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I

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Fw: Fw: Manuscript submission to Tropical Journal of Natural Product Research

1 message

Ryan Munandar <ryanmunandar0496@alumni.undip.ac.id> Wed, Jun 12, 2024 at 8:58 AM
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Best regards

Abiodun

Professor Abiodun Falodun, PhD

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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.

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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)

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Date Issued

January 16, 2023

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No.	Reviewer's comments	Correction/responses	Description
1.	*Better condense it to be one sentences:* 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	Herbs, known for their naturally active chemicals, are currently being reconsidered as a safer alternative.	See line 20-21
2.	*What it is mean?:* Each crude ethanolic extract	The GM and CA crude ethanolic extract (CEE)	See line 102
3.	*Make this statement more simple:* 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.	10 and 20 mg of GM and CA extracts and their respective fractions are dissolved in 10 mL ethanol.	See line 110-111
4.	*Please authors explain how TLC could know exactly the groups of secondary metabolites? Authors used HPTLC??:* The CEE and its fraction of CA contain secondary metabolites, such as flavonoids, terpenoids, and phenolic compounds	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.	*Authors used the change of color under UV lamp to justified the group of secondary*	The obtained spot was identified using specific spray dyeing reagents and examined under a UV	See line 113-118

	metabolites? Authors should prove this approach : 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	lamp at 254 nm and 365 nm. Flavanoids were identified using $AlCl_3$ 1%, and their presence is denoted by the change of color to blue, yellow, green, and orange. Phenolic compounds were detected using $FeCl_3$ 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	
6.	*Please check some reference that have red mark:* Nur Khusnawati N, Pramono S, Sasmito E. 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 PENGARUH EKSTRAK ETANOLIK 50% HERBA PEGAGAN (Centella asiatica	Nur Khusnawati N, Pramono S, Sasmito E. 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. Trad Med J. 2015; 20(3):164 169.	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.	*Plase make clear result of GM extract and GM Fractions, separately*	CEE: crude ethanolic extract; EAF: ethyl acetate fraction; EF: ethanolic fraction.	See line 361
8.	*Please authors put IC50 of positive control also:*	Positive control : Quersetin IC50: 4.63	See Table 3
9.	*Which active fraction that authors mean?* The Antioxidant activities of GM-CA active fraction combinations	The Antioxidant activities of GM-CA ethyl acetate fraction combination	See Table 4

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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Abstract

Herbs, known for their naturally active chemicals, are currently being reconsidered as a safer alternative. The natural substances that are known to have lots of bioactivities, such as antioxidant properties, are mangosteen *(Garcinia mangosatana* 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_{50}) 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_{50} = 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, α-mangosteen, sarcandin acetate, and achilin. In CA, 5,7,2',5'-tetrahydroxy-flavon,5-hydroxy-6,4'-dimethoxy-flavone-7-O-β-D-glucopyranose, asiaticoside, kaempferol-3,7-diglucoside, madecassoside, 3β,6β,23-trihydroxy-urs-12-en-28-oic acid, kaempferol-3-O-rutinoside, and Mahuanin, were discovered.

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

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,[1] anti-proliferation,[2] and antioxidant. [3,4]

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.[5] Meanwhile, for CA, it is reported as weak to moderate.[6] 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, [7] while CA consists of phenolic and terpenoid compounds.[8] The main active component in GM is α-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.[9] 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.[10] 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 in several compositions of ratios to obtain the best ratio with the strongest antioxidant activities as a result of synergism interaction.

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

MATERIALS AND METHODS

Procedures

Materials and chemicals

The materials used in this research include GM peel powder (Java Plant, Indonesia), CA powder (Java Plant, Indonesia), Ethanol 96% (Happy Lab, Indonesia), ethyl acetate (Bratachem, Indonesia), n-hexane (Bratachem, Indonesia), phytochemistry and TLC dyeing reagents (Merck, Germany), ethanol (Merck, Germany), Na_2SO_4, benzene (Merck, Germany), ethyl acetate (Merck, Germany), chloroform (Merck, Germany), butanol (Merck, Germany), dichloromethane (Merck, Germany), sulfuric acid (Merck, Germany), gallic acid (Sigma Aldrich, USA), Quercetin (Sigma Aldrich, Japan), DPPH (Sigma Aldrich, USA) Folin-Ciocalteu (Merck, Germany), Sodium Carbonate (Merck, Germany), Distilled water, TLC

Silica gel G_{60} F_{254} (Merck, Germany), UV-Vis Spectrophotometer (Genesys 10S), Analytical Balance (Ohaus, model PA323), UV Quartz cuvette, and rotary evaporator (Scilogex RE-100 pro).

Extraction and fractionation methods

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

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

Phytochemicals screening analysis method

Qualitative phytochemical screening analyses were performed using standard methods.[11] Silica gel G_{60} F_{254} was used as a stationary phase and activated by heating at 100 °C for 10 minutes. Furthermore, 10 and 20 mg of GM and CA extracts and their respective fractions are dissolved in 10 mL ethanol. The solution was spotted in a TLC plate and eluted using a mixture of ethyl acetate and chloroform at a 3:7 ratio for GM, as well as benzene and ethyl acetate at 6:4 for CA. The obtained spot was identified using specific spray dyeing reagents and examined under a UV lamp at 254 nm and 365 nm. Flavanoids were identified using $AlCl_3$ 1%, and their presence is denoted by the change of color to blue, yellow, green, and orange. Phenolic compounds were detected using $FeCl_3$ 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. [12,13]

LC/MSMS analysis method

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

The antioxidant activities assay method

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

$$\text{Inhibition } (\%) = \frac{Abs\ of\ Control - Abs\ sample}{Abs\ of\ control} x100\ \%$$

Total phenolic content (TPC) assay method

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

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

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

Analysis of combination index (CI)

The combination index method was used to determine the interaction of the components in the mixture. Chou tested this using inhibitory concentration (IC50) causing 50% inhibitory activitiy and CI approach.[15] Synergism, additive, and antagonism were indicated by a CI <1, 1, and I>1. The following formula was used to calculate the CI value:

$$\text{CI} = \frac{IC50\ of\ A}{ratio\ of\ A\ in\ combination\ x\ IC50\ of combination} + \frac{IC50\ of\ B}{ratio\ of\ B\ in\ combination\ x\ IC50\ of\ combination}$$

RESULTS AND DISCUSSION

Phytochemicals screening analysis

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

As shown in Figure 1, 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. The preliminary analysis determined that the extract and fractions of this natural substance contain flavonoids, phenolic compounds, and terpenoids. According to the results, the spot pattern and secondary metabolites of the CEE and EAF of CA are similar. This is due to ethanol being used as a general solvent in CEE, allowing for the extraction of substances with varying polarities. As a result, the molecules in EAF are also present in CEE. However, the spot and secondary metabolites in EF are different from both CEE and EAF. This is because only polar molecules are extracted to the EF, leading to limited spots, as many compounds have already been extracted in the EAF phase. This result aligns with TLC research conducted by Daniel, which stated that the Ethyl acetate phase of CA contains flavonoids and terpenoids.[8] CEE and EAF of GM show that they contain similar secondary metabolites, such as phenolic compounds and flavonoids, except for terpenoids. The results also showed that alkaloids are not contained in both GM and CA, and this is similar to the research performed by Djoko and Vinolina.[16,17]

LC/MSMS analysis

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

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

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

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

Antioxidant activities of GM and CA extracts and fractions

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

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

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

are the main components identified in CA and are present in extract and fractions. 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.

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

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.

Analysis of combination index (CI)

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.[21]

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 declared that there is no conflict of interest.

AUTHOR'S DECLARATION

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

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REFERENCES

1. Phan TKT, Shahbazzadeh F, Pham TTH, Kihara T. Alpha-Mangostin Inhibits The Migration and Invasion of A549 Lung Cancer Cells. Peerj. 2018; 2018(6):1–17.
2. Moongkarndi P, Kosem N, Kaslungka S, Luanratana O, Pongpan N, Neungton N. Antiproliferation, Antioxidation and Induction of Apoptosis by Garcinia Mangostana (Mangosteen) on SKBR3 Human Breast Cancer Cell Line. J Ethnopharmacol. Elsevier Ireland Ltd; 2004; 90(1):161–166.
3. Palakawong, Sophanodora P, Pisuchpen S, Phongpaichit S. Antioxidant and Antimicrobial Activities of Crude Extracts From Mangosteen (*Garcinia mangostana* L.) Parts and Some Essential Oils. Int Food Res J. 2010; 17:583-589.
4. Rahman M, Hossain S, Rahaman A, Fatima N, Nahar T, Uddin B. Antioxidant Activity of *Centella asiatica* (Linn .) Urban : Impact of Extraction Solvent Polarity. J Pharmacogn Phytochem. 2013; 1(6):27–32.
5. Kusmayadi A, Adriani L, Abun A, Muchtaridi M, Tanuwiria UH. Antioxidant Activity of Mangosteen Peel (*Garcinia mangostana* L.) Extracted Using Different Solvents At The Different Times. Drug Invent Today. 2019; 11(1):8-44.
6. Jatayu D, Nursyam H, Maizar Suryanto Hertika A. Antioxidant Effect of *Centella asiatica* Ethanolic Extract to Superoxide Dismutase (SOD) Level on Cyprinus Carpio Liver. Res J Life Sci. Brawijaya University; 2018; 5(3):163–172.
7. Obolskiy D, Pischel I, Siriwatanametanon N, Heinrich M. Garcinia Mangostana L.: A Phytochemical And Pharmacological Review. Phytother Res. 2009; 23:1047–1065.

8. Ondeko DA, Juma BF, Baraza LD, Nyongesa PK. LC-ESI/MS And GC-MS Methanol Extract Analysis, Phytochemical and Antimicrobial Activity Studies of *Centella asiatica*. Asian J Chem Sci. Sciencedomain International; 2020; 8(3):32–51.
9. Aizat WM, Jamil IN, Ahmad-Hashim FH, Noor NM. Recent Updates on Metabolite Composition and Medicinal Benefits of Mangosteen Plant. Peerj. Peerj Inc. 2019; 7.
10. Sun B, Wu L, Wu Y, Zhang C, Qin L, Hayashi M, Et Al. Therapeutic Potential of *Centella asiatica* and Its Triterpenes: A Review. Front. Pharmacol. 2020; 11.
11. Utami YP, Umar AH, Syahruni R, Kadullah I. Standardisasi Simplisia dan Ekstrak Etanol Daun Leilem (*Clerodendrum minahassae* Teisjm. & Binn.) J Pharm Med Sci. 2017; 2(1):32–39.
12. Tradit AJ, Altern C, Sea NB, Of R. 418 Flavonoid Compounds Identified in *Alchemilla* L. Species Collected in The North-Eastern Black Sea Region of Turkey. Afr J Tradit Complement Altern Med. 2012; 9(3):418–425.
13. Syarifah AL, Retnowati R, Soebiantoro S. Characterization of Secondary Metabolites Profile of Flavonoid From Salam Leaves (*Eugenia polyantha*) Using TLC And Uvspectrophotometry. Pharm Sci Res. 2019; 6(3):155–163.
14. Lim YY, Murtijaya J. Antioxidant Properties of *Phyllanthus amarus* Extracts As Affected by Different Drying Methods. LWT - Food Sci Technol. 2007; 40(9):1664–1669.
15. Chou TC. Theoretical Basis, Experimental Design, and Computerized Simulation of Synergism and Antagonism in Drug Combination Studies. Pharmacol Rev. 2006; 58(3):621–681.
16. Djoko W, Taurhesia S, Djamil R, Simanjuntak P. Standardisasi Ekstrak Etanol Herba Pegagan (*Centella asiatica*). Fak Farm Univ Pancasila Jalan Raya Lenteng Agung Srengseng Sawah, Jakarta. 2020 ;13(2):59–64.
17. Vinolina NS, Sigalingging R. Phytochemical Screening and Biomass Production of *Centella asiatica* (L.) Urb of Samosir-Indonesia Accession Cultivated On Acid Soil With Different Phosphorus Treatments. IOP Conf Ser Earth Environ Sci. 2021; 782(3):8–15.
18. Kusmayadi A, Adriani L, Abun A, Muchtaridi M, Tanuwiria UH. The Effect of Solvents and Extraction Time on Total Xanthone and Antioxidant Yields of Mangosteen Peel (Garcinia Mangostana L.) Extract. Drug Invent Today. 2018; 10(12):2572–2576.
19. Nur Khusnawati N, Pramono S, Sasmito E. 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. Trad Med J. 2015; 20(3):164-169.
20. Liu H, He R, Zheng L, Xie S. Extraction And Analysis of Chemical Constituents From Mangosteen Pericarp. In: E3S Web Of Conferences. EDP Sciences. 2021; 271.
21. Olszowy-Tomczyk M. Synergistic, Antagonistic and Additive Antioxidant Effects in The Binary Mixtures. Phytochem Rev. Springer Netherlands. 2020; 19(1):63-103.
22. Molyneux P. The Use Of The Stable Free Radical Diphenylpicryl-Hydrazyl (DPPH) For Estimating Anti-Oxidant Activity. Songklanakarin J Sci Technol. 2004; 26(5):211–219.
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.

TABLES AND FIGURES

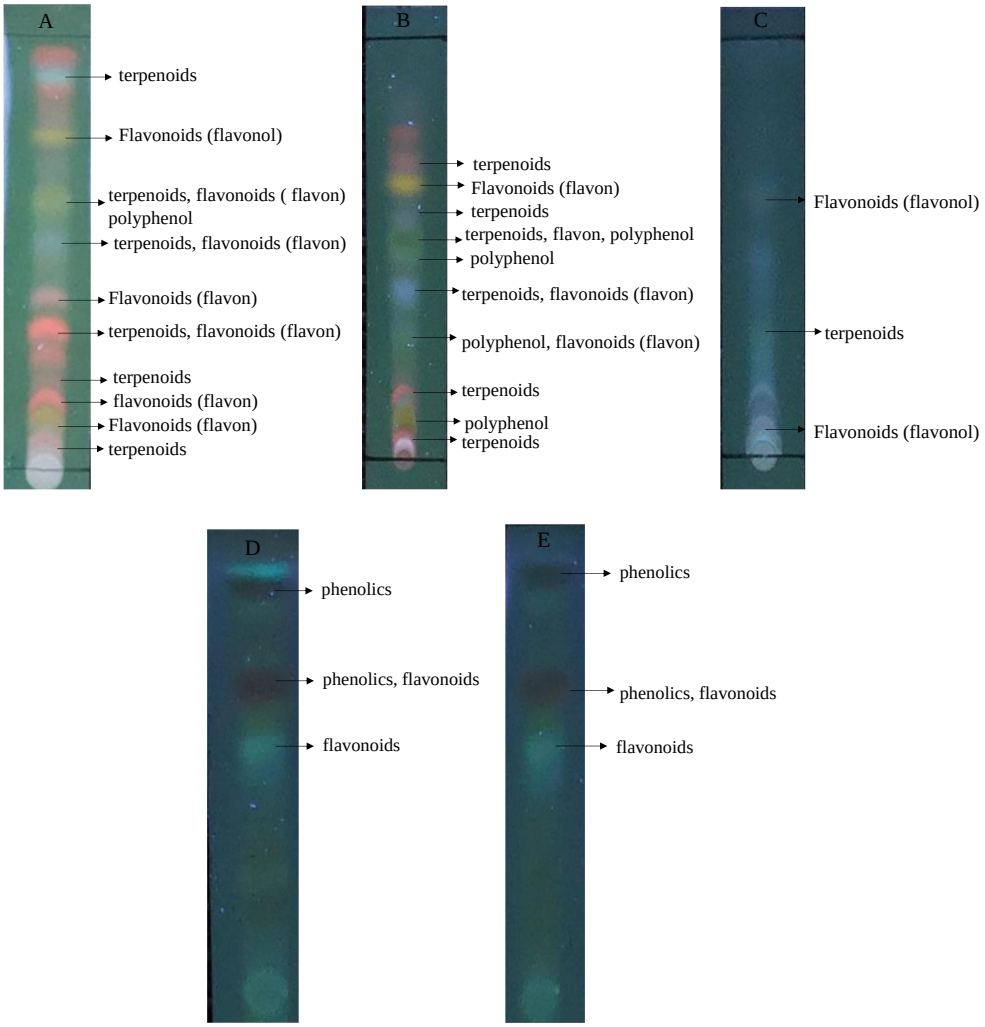

Figure 1. TLC analysis of GM and CA extracts and fractions. A) CEE of CA; B) EAF of CA; C) EF of CA; D) CEE of GM; E) EAF of GM. Identified under a UV lamp at 365 nm.

359

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	$C_{22}H_{26}O_6$ $C_{24}H_{26}O_6$	409,16	1269629	1420558	-
Archangelicin	5.11	$C_{24}H_{26}O_7$	427,17	434892	1908329	-
Biondinin A	8.35	$C_{21}H_{26}O_6$	397.16	1420338	1810666	-
α-Mangosteen	8.98	$C_{24}H_{26}O_6$	411.17	5457467	6071885	45124
Sarcandin acetate	9.21	$C_{26}H_{34}O_6$	465.22	995318	1343097	-
Achilin	3.51	$C_{15}H_{18}O_3$	247.13	-	-	72144
Candidate mass	5.97	$C_{18}H_{39}NO_3$	318.30	-	-	59745
Candidate mass	5.91	$C_{16}H_{35}NO_2$	274.27	-	-	54249
Candidate mass	4.41	$C_{17}H_{33}NO_3$	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	$C_{15}H_{10}O_6$	287.05	108001	266446	-
5-Hydroxy-6,4'-dimethoxy-flavone-7-O-β-D-glucopyranoside	3.50	$C_{23}H_{24}O_{11}$	299.12	126278	-	-
Asiaticoside	4.23	$C_{48}H_{78}O_{19}$	981.50	117774	87716	200871
Kaempferol-3,7-diglucoside	3.18	$C_{27}H_{30}O_{16}$	611.16	145253	-	161998
Madecassoside	3.97	$C_{48}H_{78}O_{20}$	997.49	85570	36480	205343
3β,6β,23-Trihydroxy-urs-12-en-28-oic acid	6.17	$C_{30}H_{48}O_5$	511.33	-	52038	-
Candidate Mass	10.19	$C_{35}H_{36}N_4O_5$	593.27	-	407065	-
Kaempferol-3-O-rutinoside	2.77	$C_{27}H_{30}O_{15}$	595.16	-	-	238532
Mahuannin F	0.49	$C_{30}H_{22}O_{10}$	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_{50}) ppm			
	Positive control	CEE	EAF	EF
Quersetin	4.63			
GM		83.98±2.50[b]	45.78±1.20[a]	72.96±14.30[b]
CA		300.88±43.78[d]	102.57±3.40[c]	494.96±25.10[d]

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 (IC50) 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[a]	64.62±2.22[b]	71.73±2.63[b]	62.00±1.67[b]	102.57±3.40[c]

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

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No	observation	Correction	Description
1.	Chemical Composition, Antioxidant Activities, and Total Phenolic Content of Combination of Mangosteen *(Garcinia mangostana* l.*)* Peel-Kodavan *(Centella asiatica* l. Urban*)* Fractions	Chemical Composition, Antioxidant Activities, and Total Phenolic Content of Combination of Mangosteen *(Garcinia mangostana* L.*)* Peel-Kodavan *(Centella asiatica* L. Urban*)* Fractions	See the title section
2.	$AlCl_3$ 1%	$AlCl_3$ 3%	See line 108
3.	$FeCl_3$ 5%	$FeCl_3$ 1%	See line 110
4.	LC/MSMS	LC-MS/MS	See Tables 1 and 2
5.	gm, ca, eaf, ic_{50}, mggae/g of tpc, cee, eaf, ef, gm-ca	GM, CA, EAF, IC_{50}, mgGAE/g of TPC, CEE, EAF, EF, GM-CA	See the conclusion section
6.	H2O	H_2O	See line 121
7.	IC50	IC_{50}	See Table 4
8.	IC50	IC_{50}	See line 162

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Original Research Article

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

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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 mangosatana* 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_{50}) 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_{50} = 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, *α*-mangosteen, samarcandin acetate, and achilin. In CA, 5,7,2',5'-tetrahydroxy-flavon, 5-hydroxy-6,4'-dimethoxy-flavone-7-*O*-*β*-D-glucopyranose, asiaticoside, kaempferol-3,7-diglucoside, madecassoside, 3β,6β,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.

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,[1] anti-proliferation,[2] and antioxidant. [3,4]

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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.[5] Meanwhile, for CA, it is reported as weak to moderate.[6] 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, [7] while CA consists of phenolic and terpenoid compounds.[8] The main active component in GM is *α*- 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.[9] 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.[10] 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

in several compositions of ratios to obtain the best ratio with the strongest antioxidant activities as a result of synergism interaction. The primary aim of this research was to investigate the hypothesis that the CEE, EAF, EF, and the combination of GM-CA active fractions exhibit different antioxidant activities, which were positively correlated with the ratio of their chemical compositions.

Materials and Methods

Materials and chemicals

The materials used in this research include GM peel powder (voucher number 8/3/2020), CA powder (voucher number 7/3/2020). The plant materials were collected from Java plant, Karanganyar, Central Java, Indonesia, in March 2020. Ethanol 96% (Happy Lab, Indonesia), ethyl acetate (Bratachem, Indonesia), n-hexane (Bratachem, Indonesia), phytochemistry and TLC dyeing reagents (Merck, Germany), ethanol (Merck, Germany), Na_2SO_4, benzene (Merck, Germany), ethyl acetate (Merck, Germany), chloroform (Merck, Germany), butanol (Merck, Germany), dichloromethane (Merck, Germany), sulfuric acid (Merck, Germany), gallic acid (Sigma Aldrich, USA), Quercetin (Sigma Aldrich, Japan), DPPH (Sigma Aldrich, USA) Folin-Ciocalteu (Merck, Germany), Sodium Carbonate (Merck, Germany), Distilled water, TLC Silica gel G_{60} F_{254} (Merck, Germany), UV-Vis Spectrophotometer (Genesys 10S), Analytical Balance (Ohaus, model PA323), UV Quartz cuvette, and rotary evaporator (Scilogex RE-100 pro).

Extraction and fractionation methods

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

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

Phytochemicals screening analysis method

Qualitative phytochemical screening analyses were performed using standard methods.[11] Silica gel G_{60} F_{254} was used as a stationary phase and activated by heating at 100 °C for 10 minutes. Furthermore, 10 and 20 mg of GM and CA extracts and their respective fractions are dissolved in 10 mL ethanol. The solution was spotted in a TLC plate and eluted using a mixture of ethyl acetate and chloroform at a 3:7 ratio for GM, as well as benzene and ethyl acetate at 6:4 for CA. The obtained spot was identified using specific spray dyeing reagents and examined under a UV lamp at 254 nm and 365 nm. Flavanoids were identified using $AlCl_3$ 3%, and their presence is denoted by the change of color to blue, yellow, green, and orange. Phenolic compounds were detected using $FeCl_3$ 1%, 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.[12,13]

LC-MS/MS analysis method

Liquid Chromatoghraphy-mass spectrometry analysis was performed on water acquity UPLC I-Class and XEVO G2-XS QTof. The instrument was operated in full scan ESI mode. The liquid chromatography conditions were a C18 column with a particle size of 1.7 µm and a 2.1 x 50 mm length. Furthermore, the eluents employed were a mixture of H_2O and 0.1% formic acid (Solvent A), as well as ACN and 0.1% formic acid (Solvent B). The injection volume was 1 µL, and the ionization type was set at ESI positive. Finally, the mass ranges from 100-1200 m/z.

The antioxidant activities assay method

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

$$\text{Inhibition}\ (\%) = \frac{Abs\ of\ Control - Abs\ sample}{Abs\ of\ control}\ x100\ \%$$

Total phenolic content (TPC) assay method

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

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

Analysis of combination index (CI)

The combination index method was used to determine the interaction of the components in the mixture. Chou tested this using inhibitory concentration (IC_{50}) causing 50% inhibitory activitiy and CI approach.[15] Synergism, additive, and antagonism were indicated by a CI <1, 1, and I>1. The following formula was used to calculate the CI value:

$$\text{CI} = \frac{IC50\ of\ A}{ratio\ of\ A\ in\ combination\ x\ IC50\ of combination} + \frac{IC50\ of\ B}{ratio\ of\ B\ in\ combination\ x\ IC50\ of\ combination}$$

Statistical analysis

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

Results and Discussion

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

As shown in Figure 1, 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. The preliminary analysis determined that the extract and fractions of this natural substance contain flavonoids, phenolic compounds, and terpenoids. According to the results, the spot pattern and secondary metabolites of the CEE and EAF of CA are similar. This is due to ethanol being used as a general solvent in CEE, allowing for the extraction of substances with varying polarities. As a result, the molecules in EAF are also present in CEE. However, the spot and secondary metabolites in EF are different from both CEE and EAF. This is because only polar molecules are extracted to the EF, leading to limited spots, as many compounds have already been extracted in the EAF phase. This result aligns with TLC research conducted by Daniel, which stated that the Ethyl acetate phase of CA contains flavonoids and terpenoids.[8] CEE and EAF of GM show that they contain similar secondary metabolites, such as phenolic compounds and flavonoids, except for terpenoids. The results also showed that alkaloids are not

contained in both GM and CA, and this is similar to the research performed by Djoko and Vinolina.[16,17]

The second analysis of chemical components, which aims to obtain the identity of compounds, was conducted using LC-MS/MS. The results for GM and CA are shown in Tables 1 and 2, respectively. The chromatogram for GM and CA are shown in Figures 2 and 3, respectively.

As shown in Table 1, GM contains 5 major compounds and 3 that are still unidentified. Those in CEE and EAF of GM are similar since ethanol is used as a general solvent. However, based on the detector counts, the quantity of the compounds in EAF is higher than in CEE. This is because they are more likely to dissolve in the moderate polarity of solvents such as ethyl acetate. *α*-mangosteen, a xanthone, is the main compound of GM and is present largely in EAF. This aligns with research conducted by Andri, which stated that xanthones are well extracted in solvents of moderate polarity.[18] In EF, the compounds are not similar to CEE and EAF. However, *α*-mangosteen is still present in EF but at a low level. This is correlated with the result of TLC, that EF of GM has a limited spot.

The components of CEE and EAF in CA are also similar, with only two different substances. However, the quantity is different based on detector counts. The 5-Hydroxy-6,4'-dimethoxy-flavone-7-*O*-*β*-D-glucopyranoside, Kaempferol-3,7-diglucoside, Kaempferol-3,7-diglucoside, and Kaempferol-3-*O*-rutinoside are only discovered in the polar phase (EF). This is because their chemical structure contains sugar moiety. This follows the research conducted by Daniel, which reported that kaempferol is only identified in the polar phase. Asiaticoside and madecassoside are the main compounds of CA, and they are discovered in all phases. However, the quantity in EF is the largest because of the sugar moiety content that makes the compounds more polar. This is consistent with the research performed by Nur, who discovered that CA contains asiaticoside and madecassoside.[19]

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

As shown in Table 3, GM shows strong antioxidant activities, with a strength order of EAF > EF > CEE. The chemical compounds responsible for its activities are the phenolic compounds, consisting of many hydroxyl groups that could be proton donors to stabilize the DPPH. EAF has very strong antioxidant activities because it contains many phenolic compounds. The primary component in GM is *α*-mangosteen, which is reported to have antioxidant activities.[20] It is discovered to be higher and lower in EAF and EF, respectively.

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

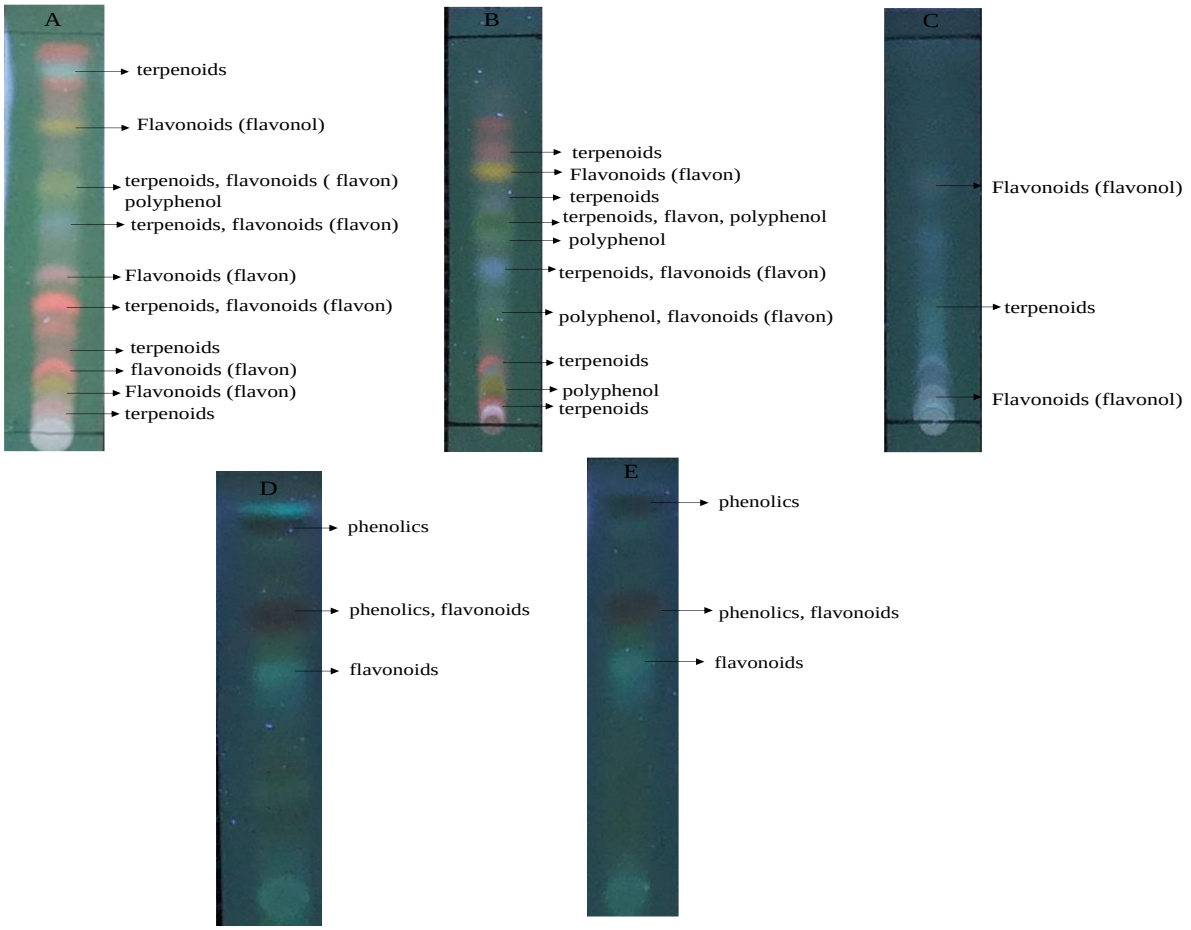


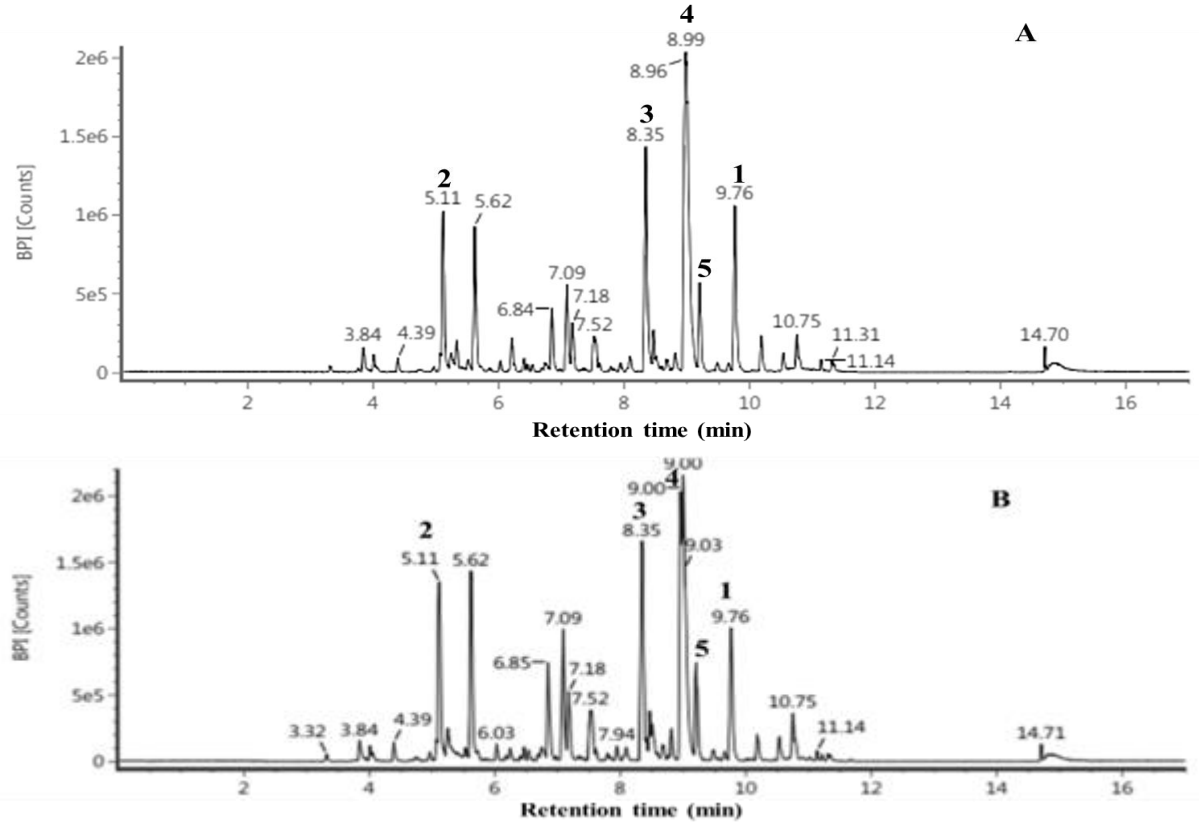
Figure 1: TLC analysis of GM and CA extracts and fractions. A) CEE of CA; B) EAF of CA; C) EF of CA; D) CEE of GM; E) EAF of GM. Identified under a UV lamp at 365 nm.

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.	*α*-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.



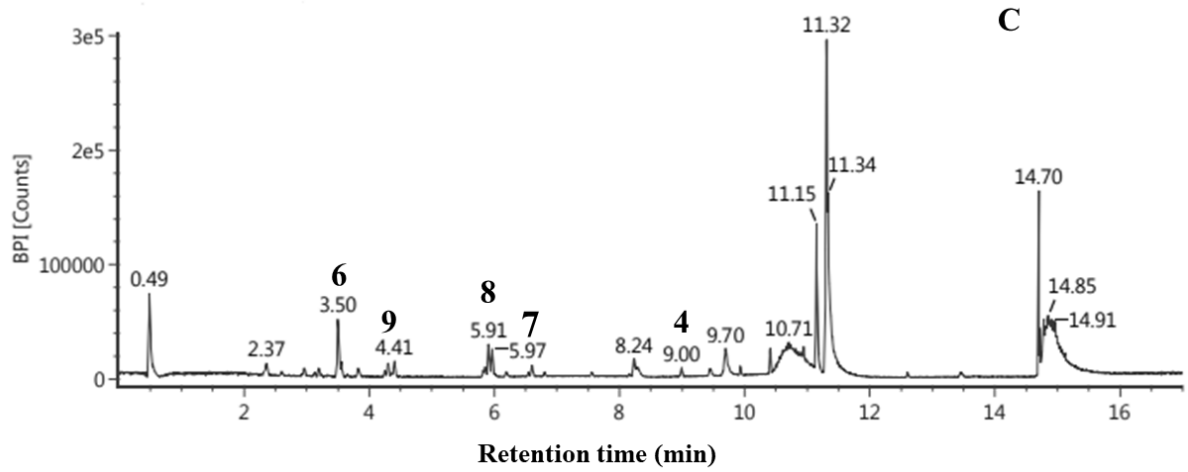


Figure 2: Chromatogram of GM extracts and fractions. A) CEE of GM; B) EAF of GM; C) EF of GM.

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	$C_{15}H_{10}O_6$	287.05	108001	266446	-
2.	5-Hydroxy-6,4'-dimethoxy-flavone-7-*O*-*β*-D-glucopyranoside	3.50	$C_{23}H_{24}O_{11}$	299.12	126278	-	-
3.	Asiaticoside	4.23	$C_{48}H_{78}O_{19}$	981.50	117774	87716	200871
4.	Kaempferol-3,7-diglucoside	3.18	$C_{27}H_{30}O_{16}$	611.16	145253	-	161998
5.	Madecassoside	3.97	$C_{48}H_{78}O_{20}$	997.49	85570	36480	205343
6.	3β,6β,23-Trihydroxy-urs-12-en-28-oic acid	6.17	$C_{30}H_{48}O_5$	511.33	-	52038	-
7.	Candidate Mass	10.19	$C_{35}H_{36}N_4O_5$	593.27	-	407065	-
8.	Kaempferol-3-*O*-rutinoside	2.77	$C_{27}H_{30}O_{15}$	595.16	-	-	238532
9.	Mahuannin F	0.49	$C_{30}H_{22}O_{10}$	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.[21]

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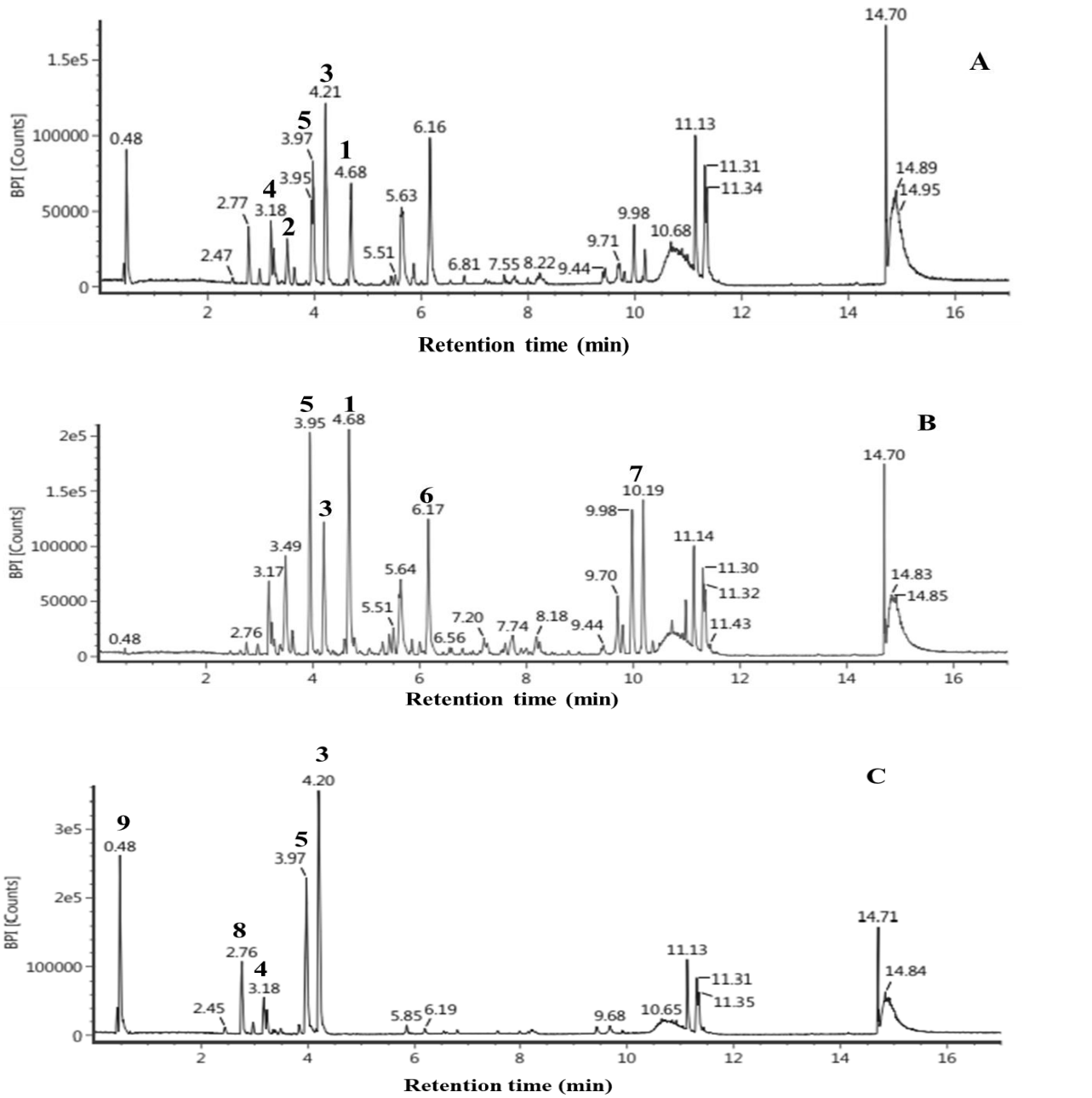

Figure 3: Chromatogram of CA extracts and fractions. A) CEE of CA; B) EAF of CA; C) EF of CA

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

Samples	Antioxidant activities (IC_{50}) ppm			
	Positive control	**CEE**	**EAF**	**EF**
Quercetin	4.63			
GM		83.98 ± 2.50[b]	45.78 ± 1.20[a]	72.96 ± 14.30[b]
CA		300.88 ± 43.78[d]	102.57 ± 3.40[c]	494.96 ± 25.10[d]

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	The ratios of GM-CA ethyl acetate fraction combination				
(IC_{50}) ppm	1:0	3:1	1:1	1:3	0:1
Value	45.78 ± 1.20[a]	64.62 ± 2.22[b]	71.73 ± 2.63[b]	62.00 ± 1.67[b]	102.57 ± 3.40[c]

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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References

1. Phan TKT, Shahbazzadeh F, Pham TTH, Kihara T. Alpha-Mangostin Inhibits The Migration and Invasion of A549 Lung Cancer Cells. Peerj. 2018; 2018(6):1–17.
2. Moongkarndi P, Kosem N, Kaslungka S, Luanratana O, Pongpan N, Neungton N. Antiproliferation, Antioxidation and Induction of Apoptosis by Garcinia Mangostana (Mangosteen) on SKBR3 Human Breast Cancer Cell Line. J Ethnopharmacol. Elsevier Ireland Ltd; 2004; 90(1):161–166.
3. Palakawong, Sophanodora P, Pisuchpen S, Phongpaichit S. Antioxidant and Antimicrobial Activities of Crude Extracts From Mangosteen (*Garcinia mangostana* L.) Parts and Some Essential Oils. Int Food Res J. 2010; 17:583-589.
4. Rahman M, Hossain S, Rahaman A, Fatima N, Nahar T, Uddin B. Antioxidant Activity of *Centella asiatica* (Linn) Urban : Impact of Extraction Solvent Polarity. J Pharmacogn Phytochem. 2013; 1(6):27–32.
5. Kusmayadi A, Adriani L, Abun A, Muchtaridi M, Tanuwiria UH. Antioxidant Activity of Mangosteen Peel (*Garcinia mangostana* L.) Extracted Using Different Solvents At The Different Times. Drug Invent Today. 2019; 11(1):8-44.
6. Jatayu D, Nursyam H, Maizar Suryanto Hertika A. Antioxidant Effect of *Centella asiatica* Ethanolic Extract to Superoxide Dismutase (SOD) Level on Cyprinus Carpio Liver. Res J Life Sci. Brawijaya University; 2018; 5(3):163–172.
7. Obolskiy D, Pischel I, Siriwatanametanon N, Heinrich M. Garcinia Mangostana L.: A Phytochemical And Pharmacological Review. Phytother Res. 2009; 23:1047–1065.
8. Ondeko DA, Juma BF, Baraza LD, Nyongesa PK. LC-ESI/MS And GC-MS Methanol Extract Analysis, Phytochemical and Antimicrobial Activity Studies of *Centella asiatica*. Asian J Chem Sci. Sciencedomain International; 2020; 8(3):32–51.
9. Aizat WM, Jamil IN, Ahmad-Hashim FH, Noor NM. Recent Updates on Metabolite Composition and Medicinal Benefits of Mangosteen Plant. Peerj. Peerj Inc. 2019; 7:1-25.
10. Sun B, Wu L, Wu Y, Zhang C, Qin L, Hayashi M, Et Al. Therapeutic Potential of *Centella asiatica* and Its Triterpenes: A Review. Front. Pharmacol. 2020; 11:1-24.
11. Utami YP, Umar AH, Syahruni R, Kadullah I. Standardisasi Simplisia dan Ekstrak Etanol Daun Leilem (*Clerodendrum minahassae* Teisjm. & Binn.) J Pharm Med Sci. 2017; 2(1):32–39.
12. Tradit AJ, Altern C, Sea NB, Of R. 418 Flavonoid Compounds Identified in *Alchemilla* L. Species Collected in The North-Eastern Black Sea Region of Turkey. Afr J Tradit Complement Altern Med. 2012; 9(3):418–425.
13. Syarifah AL, Retnowati R, Soebiantoro S. Characterization of Secondary Metabolites Profile of Flavonoid From Salam Leaves (*Eugenia polyantha*) Using TLC And Uvspectrophotometry. Pharm Sci Res. 2019; 6(3):155–163.
14. Lim YY, Murtijaya J. Antioxidant Properties of *Phyllanthus amarus* Extracts As Affected by Different Drying Methods. LWT - Food Sci Technol. 2007; 40(9):1664–1669.
15. Chou TC. Theoretical Basis, Experimental Design, and Computerized Simulation of Synergism and Antagonism in Drug Combination Studies. Pharmacol Rev. 2006; 58(3):621–681.
16. Djoko W, Taurhesia S, Djamil R, Simanjuntak P. Standardisasi Ekstrak Etanol Herba Pegagan (*Centella asiatica*). Fak Farm Univ Pancasila Jalan Raya Lenteng Agung Srengseng Sawah, Jakarta. 2020 ;13(2):59–64.
17. Vinolina NS, Sigalingging R. Phytochemical Screening and Biomass Production of *Centella asiatica* (L.) Urb of Samosir-Indonesia Accession Cultivated On Acid Soil With Different Phosphorus Treatments. IOP Conf Ser Earth Environ Sci. 2021; 782(3):8–15.
18. Kusmayadi A, Adriani L, Abun A, Muchtaridi M, Tanuwiria UH. The Effect of Solvents and Extraction Time on Total Xanthone and Antioxidant Yields of Mangosteen Peel (Garcinia Mangostana L.) Extract. Drug Invent Today. 2018; 10(12):2572–2576.
19. Nur Khusnawati N, Pramono S, Sasmito E. 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. Trad Med J. 2015; 20(3):164-169.
20. Liu H, He R, Zheng L, Xie S. Extraction And Analysis of Chemical Constituents From Mangosteen Pericarp. In: E3S Web Of Conferences. EDP Sciences. 2021; 271.
21. Olszowy-Tomczyk M. Synergistic, Antagonistic and Additive Antioxidant Effects in The Binary Mixtures. Phytochem Rev. Springer Netherlands. 2020; 19(1):63-103.
22. Molyneux P. The Use Of The Stable Free Radical Diphenylpicryl-Hydrazyl (DPPH) For Estimating Anti-Oxidant Activity. Songklanakarin J Sci Technol. 2004; 26(5):211–219.

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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