

Antioxidant Properties of Capsule Dosage Form From Mixed Extracts of *Garcinia Mangostana* Rind and *Solanum Lycopersicum* Fruit

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ABSTRACT

Several studies showed both *Garcinia mangostana* L. and *Solanum lycopersicum* L. have shown important role as natural sources of antioxidant compounds. Hence, capsule supplement from mixed extracts of *Garcinia mangostana* L. and *Solanum lycopersicum* L. was prepared. This study aims to investigate the antioxidant properties from the capsules supplement contained with mixed extracts of *Garcinia mangostana* rind (GMR) and *Solanum lycopersicum* fruit (SLF). Antioxidant activity of capsule dosage form was measured using DPPH, ABTS, and FRAP assays. In addition, the total phenolic content and total flavonoid content of the capsules preparation were also evaluated. Total phenolic content was 0.7082 ± 0.1372 mg GAE/capsule and total flavonoid content was 11.7769 ± 3.9504 μ g QE/capsule. The strong correlation observed between antioxidant capacity by ABTS method and the total phenolic contents ($R^2= 0.995$, $P<0.05$) indicated that phenolic compounds in capsule preparation related with its antioxidant activity.

Keywords: Antioxidant, DPPH, ABTS, FRAP, *Garcinia mangostana*, *Solanum lycopersicum*

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(Received 13 February 2020, accepted 17 April 2020)

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INTRODUCTION

Antioxidant is a molecule that has the ability to protect organisms from damage caused by free radical-induced oxidative stress. Oxidative stress is considered to be linked with numerous degenerative diseases such as cancer, cardiovascular disease, Alzheimer's disease and Parkinson [1-3]. Human body can balance the oxidative state by synthesizing glutathione and enzymes (e.g., catalase and superoxide dismutase) which produced internally, or taking exogenous antioxidants like the vitamin C, vitamin E, carotenoids, and polyphenols [4-5]. Taking dietary antioxidant supplementation has been found to be a promising method of countering the effects of oxidative stress [6-9]. Natural products began to receive much attention as sources of safe antioxidants nowadays [10-12]. Some species of medicinal plants have antioxidant and pharmacological activities which related to the existence of phenolic compounds [13-14].

Mangosteen (*Garcinia mangostana* L., family Guttiferae) is known as "the queen of tropical fruits" because of its tasty flavor. The pericarp of mangosteen has been used traditionally by Southeast Asian for treating diarrhea, skin infection and wounds, amoebic dysentery, etc [15-16]. *G. mangostana* rind (GMR) contains a lot of water soluble antioxidant compounds. Various kinds of xanthenes, such as prenylated and oxygenated xanthenes in GMR had been proven to have strong antioxidant activity [17-19]. Tomato (*Solanum lycopersicum* L., family Solanaceae) is one of the most consumed vegetables worldwide. *S. lycopersicum* fruit (SLF) are considered as important sources of dietary antioxidants, such as carotenoids, in particular α -carotene, β -carotene, lycopene, lutein, and cryptoxanthin [20-21]. Anthocyanins, the flavonoid constituents in highly pigmented fruits including tomato, have been reported to possess potential antioxidant, anti-inflammatory, anticancer, and antidiabetic activity [22-24].

GMR and SLF each has been widely investigated for their antioxidant activities, yet the antioxidant properties of mixed extracts of GMR and SLF has not been reviewed. Due to the widely marketed nutritional supplements containing GMR, we are challenged to create capsule dosage form as supplements prepared from both plant extracts. This study aims to investigate the antioxidant properties from the capsules supplement contained with mixed extracts of *G. mangostana* rind (GMR) and *S. lycopersicum* fruit (SLF), also its total phenolic and flavonoid contents. Several methods were applied to measure antioxidant capacity, including 2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and ferric reducing antioxidant power (FRAP).

METHODOLOGY

Chemical and Reagents

Aluminium chloride hexahydrate, Folin-Ciocalteu's phenol reagent, ascorbic acid, sodium carbonate, quercetin, and gallic acid were purchased from Merck Millipore. Dimethylsulfoxide (DMSO) and AR grade methanol from Sigma-Aldrich. Aerocyl and amyllum manihot for preparation of GMR and SLF capsules were purchased from local industries. The commercial grade solvents were used for extraction.

Plant Material

The *G. mangostana* and *S. lycopersicum* fruit were collected from Gatutkaca, a local Jamu (Indonesian traditional medicine) industry and identified by Eling Purwantoyo, M.Si. (Department of Biology, Universitas Negeri Semarang). The voucher specimen (204579) was deposited at the Biology Laboratory, Department of Biology, Universitas Negeri Semarang, Indonesia.

Preparation of Plant Extracts

Extraction of *G. mangostana* and *S. lycopersicum* using hydroalcoholic solvent was based on previous studies which performed hydroalcoholic extractions to obtain antioxidant compounds [25-26]. The dried pericarp of *G. mangostana* (1000 g) were macerated using 70% ethanol/water (1:3) at room temperature for 72 h. After filtration, the filtrate was concentrated followed with the addition of aerocyl (30 g). From this process, 50 g of condensed extract was obtained. For the *S. lycopersicum* extract, fresh fruits of tomatoes (1000 g) were crushed and blended using 70% ethanol/water (1:1). After filtration, the filtrate was concentrated followed with the addition of aerocyl (20 g). 65 g of condensed tomato extracts were obtained.

Preparation of Capsule Contains Mixed Extracts of GMR and SLF

The condensed extract of GMR were added with 1500 g filling agents (amyllum manihot) to produce dry powder of GMR extracts (1448 g). The same procedure was also conducted to the condensed extract of SLF. 850 g filling agents (amyllum manihot) were added to produce dry powder of SLF extracts (774 g). Both dried extracts were mixed at a ratio of GMR:SLF (2:1) and capsulated with number 0 capsule shell (average weight 450 mg/capsule).

DPPH-radical Scavenging Activity Assay

The diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay was performed based on the method of Zongo (2010) with slight modification [27]. In the microplate well, 10, 20, 30, 40, 50 μ L of the capsule powder (50 mg/mL in DMSO)

or standard solution was mixed with 100 μL of the DPPH-radical (100 $\mu\text{g}/\text{mL}$ in methanol) and left to stand at room temperature for 15 min in the dark. The absorbance was measured at 517 nm. Ascorbic acid (Vitamin C) was used as references. This experiment was conducted in triplicates.

ABTS Assay

In the ABTS free radical assay, the method of Jemli (2015) was adopted with minor changes [28]. Briefly, ABTS reagent solution was freshly prepared by mixing 2 mM of ABTS solution with 70 mM of potassium persulfate, stored in the dark at room temperature for 16 h before use. ABTS \cdot + solution was then diluted with 80% methanol to obtain an absorbance reading of 0.700 ± 0.005 at 743 nm. The 100 μL of sample solution with various concentration was added to 100 μL of ABTS solution. The absorbance was measured at 734 nm after 1 minutes of mixture reaction. All the measurements were carried out three times repetition. A standard curve was obtained by using ascorbic acid standard solution at various concentrations (ranging from 25 to 125 g/mL). The scavenging activity of different concentrations of sample against ABTS radical were also measured to calculate IC_{50} , and the procedure was similar to the DPPH scavenging method described above.

FRAP Assay

FRAP was measured by spectrophotometric assay as previously described [28]. 100 μL of sample at different concentration, 100 μL of phosphate buffer (0.2 M, pH 6.6), and 100 μL of potassium ferricyanide $\text{K}_3\text{Fe}(\text{CN})_6$ (1%) were mixed and incubated at 50°C for 20 min, to reduce ferricyanide into ferrocyanide. The reaction was stopped by adding 100 μL of 10% (w/v) trichloroacetic acid followed by centrifugation at 3000 rpm for 10 min. Lastly, 100 μL of the top layer was mixed with 100 μL of distilled water and 25 μL of ferric chloride solution (0.1%) and the absorbance at 710 nm was calculated by plotting absorbance against the corresponding sample concentration. All the determinations were performed triplicates. Ascorbic acid (vitamin C) was used as a reference compound.

Total Phenolic Contents

Folin–Ciocalteu’s method with slight modification was applied to determine the total phenolic content [29]. In a 96-well plate, 12 μL of capsule powder solutions (250 $\mu\text{g}/\text{mL}$ in DMSO) or standard gallic acid solutions were added, followed by 50 μL of DI water and 12 μL of Folin-Ciocalteu (50%, v/v in DI water). After 10 min, 125 μL of 7% Na_2CO_3 and 100 μL of DI water were added. The mixture was allowed to stand for 15 min at 45°C and the absorbance was determined at 765 nm. Total phenolic content was calculated from gallic acid standard curve with linear relation of $r^2=0.9727$. Data were expressed as mg of gallic equivalent (GAE) per capsule.

Total Flavonoid Contents

In order to investigate the total flavonoid content, a colorimetric method was applied [27]. In a 96-well plate, 100 μL of the capsule powder (100 $\mu\text{g}/\text{mL}$ in DMSO) or standard quercetin solutions and 100 μL of 2 % AlCl_3 in methanol were added and mixed thoroughly. The reaction mixture was kept at room temperature for 15 min and the absorbance was recorded at 435 nm. The total flavonoid content was calculated using quercetin standard curve with linear relation of $R^2=0.9936$. Data were expressed as mg quercetin equivalent (QE) per capsule.

RESULT AND DISCUSSION

According to previous studies, *G. mangostana* rind contains phytochemicals such as xanthenes, terpenes, anthocyanins, tannins, and phenols, which exert numerous biological effects, including antioxidant activity [30-31]. It is believed that antioxidants can help to overcome oxidative damage in human body, which is associated with many degenerative diseases such as atherosclerosis, coronary heart diseases, aging, and cancer [32-33]. *S. lycopersicum* fruit, which is being widely consumed either fresh or processed in products, possess carotenoids, such as lycopene and β -carotene that are apparently the main tomato micro constituents that responsible for the effect of tomato product on antioxidant activity [34]. Different solvent was used in the extraction of GMR and SLF. The extraction of GMR and SLF by using 70% ethanol/water 1:3 and 1:1, respectively, was conducted according to the polarity of major compounds contained in GMR, which is xanthone, and SLF, which is carotenoid. In this study, the antioxidant activity of capsule dosage form prepared from mixed extracts of GMR and SLF was being determined.

Total Phenolic Contents

It is important to measure the total phenolic compounds correctly in such medicinal plants, the better to assess their antioxidant capacity. Under the basic reaction conditions, a phenol loses an H^+ ion to produce a phenolate ion, which reduces Folin-Ciocalteu reagent [35]. The change is monitored spectrophotometrically. Results of Total Phenolic Contents (TPC) determination by Folin-Ciocalteu method are summarized in Table 1. The greater amount signifies the presence of different constituents having phenolic moiety in their structures. The phenolic content with respect to gallic acid was found to be 0.7082 ± 0.1372 (mg Gallic Acid Equivalent/capsule).

Table 1. The Total Phenolic Content of Capsule Preparation from Mixed Extract of *G. mangostana* rind (GMR) and *S. lycopersicum* fruit (SLF)

| Sample | Equation | R ² | TPC (mg GAE/capsule) | Mean TPC (mg GAE/capsule) |
|---|------------------------|----------------|-------------------------|------------------------------|
| Capsule (1 st repetition) | $y = 4.1289x + 0.029$ | 0.9863 | 0.8588 | |
| Capsule (2 nd repetition) | $y = 4.07x + 0.0346$ | 0.9987 | 0.6643 | |
| Capsule (3 rd repetition) | $y = 3.8011x + 0.0854$ | 0.9596 | 0.6241 | 0.7082 ± 0.1372 |
| Capsule (4 th repetition) | $y = 4.2799x + 0.0458$ | 0.9438 | 0.7974 | |
| Capsule (5 th repetition) | $y = 4.2376x - 0.0213$ | 0.9343 | 0.9564 | |

Total Flavonoid Contents

The Total Flavonoids Content (TFC) of the capsule dosage form was determined by a colorimetric assay using quercetin as standard (Table 2). The greater amount signifies the presence of more flavonoids moieties in the constituents. The flavonoid content with respect to quercetin was found to be 11.7769 ± 3.9504 (μg Quercetin Equivalent/capsule).

Table 2. The Total Flavonoid Content of Capsule Preparation from Mixed Extract of *G. mangostana* rind (GMR) and *S. lycopersicum* fruit (SLF)

| Sample | Equation | R ² | TFC (μg QE/capsule) | Mean TPC (μg QE/capsule) |
|---|------------------------|----------------|------------------------------------|---|
| Capsule (1 st repetition) | $y = 0.0155x + 0.1738$ | 0.9453 | 11.5552 | |
| Capsule (2 nd repetition) | $y = 0.0162x + 0.1863$ | 0.9885 | 6.4782 | |
| Capsule (3 rd repetition) | $y = 0.0156x + 0.175$ | 0.9726 | 12.8523 | 11.7769 ± 3.9504 |
| Capsule (4 th repetition) | $y = 0.0173x + 0.1334$ | 0.9780 | 17.4097 | |
| Capsule (5 th repetition) | $y = 0.0154x + 0.1524$ | 0.9752 | 10.5889 | |

Most antioxidant activities from plant sources correlate with phenolic and flavonoid contents. The next section discuss about the antioxidant activity and the correlation between phenolic and flavonoid contents; and antioxidant activity.

Antioxidant Activity

In this study, the antioxidant activities were determined by *in vitro* assays, including 2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and ferric reducing antioxidant power (FRAP). All three assays are based on the reaction of electron transfer, where the color change would serve as an indication of the antioxidant's ability to reduce radicals [36].

Table 3. IC₅₀ Values of Capsule Preparation from Mixed Extract of *G. mangostana* rind (GMR) and *S. lycopersicum* fruit (SLF)

| Assays | Sample (mg/mL) | Positive control (Vitamin C) (µg/mL) |
|--------|------------------|--------------------------------------|
| DPPH | 5.8837 ± 1.4586 | 184.7211 ± 9.1777 |
| ABTS | 6.8098 ± 2.8832 | 83.6069 ± 8.2220 |
| FRAP | 13.7393 ± 2.3856 | 80.6294 ± 9.5560 |

Values represent means ± SD (standard deviations) for triplicates experiment

As summarized in Table 3, the measurement using DPPH method resulted the lowest IC₅₀ for capsule dosage form (sample), whereas FRAP method resulted the lowest IC₅₀ for standard reference (ascorbic acid). Therefore, it is possible that capsule contain mixed extracts of SLF and GMR exhibit different antioxidant mechanism than ascorbic acid. Vitamin C acts as a scavenger of ROS and by one-electron reduction of lipid hydroperoxyl radicals via the vitamin E redox cycle, hence it has the ability to protect against lipid peroxidation (radical chain reaction) [37-39].

Since the capsules were prepared from mixing 50 g of GMR extracts and 65 g of SLF extracts and filling agents, we calculated the estimation of the IC₅₀ value of each extracts that may contribute to the antioxidant activity (Table 4).

Table 4. Estimation of IC₅₀ Value of Each Extracts

| Assays | GMR extract (µg/mL) | SLF extract (µg/mL) |
|--------|---------------------|---------------------|
| DPPH | 200.10 | 100.50 |
| ABTS | 231.60 | 115.80 |
| FRAP | 467.27 | 233.63 |

It has been proposed that samples with IC_{50} lower than 50 $\mu\text{g}/\text{mL}$ are very strong antioxidants, with 50-100 $\mu\text{g}/\text{mL}$ are strong, with 100-250 $\mu\text{g}/\text{mL}$ are moderate, with IC_{50} greater than 250 $\mu\text{g}/\text{mL}$ are weak antioxidants, and with IC_{50} greater than 500 $\mu\text{g}/\text{mL}$ are inactive [40]. Meanwhile, Molyneux (2004) stated that IC_{50} of 200-1000 $\mu\text{g}/\text{mL}$ is less active but still has an antioxidant potential [41]. Thus, generally, GMR and SLF extracts are considered to have moderate antioxidant activity.

In addition, we conducted the correlation analysis of the values of total antioxidant capacity obtained by three assay methods, also the correlation analysis of the TFC and TPC to the antioxidant capacity. As shown in Table 5, TPC and ABTS assay indicated a strong correlation ($R^2= 0.995$).

Table 5. Correlation Coefficient (R^2) among Antioxidant Assays and Total Phenolic and Flavonoid Contents

| | DPPH | ABTS | FRAP |
|------|-------|--------|-------|
| ABTS | 0.182 | – | – |
| FRAP | 0.395 | 0.190 | – |
| TPC | 0.133 | 0.995* | 0.255 |
| TFC | 0.358 | 0.221 | 0.217 |

*Correlation is significant at $P < 0.05$

The strong correlation between TPC and antioxidant capacity of ABTS assay shown that phenolic content of GMR and SLF capsule was responsible for its antioxidant activity. The present study revealed that strong correlation between total phenolic and ABTS assay was in agreement with previous studies [42-44]. The ABTS assay is based on the generation of a blue/green $ABTS^{\cdot+}$, which is applicable to both hydrophilic and lipophilic antioxidant systems. The previous investigation by Floegel (2011) stated that the high-pigmented and hydrophilic antioxidants were better reflected by ABTS assay [42]. There was no correlation between antioxidant activity as determined by DPPH, ABTS, and FRAP assays ($R^2= 0.182$ to 0.395 , $P>0.05$). This might be due to the potential of an antioxidant against free radicals of DPPH and ABTS and inevitably does not equal with its ability to reduce ferric to ferrous [45].

Therefore, based on the finding of this study, capsule dosage form containing mixed extracts of GMR and SLF possesses *in vitro* antioxidant potential. Capsule dosage form of mixed GMR and SLF extracts would be an interesting subject to be further investigated for its *in vivo* antioxidant activity study in animal models. Further experiments needed to obtain a standardized natural-based supplement for combating harmful effects of oxidative stress in human body.

ACKNOWLEDGEMENT

This research is supported by grant funds under PDUPT program (DIPA 042.01.2.400899/2019) by the Directorate of Research and Public Service, Ministry of Research, Technology, and Higher Education, Republic of Indonesia. The authors also gratefully acknowledge support from the Production Laboratory of PJ Gatutkaca (Hj Widatik and Dr. Anton B.H.) and all other parties involved in this study.

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