution was spotted in a TLC plate and eluted using a mixture  
112 of ethyl acetate and chloroform at a 3:7 ratio for GM, as well as benzene and ethyl acetate at  
113 6:4 for CA. The obtained spot was identified using specific spray dyeing reagents and examined  
114 under a UV lamp at 254 nm and 365 nm. Flavanoids were identified using AlCl<sub>3</sub> 1%, and their  
115 presence is denoted by the change of color to blue, yellow, green, and orange. Phenolic  
116 compounds were detected using FeCl<sub>3</sub> 5%, and their presence is denoted by the change of color  
117 to black. Additionally, terpenoids were identified by the lieberman-burchad reagent, and their  
118 presence is denoted by the change of color to pink.<sup>12,13</sup>

119 *LC/MSMS analysis method*

120 Liquid Chromatography-mass spectrometry analysis was performed on water acquity  
121 UPLC I-Class and XEVO G2-XS QToF. The instrument was operated in full scan ESI mode.  
122 The liquid chromatography conditions were a C18 column with a particle size of 1.7 µm and a  
123 2.1 x 50 mm length. Furthermore, the eluents employed were a mixture of H2O and 0.1%  
124 formic acid (Solvent A), as well as ACN and 0.1% formic acid (Solvent B). The injection  
125 volume was 1 µL, and the ionization type was set at ESI positive. Finally, the mass ranges from  
126 100-1200 m/z.

127 *The antioxidant activities assay method*

128 The scavenging of DPPH free radical was used for measuring the antioxidant activities of  
129 extracts, fractions, and combinations, with the ratios of GM-CA being 3:1, 1:1, and 1:3 %.  
130 About 25 mg of each sample was dissolved in 25 mL methanol to obtain the stock solution at  
131 the concentration of 1000 ppm. Subsequently, the stock solution was diluted with methanol to  
132 obtain a series of concentrations of each sample. About 3.5 mL of each sample solution was  
133 thoroughly mixed with freshly prepared 1.5 mL of DPPH 0.4 mM and kept for 30 minutes in  
134 the dark at room temperature, respectively. The amount of reaction mixture was determined  
135 using a UV-Vis spectrophotometer at 517 nm. The antioxidant activities expressed as IC<sub>50</sub> and  
136 Quercetin serve as standard antioxidants. Furthermore, the experiments were repeated 3 times  
137 and reported as Mean ± SD.<sup>14</sup> The percentage of inhibition was calculated by the following  
138 formula:

139

$$140 \quad \text{Inhibition (\%)} = \frac{\text{Abs of Control} - \text{Abs sample}}{\text{Abs of control}} \times 100 \%$$

141 *Total phenolic content (TPC) assay method*

142 About 25 mg of gallic acid and each sample were dissolved in 25 mL methanol, respectively,  
143 to obtain 1000 ppm of stock solutions. Approximately 0.5 mL of 500 ppm solution was placed

144 in a vial, followed by adding 2.5 mL distilled water and 2.5 mL of Folin-Ciocalteu reagent,  
145 respectively. The mixture was thoroughly mixed and allowed to incubate for 15 minutes.  
146 Furthermore, about 2.5 mL of 7.5% sodium carbonate solution was added, mixed, and  
147 incubated for 30 minutes in the dark. The absorbance of the mixture was measured at 756 nm.  
148 The gallic acid curve was used as a calibration curve, while the TPC is represented as gallic  
149 acid equivalents (GAE). The experiments were repeated 3 times, and the results were expressed  
150 as Mean  $\pm$  SD.<sup>14</sup> The following formula was used to calculate TPC:

$$151 \quad \text{TPC} \left( \frac{\text{mgGAE}}{\text{g of dried extract}} \right) = \frac{\text{concentration of total phenols} \left( \frac{\text{mg}}{\text{L}} \right)}{\text{concentration of extract} \left( \frac{\text{g}}{\text{L}} \right)}$$

152 *Analysis of combination index (CI)*

153 The combination index method was used to determine the interaction of the components  
154 in the mixture. Chou tested this using inhibitory concentration (IC<sub>50</sub>) causing 50% inhibitory  
155 activity and CI approach.<sup>15</sup> Synergism, additive, and antagonism were indicated by a CI <1, 1,  
156 and I>1. The following formula was used to calculate the CI value:

$$157$$
$$158 \quad \text{CI} = \frac{\text{IC}_{50} \text{ of } A}{\text{ratio of } A \text{ in combination} \times \text{IC}_{50} \text{ of combination}}$$
$$159 \quad + \frac{\text{IC}_{50} \text{ of } B}{\text{ratio of } B \text{ in combination} \times \text{IC}_{50} \text{ of combination}}$$

## 160 **RESULTS AND DISCUSSION**

161 *Phytochemicals screening analysis*

162 Phytochemical screening analysis was performed using the thin-layer chromatography  
163 (TLC) method. It aimed to identify the phytochemical compounds in each sample and obtain  
164 their spot profiles. This analysis evaluated the CEE and EAF of GM, as well as the CEE, EAF,  
165 and EF of CA. The results of the analysis are shown in Figure 1.

166 As shown in Figure 1, The CEE and its fraction of CA displayed a spot of a different color.  
167 Further analysis was conducted using specific spray reagents as preliminary identification of  
168 the chemical compound groups, such as flavonoids, terpenoids, and phenolic compounds. The  
169 preliminary analysis determined that the extract and fractions of this natural substance contain  
170 flavonoids, phenolic compounds, and terpenoids. According to the results, the spot pattern and  
171 secondary metabolites of the CEE and EAF of CA are similar. This is due to ethanol being used  
172 as a general solvent in CEE, allowing for the extraction of substances with varying polarities.  
173 As a result, the molecules in EAF are also present in CEE. However, the spot and secondary  
174 metabolites in EF are different from both CEE and EAF. This is because only polar molecules  
175 are extracted to the EF, leading to limited spots, as many compounds have already been  
176 extracted in the EAF phase. This result aligns with TLC research conducted by Daniel, which  
177 stated that the Ethyl acetate phase of CA contains flavonoids and terpenoids.<sup>8</sup> CEE and EAF  
178 of GM show that they contain similar secondary metabolites, such as phenolic compounds and  
179 flavonoids, except for terpenoids. The results also showed that alkaloids are not contained in  
180 both GM and CA, and this is similar to the research performed by Djoko and Vinolina.<sup>16,17</sup>

#### 181 *LC/MSMS analysis*

182 The second analysis of chemical components, which aims to obtain the identity of  
183 compounds, was conducted using LC-MS/MS. The results for GM and CA are shown in Tables  
184 1 and 2, respectively.

185 As shown in table 1, GM contains 5 major compounds and 3 that are still unidentified. Those  
186 in CEE and EAF of GM are similar since ethanol is used as a general solvent. However, based  
187 on the detector counts, the quantity of the compounds in EAF is higher than in CEE. This is  
188 because they are more likely to dissolve in the moderate polarity of solvents such as ethyl  
189 acetate.  $\alpha$ -mangosteen, a xanthone, is the main compound of GM and is present largely in EAF.  
190 This aligns with research conducted by Andri, which stated that xanthones are well extracted  
191 in solvents of moderate polarity.<sup>18</sup> In EF, the compounds are not similar to CEE and EAF.



192 However,  $\alpha$ -mangosteen is still present in EF but at a low level. This is correlated with the  
193 result of TLC, that EF of GM has a limited spot.

194 The components of CEE and EAF in CA are also similar, with only two different  
195 substances. However, the quantity is different based on detector counts. The 5-Hydroxy-6,4'-  
196 dimethoxy-flavone-7-O- $\beta$ -D-glucopyranoside, Kaempferol-3,7-diglucoside, Kaempferol-3,7-  
197 diglucoside, and Kaempferol-3-O-rutinoside are only discovered in the polar phase (EF). This  
198 is because their chemical structure contains sugar moiety. This follows the research conducted  
199 by Daniel, which reported that kaempferol is only identified in the polar phase. Asiaticoside  
200 and madecassoside are the main compounds of CA, and they are discovered in all phases.  
201 However, the quantity in EF is the largest because of the sugar moiety content that makes the  
202 compounds more polar. This is consistent with the research performed by Nur, who discovered  
203 that CA contains asiaticoside and madecassoside.<sup>19</sup>

#### 204 *Antioxidant activities of GM and CA extracts and fractions*

205 This research evaluated the antioxidant activities from the extract and fractions from both  
206 GM and CA using the DPPH method. The result of antioxidant activities is shown in Table 3.

207 As shown in Table 3, GM shows strong antioxidant activities, with a strength order of EAF  
208 > EF > CEE. The chemical compounds responsible for its activities are the phenolic  
209 compounds, consisting of many hydroxyl groups that could be proton donors to stabilize the  
210 DPPH. EAF has very strong antioxidant activities because it contains many phenolic  
211 compounds. The primary component in GM is  $\alpha$ -mangosteen, which is reported to have  
212 antioxidant activities.<sup>20</sup> It is discovered to be higher and lower in EAF and EF, respectively.

213 In CA, EAF shows moderate antioxidant activities, while in CEE and EF, it is known to be  
214 weak. Based on the LC-MS/MS analysis, the highest activities are identified in EAF, as it  
215 contains flavon 5,7,2',5'-Tetrahydroxy-flavone, a free aglycon flavonoid that probably  
216 possesses antioxidant activities. However, The compound is not contained in EF of CA, as it  
217 cannot dissolve in polar solvent due to its chemical structure. Asiaticoside and madecassoside

218 are the main components identified in CA and are present in extract and fractions. However,  
219 the amount in EF is larger than in EAF and CEE since their chemical structures contain the  
220 polar sugar moiety. The EF of this natural substance contains flavonoids, such as Kaempferol-  
221 3-O-rutinoside, Kaempferol-3,7-diglucoside, Asiaticoside, and madecassoside. However, it  
222 shows weak antioxidant activities. Meanwhile, in EAF, there is a low amount of asiaticoside  
223 and madecassoside, but it still has strong antioxidant activities, which may be due to  
224 compounds such as 5,7,2',5'-Tetrahydroxy-flavone.

225 It was discovered that EAF exhibit the strongest antioxidant activities in both GM and CA.  
226 Furthermore, the antioxidant activities of the combination of EAF from GM and CA were  
227 evaluated.

#### 228 *Antioxidant activities and TPC of the combination of GM-CA active fractions*

229 The EAF shows the strongest antioxidant activities in both GM and CA. Furthermore, the  
230 active fractions are combined and made in several ratios, with the antioxidant activities being  
231 evaluated, which is expected to increase due to the synergism of secondary metabolites. The  
232 results of antioxidant activities and TPC from the GM-CA combination are shown in Tables 4  
233 and 5, respectively.

234 Table 4 shows that the combinations have strong antioxidant activities, with the strength  
235 order being 1:3 > 3:1 > 1:1. The antioxidant activities correlate with the TPC because it is  
236 attributed to phenolic compounds. The ratio combination of 1:3 shows the strongest antioxidant  
237 activities because the TPC is also high. However, when GM is combined with CA, the TPC of  
238 the combination changes. The ratio of 3:1 should be the strongest antioxidant activities and the  
239 highest content of phenolic compounds because GM is larger. However, the results show that  
240 1:3 possess the highest TPC. This may be due to the interaction of components in combination.

241 *Analysis of combination index (CI)*

242 The CI analysis showed the interaction between the components in the mixture. The  
243 interaction between GM and CA can be positive or negative. It can be influenced by several  
244 factors, such as the composition of the reaction mixture, the structure of the antioxidant, the  
245 neutralization of radical mechanics, and the concentration of the molar ratio. Table 6 shows the  
246 result of the CI analysis.

247 Table 6 shows that each combination has a different value of CI. The combination of GM-  
248 CA at 3:1 exhibit antagonism interaction (index 1.22), implying an adverse interaction between  
249 the components.

250 The combination GM-CA 1:1 also shows antagonism interaction (index 1.13). It has an  
251 adverse interaction between the components, which is not strong as the 3:1 combination.

252 The GM-CA 1:3 combination shows synergism interaction (index 0.79), meaning that GM  
253 and CA at the ratio of 1:3 exhibit greater antioxidant activities than the ratio of 1:1 and 3:1.

254 The TPC influences antioxidant activities, and the formation of dimers or new molecules  
255 with increased activities can explain synergistic interactions of phenolic compounds.  
256 Antagonistic interactions may be caused by polymerization, resulting in decreased activities.<sup>21</sup>

257 **CONCLUSION**

258 The different polarity of the solvent influences the antioxidant activities. Ethyl acetate fractions  
259 from both GM and CA show the strongest activities. The combination of EAF at 1:3 shows  
260 synergistic interaction, strong antioxidant activities  $IC_{50} = 62.00$  ppm, and 132.38 mgGAE/g

261 of TPC. This research supported the hypothesis that CEE, EAF, EF, and GM-CA have different  
262 activities and correlate with chemical components' composition.

### 263 **CONFLICT OF INTEREST**

264 The authors declared that there is no conflict of interest.

### 265 **AUTHOR'S DECLARATION**

266 The authors hereby declare that the work presented in this article are original and that any  
267 liability for claims relating to the content of this article will be borne by them.

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- 337

339 **TABLES AND FIGURES**

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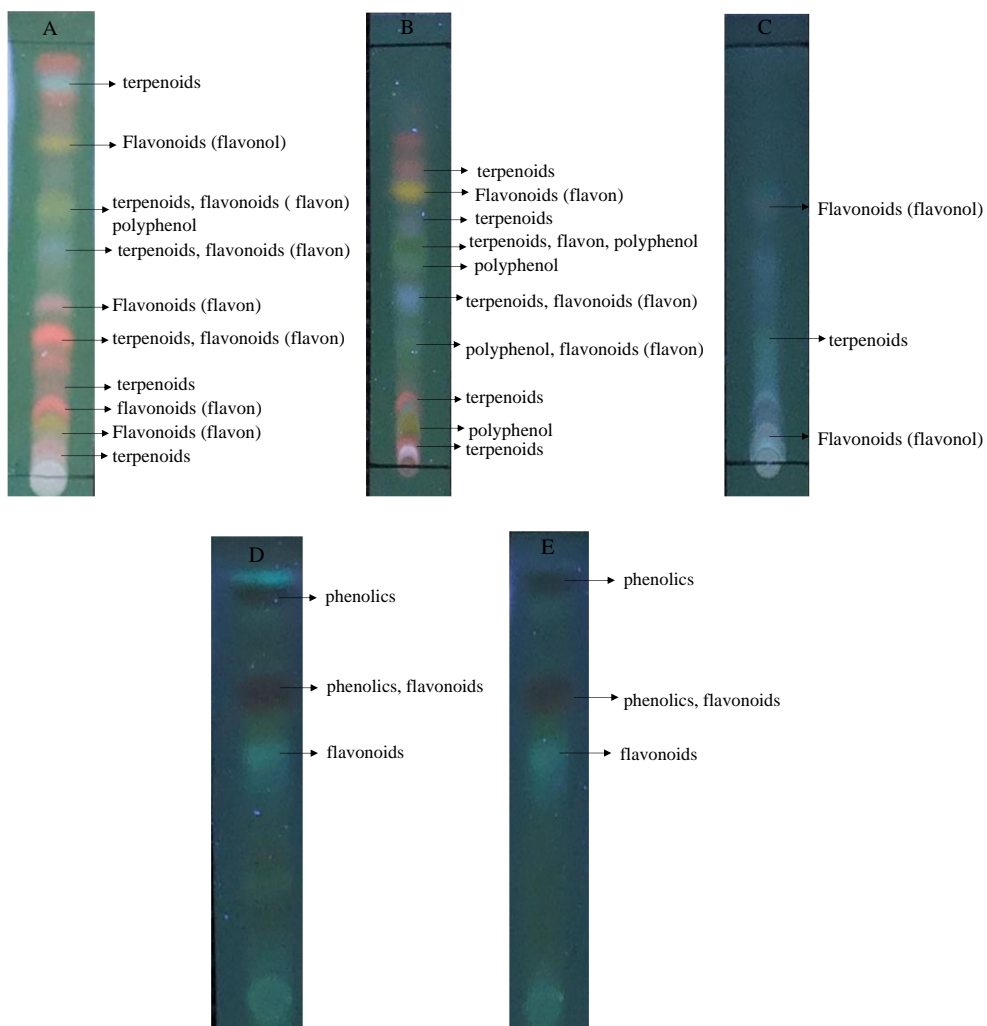
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355 **Figure**



360 Table 1. The LC/MSMS analysis of chemical compounds of GM extract and fractions.

Compounds	Rt (min)	Chemical formula	m/z	Detector counts		
				CEE	EAF	EF
+/-) Gomisin M2	9.77	C <sub>22</sub> H <sub>26</sub> O <sub>6</sub> C <sub>24</sub> H <sub>26</sub> O <sub>6</sub>	409,16	1269629	1420558	-
Archangelicin	5.11	C <sub>24</sub> H <sub>26</sub> O <sub>7</sub>	427,17	434892	1908329	-
Biondinin A	8.35	C <sub>21</sub> H <sub>26</sub> O <sub>6</sub>	397.16	1420338	1810666	-
$\alpha$ - Mangosteen	8.98	C <sub>24</sub> H <sub>26</sub> O <sub>6</sub>	411.17	5457467	6071885	45124
Sarcandin acetate	9.21	C <sub>26</sub> H <sub>34</sub> O <sub>6</sub>	465.22	995318	1343097	-
Achilin	3.51	C <sub>15</sub> H <sub>18</sub> O <sub>3</sub>	247.13	-	-	72144
Candidate mass	5.97	C <sub>18</sub> H <sub>39</sub> NO <sub>3</sub>	318.30	-	-	59745
Candidate mass	5.91	C <sub>16</sub> H <sub>35</sub> NO <sub>2</sub>	274.27	-	-	54249
Candidate mass	4.41	C <sub>17</sub> H <sub>33</sub> NO <sub>3</sub>	300.25	-	-	52493

361 Note : Rt = retention time, m/z= mass/charge number of ion

362 CEE: crude ethanolic extract; EAF: ethyl acetate fraction; EF: ethanolic fraction.

363 Table 2. The LC/MSMS analysis of chemical compounds of CA extract and fractions.

Compounds	Rt (min)	Chemical formula	m/z	Detector counts		
				CEE	EAF	EF
5,7,2',5'- Tetrahydroxy- flavone	4.68	C <sub>15</sub> H <sub>10</sub> O <sub>6</sub>	287.05	108001	266446	-
5-Hydroxy-6,4'- dimethoxy- flavone-7-O-β- D- glucopyranoside	3.50	C <sub>23</sub> H <sub>24</sub> O <sub>11</sub>	299.12	126278	-	-
Asiaticoside	4.23	C <sub>48</sub> H <sub>78</sub> O <sub>19</sub>	981.50	117774	87716	200871
Kaempferol- 3,7-diglucoside	3.18	C <sub>27</sub> H <sub>30</sub> O <sub>16</sub>	611.16	145253	-	161998
Madecassoside	3.97	C <sub>48</sub> H <sub>78</sub> O <sub>20</sub>	997.49	85570	36480	205343
3β,6β,23- Trihydroxy-urs- 12-en-28-oic acid	6.17	C <sub>30</sub> H <sub>48</sub> O <sub>5</sub>	511.33	-	52038	-
Candidate Mass	10.19	C <sub>35</sub> H <sub>36</sub> N <sub>4</sub> O <sub>5</sub>	593.27	-	407065	-
Kaempferol-3- O-rutinoside	2.77	C <sub>27</sub> H <sub>30</sub> O <sub>15</sub>	595.16	-	-	238532
Mahuannin F	0.49	C <sub>30</sub> H <sub>22</sub> O <sub>10</sub>	543.13	-	-	492063

364 Note: Rt = retention time, m/z= mass/charge number of ion

365 CEE: crude ethanolic extract; EAF: ethyl acetate fraction; EF: ethanolic fraction.

366 Table 3. The Antioxidant activities of GM and CA extract and fractions



Samples	Antioxidant activities (IC <sub>50</sub> ) ppm			
	Positive control	CEE	EAF	EF
Quersetin	4.63			
GM		83.98±2.50 <sup>b</sup>	45.78±1.20 <sup>a</sup>	72.96±14.30 <sup>b</sup>
CA		300.88±43.78 <sup>d</sup>	102.57±3.40 <sup>c</sup>	494.96±25.10 <sup>d</sup>

Note: a = very strong; b= strong; c= moderate; d= weak 22,23

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368 Table 4. The Antioxidant activities of GM-CA ethyl acetate fraction combination

Antioxidant activities (IC <sub>50</sub> ) ppm	The ratios of GM-CA ethyl acetate fraction combination				
	1:0	3:1	1:1	1:3	0:1
Value	45.78±1.20 <sup>a</sup>	64.62±2.22 <sup>b</sup>	71.73±2.63 <sup>b</sup>	62.00±1.67 <sup>b</sup>	102.57±3.40 <sup>c</sup>

Note: a = very strong; b= strong; c= moderate; d= weak 22,23

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370 Table 5. Total phenolics content GM-CA ethyl acetate fraction combination

TPC (mgGAE/g)	The ratios of GM-CA ethyl acetate fraction combination				
	1:0	3:1	1:1	1:3	0:1
Value	175.33±4.45	116.07±3.24	109.75±2.47	132.38±21.06	102.78±14,16

371

372 Table 6. The combination index analysis of combinations

Combination Index			
GM-CA Combination ratio	Value	Description	
1:3	0.79	Synergism	
1:1	1.13	Antagonism	
3:1	1.22	Antagonism	

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No	observation	Correction	Description
1.	Chemical Composition, Antioxidant Activities, and Total Phenolic Content of Combination of Mangosteen ( <i>Garcinia mangostana</i> L.) Peel-Kodavan ( <i>Centella asiatica</i> L. Urban) Fractions	Chemical Composition, Antioxidant Activities, and Total Phenolic Content of Combination of Mangosteen ( <i>Garcinia mangostana</i> L.) Peel-Kodavan ( <i>Centella asiatica</i> L. Urban) Fractions	See the title section
2.	AlCl <sub>3</sub> 1%	AlCl <sub>3</sub> 3%	See line 108
3.	FeCl <sub>3</sub> 5%	FeCl <sub>3</sub> 1%	See line 110
4.	LC/MSMS	LC-MS/MS	See Tables 1 and 2
5.	gm, ca, eaf, ic <sub>50</sub> , mggae/g of tpc, cee, eaf, ef, gm-ca	GM, CA, EAF, IC <sub>50</sub> , mgGAE/g of TPC, CEE, EAF, EF, GM-CA	See the conclusion section
6.	H <sub>2</sub> O	H <sub>2</sub> O	See line 121
7.	IC <sub>50</sub>	IC <sub>50</sub>	See Table 4
8.	IC <sub>50</sub>	IC <sub>50</sub>	See line 162



## Chemical Composition, Antioxidant Activities, and Total Phenolic Content of Combination of Mangosteen (*Garcinia mangostana* L.) Peel-Kodavan (*Centella asiatica* L. Urban) Fractions

Khairul Anam<sup>1,2</sup>, Ryan Munandar<sup>1,\*</sup>, Octavia N. Wulandari<sup>1</sup>, Aninda B. Lestari<sup>1</sup>, Rivany E. Farada<sup>1</sup>, Dwi Hudyanti<sup>1</sup>, Agustina L.N. Aminin<sup>1</sup>.

<sup>1</sup>Department of Chemistry, Faculty of Science and Mathematics, Universitas Diponegoro. Jl. Prof. Jacob Rais, Tembalang Semarang -50275, Central Java, Indonesia.

<sup>2</sup>Department of Pharmacy, Faculty of Medicine, Universitas Diponegoro, Jl. Prof Soedharto, SH. Tembalang Semarang, Central Java, Indonesia

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### ABSTRACT

Herbs, known for their naturally active chemicals, are currently being reconsidered as a safer alternative medication. The natural substances that are known to have lots of bioactivities, such as antioxidant properties, are mangosteen (*Garcinia mangostana* L.) (GM) and kodavan (*Centella asiatica* L. Urban) (CA). However, the antioxidant activities of the GM-CA combination have not been previously reported. It is essential to investigate the properties of the active fractions to determine which fraction possesses the best antioxidant activities. Therefore, this research aimed to determine the chemical composition and evaluate the antioxidant activities (IC<sub>50</sub>) and total phenolic content (TPC) of GM, CA, and their combination. The combination is expected to exhibit a synergistic effect and an increase in antioxidant activities. GM and CA were percolated using ethanol and successively partitioned by n-hexane and ethyl acetate. Antioxidant activities and TPC were evaluated using DPPH and Folin Ciocalteu methods, respectively. Chemical components were determined through LC-MS/MS analysis and phytochemical screening. The combination of ethyl acetate fractions (EAF) of GM-CA in a 1:3 ratio indicates synergistic interaction, strong antioxidant activities IC<sub>50</sub> = 62.00 ppm, and 132.38 mgGAE/g of TPC. Phytochemical screening showed the presence of flavonoids, terpenoids, and polyphenols. LC-MS/MS identified several compounds in GM, such as (+/-) gomisin M2, archangelicin, biodinin A,  $\alpha$ -mangosteen, samarandin acetate, and achilin. In CA, 5,7,2',5'-tetrahydroxy-flavon-5-hydroxy-6,4'-dimethoxy-flavone-7-O- $\beta$ -D-glucopyranose, asiaticoside, kaempferol-3,7-diglucoside, madecassoside, 3 $\beta$ ,6 $\beta$ ,23-trihydroxy-urs-12-en-28-oic acid, kaempferol-3-O-rutinoside, and mahuannin F, were determined

**Keywords:** Antioxidant, *Centella asiatica* L. Urban, *Garcinia mangostana* L., Phytochemistry, Total phenolic content.

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### 1 Introduction

The use of herbs as traditional medicine is increasing as they are being reconsidered by people worldwide. Their advantages include easy accessibility, inexpensiveness, and safer due to the natural source. Herbs are composed of various mixtures of ingredients that possess different chemical compositions. Combining two natural ingredients with different chemical content is expected to produce a better therapeutic effect through the synergistic interaction of the various active components.

Mangosteen (*Garcinia mangostana* L.) (GM) and Kodavan (*Centella asiatica* L. Urban) (CA) are widely spread in Indonesia, India, and many other Southeast Asia countries. These herbs have been a natural medicine due to their bioactivities. GM and CA have been reported to possess several bioactive properties, such as anti-cancer,<sup>1</sup> anti-proliferation,<sup>2</sup> and antioxidant.<sup>3,4</sup>

\*Corresponding author. E mail: [ryanmunandar0496@students.undip.ac.id](mailto:ryanmunandar0496@students.undip.ac.id)  
Tel: +62895365074432

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Antioxidants protect the body from free radicals caused by unhealthy lifestyles and air pollution. An excess of free radicals in the body can lead to damage to the cells and tissues. Andri reported that GM's acetone and ethyl acetate extracts have strong and moderate antioxidant activities, respectively.<sup>5</sup> Meanwhile, for CA, it is reported as weak to moderate.<sup>6</sup> However, the antioxidant activities from the combination of GM and CA (GM-CA) active fractions have not been evaluated previously. Evaluating the antioxidant activities and total phenolic content (TPC) from the combination of GM-CA active fraction is of great interest. The combination could enhance its antioxidant activities due to the synergism of its secondary metabolites. Furthermore, it is also expected to have anti-bacterial and immunomodulatory activities. GM is reported to contain secondary metabolites, such as phenolic compounds,<sup>7</sup> while CA consists of phenolic and terpenoid compounds.<sup>8</sup> The main active component in GM is  $\alpha$ -mangosteen, which belongs to the xanthone group, a class of polyphenols, with a chemical structure consisting of a C6-C1-C6 backbone. It is discovered in large amounts in GM's pericarp and possesses many biological activities.<sup>9</sup> Asiaticoside, a pentacyclic triterpene of the ursane class, is one of the main components identified in CA. It is a triterpene glycoside with glucose attached to its C-28 (ring E) and possesses many biological activities.<sup>10</sup> These secondary metabolites are expected to exhibit synergistic effects and the strongest antioxidant activities. Previous studies have investigated the antioxidant activities of ethanolic extracts. In this research, GM and CA were examined from the different polarity of the solvents, such as crude ethanolic extract (CEE), ethyl acetate fraction (EAF), and an ethanolic fraction (EF), to determine which fraction has the highest level. GM-CA combination will be made

59 in several compositions of ratios to obtain the best ratio with the  
60 strongest antioxidant activities as a result of synergism interaction.

61 The primary aim of this research was to investigate the hypothesis that  
62 the CEE, EAF, EF, and the combination of GM-CA active fractions  
63 exhibit different antioxidant activities, which were positively correlated  
64 with the ratio of their chemical compositions.

## 66 Materials and Methods

### 67 Materials and chemicals

68 The materials used in this research include GM peel powder (voucher  
69 number 8/3/2020), CA powder (voucher number 7/3/2020). The plant  
70 materials were collected from Java plant, Karanganyar, Central Java,  
71 Indonesia, in March 2020. Ethanol 96% (Happy Lab, Indonesia), ethyl  
72 acetate (Bratachem, Indonesia), n-hexane (Bratachem, Indonesia),  
73 phytochemistry and TLC dyeing reagents (Merck, Germany), ethanol  
74 (Merck, Germany), Na<sub>2</sub>SO<sub>4</sub>, benzene (Merck, Germany), ethyl acetate  
75 (Merck, Germany), chloroform (Merck, Germany), butanol (Merck,  
76 Germany), dichloromethane (Merck, Germany), sulfuric acid (Merck,  
77 Germany), gallic acid (Sigma Aldrich, USA), Quercetin (Sigma  
78 Aldrich, Japan), DPPH (Sigma Aldrich, USA) Folin-Ciocalteu (Merck,  
79 Germany), Sodium Carbonate (Merck, Germany), Distilled water, TLC  
80 Silica gel G<sub>60</sub> F<sub>254</sub> (Merck, Germany), UV-Vis Spectrophotometer  
81 (Genesys 10S), Analytical Balance (Ohaus, model PA323), UV Quartz  
82 cuvette, and rotary evaporator (Scilogex RE-100 pro).

### 84 Extraction and fractionation methods

85 The GM and CA dried powdered plant material (5kg) were each  
86 percolated with Ethanol 96% at a ratio of 1:4 (w/v) continuously for 7  
87 days at room temperature. Following this, filtration was performed, and  
88 the solvent was removed using a rotary evaporator to obtain the crude  
89 ethanolic extract (CEE)

90 The GM and CA crude ethanolic extract (CEE) were each dissolved in  
91 ethanol 1:4 (w/v), partitioned by n-hexane 1:1 (v/v), and separated.  
92 Distilled water was then added to the ethanolic phase 1:1 (v/v) and  
93 successively partitioned by ethyl acetate 1:1 (v/v) to obtain ethyl acetate  
94 fraction (EAF). The residue of the last partition is known as the  
95 ethanolic fraction (EF). Finally, all the fractions are filtered, and their  
96 solvent is removed using a rotary evaporator.

### 98 Phytochemicals screening analysis method

99 Qualitative phytochemical screening analyses were performed using  
100 standard methods.<sup>11</sup> Silica gel G<sub>60</sub> F<sub>254</sub> was used as a stationary phase  
101 and activated by heating at 100 °C for 10 minutes. Furthermore, 10 and  
102 20 mg of GM and CA extracts and their respective fractions are  
103 dissolved in 10 mL ethanol. The solution was spotted in a TLC plate  
104 and eluted using a mixture of ethyl acetate and chloroform at a 3:7 ratio  
105 for GM, as well as benzene and ethyl acetate at 6:4 for CA. The obtained  
106 spot was identified using specific spray dyeing reagents and examined  
107 under a UV lamp at 254 nm and 365 nm. Flavanoids were identified  
108 using AlCl<sub>3</sub> 3%, and their presence is denoted by the change of color to  
109 blue, yellow, green, and orange. Phenolic compounds were detected  
110 using FeCl<sub>3</sub> 1%, and their presence is denoted by the change of color to  
111 black. Additionally, terpenoids were identified by the lieberman-  
112 burchad reagent, and their presence is denoted by the change of color to  
113 pink.<sup>12,13</sup>

### 115 LC-MS/MS analysis method

116 Liquid Chromatography-mass spectrometry analysis was performed  
117 on water acuity UPLC I-Class and XEVO G2-XS QToF. The  
118 instrument was operated in full scan ESI mode. The liquid  
119 chromatography conditions were a C18 column with a particle size of  
120 1.7 µm and a 2.1 x 50 mm length. Furthermore, the eluents employed  
121 were a mixture of H<sub>2</sub>O and 0.1% formic acid (Solvent A), as well as  
122 ACN and 0.1% formic acid (Solvent B). The injection volume was 1  
123 µL, and the ionization type was set at ESI positive. Finally, the mass  
124 ranges from 100-1200 m/z.

### 126 The antioxidant activities assay method

127 The scavenging of DPPH free radical was used for measuring the  
128 antioxidant activities of extracts, fractions, and combinations, with the  
129 ratios of GM-CA being 3:1, 1:1, and 1:3 %. About 25 mg of each sample  
130 was dissolved in 25 mL methanol to obtain the stock solution at the

131 concentration of 1000 ppm. Subsequently, the stock solution was  
132 diluted with methanol to obtain a series of concentrations of each  
133 sample. About 3.5 mL of each sample solution was thoroughly mixed  
134 with freshly prepared 1.5 mL of DPPH 0.4 mM and kept for 30 minutes  
135 in the dark at room temperature, respectively. The amount of reaction  
136 mixture was determined using a UV-Vis spectrophotometer at 517 nm.  
137 The antioxidant activities expressed as IC<sub>50</sub> and Quercetin serve as  
138 standard antioxidants. Furthermore, the experiments were repeated 3  
139 times and reported as Mean ± SD.<sup>14</sup> The percentage of inhibition was  
140 calculated by the following formula:

$$141 \text{ Inhibition (\%)} = \frac{\text{Abs of Control} - \text{Abs sample}}{\text{Abs of control}} \times 100 \%$$

### 144 Total phenolic content (TPC) assay method

145 About 25 mg of gallic acid and each sample were dissolved in 25 mL  
146 methanol, respectively, to obtain 1000 ppm of stock solutions.  
147 Approximately 0.5 mL of 500 ppm solution was placed in a vial,  
148 followed by adding 2.5 mL distilled water and 2.5 mL of Folin-  
149 Ciocalteu reagent, respectively. The mixture was thoroughly mixed and  
150 allowed to incubate for 15 minutes. Furthermore, about 2.5 mL of 7.5%  
151 sodium carbonate solution was added, mixed, and incubated for 30  
152 minutes in the dark. The absorbance of the mixture was measured at  
153 756 nm. The gallic acid curve was used as a calibration curve, while the  
154 TPC is represented as gallic acid equivalents (GAE). The experiments  
155 were repeated 3 times, and the results were expressed as Mean ± SD.<sup>14</sup>  
156 The following formula was used to calculate TPC:

$$157 \text{ TPC } \left( \frac{\text{mgGAE}}{\text{g of dried extract}} \right) = \frac{\text{concentration of total phenols } \left( \frac{\text{mg}}{\text{L}} \right)}{\text{concentration of extract } \left( \frac{\text{g}}{\text{L}} \right)}$$

### 159 Analysis of combination index (CI)

160 The combination index method was used to determine the interaction of  
161 the components in the mixture. Chou tested this using inhibitory  
162 concentration (IC<sub>50</sub>) causing 50% inhibitory activity and CI approach.<sup>15</sup>  
163 Synergism, additive, and antagonism were indicated by a CI <1, 1, and  
164 >1. The following formula was used to calculate the CI value:

$$165 \text{ CI} = \frac{\text{IC}_{50} \text{ of A}}{\text{ratio of A in combination} \times \text{IC}_{50} \text{ of combination}} + \frac{\text{IC}_{50} \text{ of B}}{\text{ratio of B in combination} \times \text{IC}_{50} \text{ of combination}}$$

### 170 Statistical analysis

171 All experiments were performed in triplicates and results were  
172 presented as mean ± SD.

## 173 Results and Discussion

174 Phytochemical screening analysis was performed using the thin-layer  
175 chromatography (TLC) method to identify the phytochemical  
176 compounds in each sample and obtain their spot profiles. This analysis  
177 evaluated the CEE and EAF of GM, as well as the CEE, EAF, and EF  
178 of CA. The results of the analysis are shown in Figure 1.

179 As shown in Figure 1, The CEE and its fraction of CA displayed a spot  
180 of a different color. Further analysis was conducted using specific spray  
181 reagents as preliminary identification of the chemical compound  
182 groups, such as flavonoids, terpenoids, and phenolic compounds. The  
183 preliminary analysis determined that the extract and fractions of this  
184 natural substance contain flavonoids, phenolic compounds, and  
185 terpenoids. According to the results, the spot pattern and secondary  
186 metabolites of the CEE and EAF of CA are similar. This is due to  
187 ethanol being used as a general solvent in CEE, allowing for the  
188 extraction of substances with varying polarities. As a result, the  
189 molecules in EAF are also present in CEE. However, the spot and  
190 secondary metabolites in EF are different from both CEE and EAF. This  
191 is because only polar molecules are extracted to the EF, leading to  
192 limited spots, as many compounds have already been extracted in the  
193 EAF phase. This result aligns with TLC research conducted by Daniel,  
194 which stated that the Ethyl acetate phase of CA contains flavonoids and  
195 terpenoids.<sup>8</sup> CEE and EAF of GM show that they contain similar  
196 secondary metabolites, such as phenolic compounds and flavonoids,  
197 except for terpenoids. The results also showed that alkaloids are not

198 contained in both GM and CA, and this is similar to the research  
 199 performed by Djoko and Vinolina.<sup>16,17</sup>  
 200 The second analysis of chemical components, which aims to obtain the  
 201 identity of compounds, was conducted using LC-MS/MS. The results  
 202 for GM and CA are shown in Tables 1 and 2, respectively. The  
 203 chromatogram for GM and CA are shown in Figures 2 and 3,  
 204 respectively.

205 As shown in Table 1, GM contains 5 major compounds and 3 that are  
 206 still unidentified. Those in CEE and EAF of GM are similar since  
 207 ethanol is used as a general solvent. However, based on the detector  
 208 counts, the quantity of the compounds in EAF is higher than in CEE.  
 209 This is because they are more likely to dissolve in the moderate polarity  
 210 of solvents such as ethyl acetate.  $\alpha$ -mangosteen, a xanthone, is the main  
 211 compound of GM and is present largely in EAF. This aligns with  
 212 research conducted by Andri, which stated that xanthones are well  
 213 extracted in solvents of moderate polarity.<sup>18</sup> In EF, the compounds are  
 214 not similar to CEE and EAF. However,  $\alpha$ -mangosteen is still present in  
 215 EF but at a low level. This is correlated with the result of TLC, that EF  
 216 of GM has a limited spot.

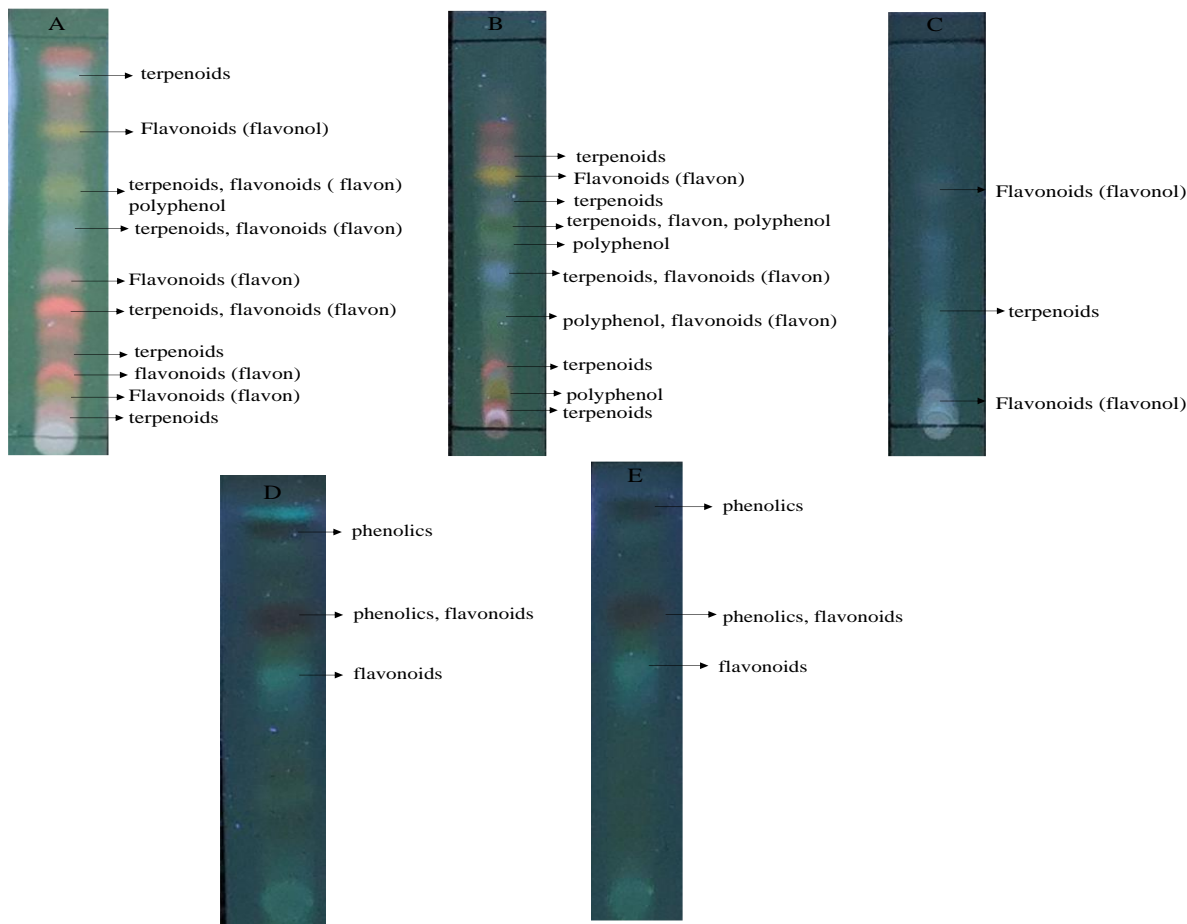
217 The components of CEE and EAF in CA are also similar, with only two  
 218 different substances. However, the quantity is different based on  
 219 detector counts. The 5-Hydroxy-6,4'-dimethoxy-flavone-7-O- $\beta$ -D-  
 220 glucopyranoside, Kaempferol-3,7-diglucoside, Kaempferol-3,7-  
 221 diglucoside, and Kaempferol-3-O-rutinoside are only discovered in the  
 222 polar phase (EF). This is because their chemical structure contains sugar  
 223 moiety. This follows the research conducted by Daniel, which reported

224 that kaempferol is only identified in the polar phase. Asiaticoside and  
 225 madecassoside are the main compounds of CA, and they are discovered  
 226 in all phases. However, the quantity in EF is the largest because of the  
 227 sugar moiety content that makes the compounds more polar. This is  
 228 consistent with the research performed by Nur, who discovered that CA  
 229 contains asiaticoside and madecassoside.<sup>19</sup>

230 This research evaluated the antioxidant activities from the extract and  
 231 fractions from both GM and CA using the DPPH method. The result of  
 232 antioxidant activities is shown in Table 3.

233 As shown in Table 3, GM shows strong antioxidant activities, with a  
 234 strength order of EAF > EF > CEE. The chemical compounds  
 235 responsible for its activities are the phenolic compounds, consisting of  
 236 many hydroxyl groups that could be proton donors to stabilize the  
 237 DPPH. EAF has very strong antioxidant activities because it contains  
 238 many phenolic compounds. The primary component in GM is  $\alpha$ -  
 239 mangosteen, which is reported to have antioxidant activities.<sup>20</sup> It is  
 240 discovered to be higher and lower in EAF and EF, respectively.

241 In CA, EAF shows moderate antioxidant activities, while in CEE and  
 242 EF, it is known to be weak. Based on the LC-MS/MS analysis, the  
 243 highest activities are identified in EAF, as it contains flavon 5,7,2',5'-  
 244 Tetrahydroxy-flavone, a free aglycon flavonoid that probably possesses  
 245 antioxidant activities. However, the compound is not contained in EF  
 246 of CA, as it cannot dissolve in polar solvent due to its chemical  
 247 structure. Asiaticoside and madecassoside are the main components  
 248 identified in CA and are present in extract and fractions.



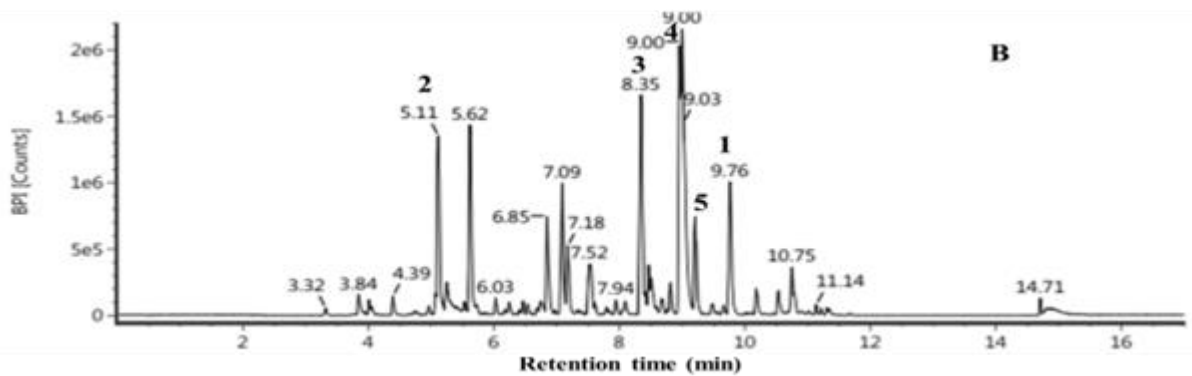
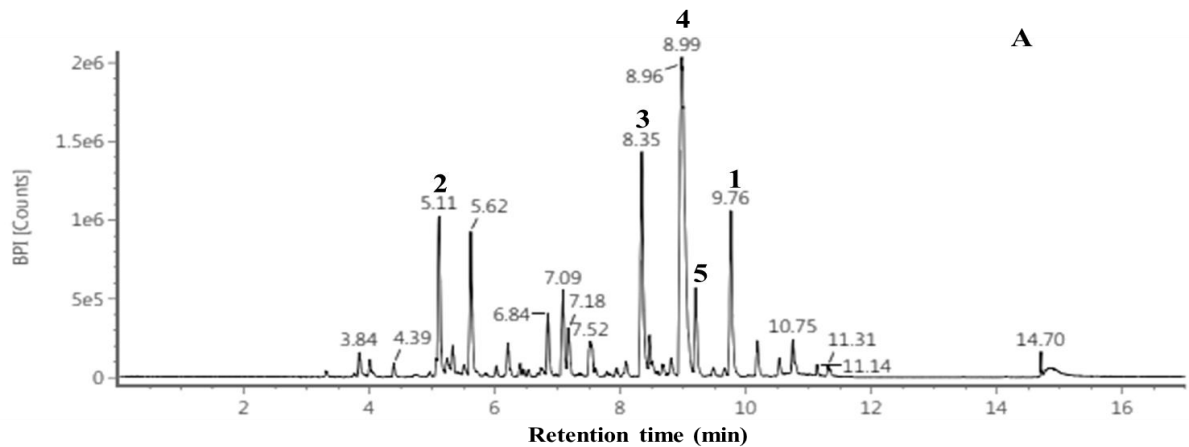
**Figure 1:** TLC analysis of GM and CA extracts and fractions. A) CEE of CA; B) EAF of CA; C) EF of CA; D) CEE of GM; E) EAF of GM. Identified under a UV lamp at 365 nm.

**Table 1:** The LC-MS/MS analysis of chemical compounds of GM extract and fractions.

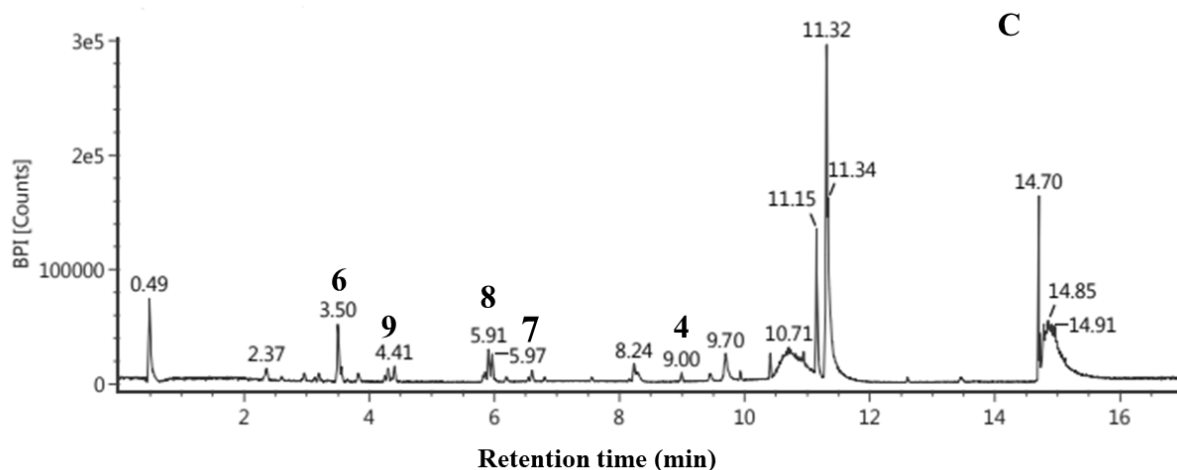
No	Compounds	Rt (min)	Chemical formula	m/z	Detector counts		
					CEE	EAF	EF

1.	+/-) Gomisin M2	9.77	$C_{22}H_{26}O_6$ $C_{24}H_{26}O_6$	409,16	1269629	1420558	-
2.	Archangelicin	5.11	$C_{24}H_{26}O_7$	427,17	434892	1908329	-
3.	Biondinin A	8.35	$C_{21}H_{26}O_6$	397.16	1420338	1810666	-
4.	$\alpha$ - Mangosteen	8.98	$C_{24}H_{26}O_6$	411.17	5457467	6071885	45124
5.	Samarcandin acetate	9.21	$C_{26}H_{34}O_6$	465.22	995318	1343097	-
6.	Achilin	3.51	$C_{15}H_{18}O_3$	247.13	-	-	72144
7.	Candidate mass	5.97	$C_{18}H_{39}NO_3$	318.30	-	-	59745
8.	Candidate mass	5.91	$C_{16}H_{35}NO_2$	274.27	-	-	54249
9.	Candidate mass	4.41	$C_{17}H_{33}NO_3$	300.25	-	-	52493

Note : Rt = retention time, m/z= mass/charge number of ion  
CEE: crude ethanolic extract; EAF: ethyl acetate fraction; EF: ethanolic fraction.







**Figure 2:** Chromatogram of GM extracts and fractions. A) CEE of GM; B) EAF of GM; C) EF of GM.

**Table 2:** The LC-MS/MS analysis of chemical compounds of CA extract and fractions.

No.	Compounds	Rt (min)	Chemical formula	m/z	Detector counts		
					CEE	EAF	EF
1.	5,7,2',5'-Tetrahydroxy-flavone	4.68	C <sub>15</sub> H <sub>10</sub> O <sub>6</sub>	287.05	108001	266446	-
2.	5-Hydroxy-6,4'-dimethoxy-flavone-7-O-β-D-glucopyranoside	3.50	C <sub>23</sub> H <sub>24</sub> O <sub>11</sub>	299.12	126278	-	-
3.	Asiaticoside	4.23	C <sub>48</sub> H <sub>78</sub> O <sub>19</sub>	981.50	117774	87716	200871
4.	Kaempferol-3,7-diglucoside	3.18	C <sub>27</sub> H <sub>30</sub> O <sub>16</sub>	611.16	145253	-	161998
5.	Madecassoside	3.97	C <sub>48</sub> H <sub>78</sub> O <sub>20</sub>	997.49	85570	36480	205343
6.	3β,6β,23-Trihydroxy-urs-12-en-28-oic acid	6.17	C <sub>30</sub> H <sub>48</sub> O <sub>5</sub>	511.33	-	52038	-
7.	Candidate Mass	10.19	C <sub>35</sub> H <sub>36</sub> N <sub>4</sub> O <sub>5</sub>	593.27	-	407065	-
8.	Kaempferol-3-O-rutinoside	2.77	C <sub>27</sub> H <sub>30</sub> O <sub>15</sub>	595.16	-	-	238532
9.	Mahuannin F	0.49	C <sub>30</sub> H <sub>22</sub> O <sub>10</sub>	543.13	-	-	492063

Note: Rt = retention time, m/z = mass/charge number of ion  
CEE: crude ethanolic extract; EAF: ethyl acetate fraction; EF: ethanolic fraction.

However, the amount in EF is larger than in EAF and CEE since their chemical structures contain the polar sugar moiety. The EF of this natural substance contains flavonoids, such as Kaempferol-3-O-rutinoside, Kaempferol-3,7-diglucoside, Asiaticoside, and madecassoside. However, it shows weak antioxidant activities. Meanwhile, in EAF, there is a low amount of asiaticoside and madecassoside, but it still has strong antioxidant activities, which may be due to compounds such as 5,7,2',5'-Tetrahydroxy-flavone.

It was discovered that EAF exhibit the strongest antioxidant activities in both GM and CA. Furthermore, the antioxidant activities of the combination of EAF from GM and CA were evaluated.

The EAF shows the strongest antioxidant activities in both GM and CA. Furthermore, the active fractions are combined and made in several ratios, with the antioxidant activities being evaluated, which is expected to increase due to the synergism of secondary metabolites. The results of antioxidant activities and TPC from the GM-CA combination are shown in Tables 4 and 5, respectively.

Table 4 shows that the combinations have strong antioxidant activities, with the strength order being 1:3 > 3:1 > 1:1. The antioxidant activities correlate with the TPC because it is attributed to phenolic compounds. The ratio combination of 1:3 shows the strongest antioxidant activities because the TPC is also high. However, when GM is combined with CA, the TPC of the combination changes. The ratio of 3:1 should be the strongest antioxidant activities and the highest content of phenolic

compounds because GM is larger. However, the results show that 1:3 possess the highest TPC. This may be due to the interaction of components in combination.

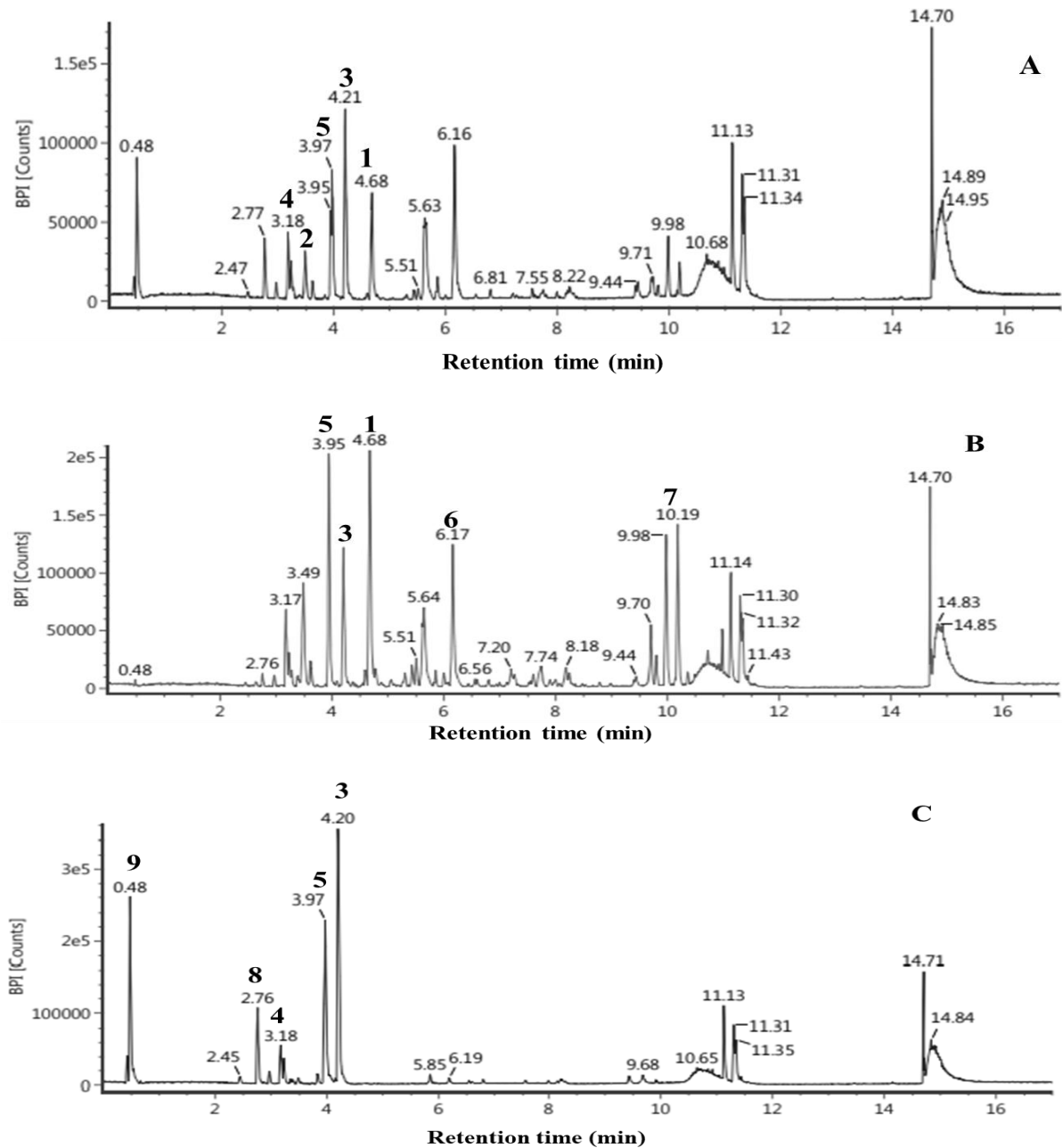
The CI analysis showed the interaction between the components in the mixture. The interaction between GM and CA can be positive or negative. It can be influenced by several factors, such as the composition of the reaction mixture, the structure of the antioxidant, the neutralization of radical mechanics, and the concentration of the molar ratio. Table 6 shows the result of the CI analysis.

Table 6 shows that each combination has a different value of CI. The combination of GM-CA at 3:1 exhibit antagonism interaction (index 1.22), implying an adverse interaction between the components.

The combination GM-CA 1:1 also shows antagonism interaction (index 1.13). It has an adverse interaction between the components, which is not strong as the 3:1 combination.

The GM-CA 1:3 combination shows synergism interaction (index 0.79), meaning that GM and CA at the ratio of 1:3 exhibit greater antioxidant activities than the ratio of 1:1 and 3:1.

The TPC influences antioxidant activities, and the formation of dimers or new molecules with increased activities can explain synergistic interactions of phenolic compounds. Antagonistic interactions may be caused by polymerization, resulting in decreased activities.<sup>21</sup>



**Figure 3:** Chromatogram of CA extracts and fractions. A) CEE of CA; B) EAF of CA; C) EF of CA

**Table 3:** The Antioxidant activities of GM and CA extract and fractions

Samples	Antioxidant activities (IC <sub>50</sub> ) ppm			
	Positive control	CEE	EAF	EF
Quercetin	4.63			
GM		83.98 ± 2.50 <sup>b</sup>	45.78 ± 1.20 <sup>a</sup>	72.96 ± 14.30 <sup>b</sup>
CA		300.88 ± 43.78 <sup>d</sup>	102.57 ± 3.40 <sup>c</sup>	494.96 ± 25.10 <sup>d</sup>

Note: a = very strong; b = strong; c = moderate; d = weak 22,23

**Table 4:** The Antioxidant activities of GM-CA ethyl acetate fraction combination

Antioxidant activities (IC <sub>50</sub> ) ppm	The ratios of GM-CA ethyl acetate fraction combination				
	1:0	3:1	1:1	1:3	0:1
Value	45.78 ± 1.20 <sup>a</sup>	64.62 ± 2.22 <sup>b</sup>	71.73 ± 2.63 <sup>b</sup>	62.00 ± 1.67 <sup>b</sup>	102.57 ± 3.40 <sup>c</sup>

Note: a = very strong; b = strong; c = moderate; d = weak 22,23

**Table 5:** Total phenolics content GM-CA ethyl acetate fraction combination

TPC (mgGAE/g)	The ratios of GM-CA ethyl acetate fraction combination				
	1:0	3:1	1:1	1:3	0:1
Value	175.33 ± 4.45	116.07 ± 3.24	109.75 ± 2.47	132.38 ± 21.06	102.78 ± 14,16

**Table 6:** The combination index analysis of combinations

Combination Index		
GM-CA Combination ratio	Value	Description
1:3	0.79	Synergism
1:1	1.13	Antagonism
3:1	1.22	Antagonism

## Conclusion

The different polarity of the solvent influences the antioxidant activities. Ethyl acetate fractions from both GM and CA show the strongest activities. The combination of EAF at 1:3 shows synergistic interaction, strong antioxidant activities  $IC_{50} = 62.00$  ppm, and 132.38 mgGAE/g of TPC. This research supported the hypothesis that CEE, EAF, EF, and GM-CA have different activities and correlate with chemical components' composition.

## Conflict of Interest

The authors declare no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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23. Sukandar D, Nurbayti S, Rudiana T, Husna TW. Isolation and Structure Determination of Antioxidants Active Compounds From Ethyl Acetate Extract of Heartwood

Namnam (*Cynometra cauliflora* L.). J. Kim. Terap. Indones. 2017; 19(1):11–17.

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