Antiobesity Properties of Star Fruits Extract and its Combination with Toddalia Aculeata Leaves Extract

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Abstract. The existing pharmacological therapy of obesity often causes side effects. Therefore, this study aimed to explore active compounds derived from star fruits and Toddallia acuelata leaves extracts for development of obesity therapy. From phytochemical screening test, it was found that star fruits extract (SFE) contained alkaloids, phenolics, and flavonoids while T. acuelata leaves extract (TLE) contained alkaloids, phenolics, saponins, and steroids. Based on the in-silico study confirmed with a chemical analysis using the Liquid Chromatography-Mass Spectrometry (LC-MS) technique, SFE contained Epicathecine Gallate (tannin derivative). Furthermore, the highest antioxidant activity was detected in pure TLE (58.5%) followed by combination of 25: 75% SFE and TLE (46.81%). In conclusion, TLE and combination of 25:75% star fruits and T. acuelata extracts become a potential obesity therapy. Further research is required for the investigation of pharmacological properties of those extracts.

Keywords: Star fruits; T. Aculeata; Obesity

1 Introduction

Covid-19 pandemic causes obesity prevalence increases because most people stay at home, increase daily food intake, and decrease physical activity [1]. Obesity is a major risk factor for chronic diseases such as Type 2 Diabetes Mellitus and cardiovascular disease which is one of the causes of premature death worldwide [2]. Pharmacological therapy is often given to obese patients because non-pharmacological therapy in the form of a low-calorie diet and increased physical activity in the long term often fail [3]. However, giving Orlistat (lipase inhibitor) is less effective in reducing body weight and often causes side effects in the form of increased heart rate and blood pressure, as well as gastrointestinal symptoms [4]. Therefore, the use of phytochemicals become an alternative therapy for obesity [5].

Several previous studies have used herbal plants such as green tea leaves are alternatively used for obesity therapy but the result studies are debatable as in the study of Hardani et al. [6], so it is necessary to find alternatives to obesity therapy from other herbal plants. Star fruits (Averrhoa carambola) which is a member from the Oxalidaceae family and T. aculeata as a member from the Rutaceae family, are widely found in tropical countries such as Asia [7], [8]. The characteristics of star fruits can be seen in its shape which resembles a five-pointed star with a bar measuring 1 cm and containing five seeds in each fruit cell [9]. T. aculeata has glossy green leaves, tastes bitterry and minty, which also produces essential oil that warms and has a calming aroma [8]. Based on previous studies, star fruit and T. acuelata leaves were used to treat hypercholesterolemia and various diseases respectively [8], [9].

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Both of these plants have not been widely used for their antiobesity potential even though it is known that they contain various phytochemicals that have the potential as antiobesity [7], [8]. Star fruits contain Epicatechin gallate (flavonoids) and T. acuelata leaves contain dictamine (alkaloid quinolone) that they have antihyperlipidemic and antioxidant activity [10], [11]. So far combination of both extracts has not been studied for its antioxidant activity. Therefore, the objectives of this study were to identify phytochemical content and antioxidant activity of star fruits and T. acuelata leaves extracts and their combinations.

2 Method

2.1 Materials

All reagents used in this research study were purchased from Merck KgaA (Darmstadt, Germany) otherwise further stated. The standard compound, Epicatechin Gallate (ECG) was obtained from Sigma-Aldrich (Burlington, USA). Fresh star fruits and T. acuelata leaves were provided by farmers who came from Tempuran Village, Demak and Tawangmangu respectively. The reagents consist of methanol solution, ethanol solution, aqua sterile, Whatmaan filter paper, H2SO4 solution, Dragendorff reagent, Mg powder.36% HCl solution, glacial acetic acid solution, 1% FeCl3 solution, formic acid solution, acetonitrile solution, and DPPH.

2.2 Methods

Fruits and Leaves Extraction

Picked star fruits and T. acuelata leaves immediately were washed with tap water and then star fruits were cut in to the small pieces with 0.5 cm thickness. Furthermore, chopped star fruits and T. acuelata leaves were dried 2 days during the day and followed by drying in the oven at 1050C for 24 hours. Once both materials have completely dried, star fruits and T. acuelata leaves were separately grounded and sieved with the 80-mesh filter.

For extraction of star fruits, we modified the maceration method developed by Aladaileh et al. [6]. In brief, 100 g star fruits powder was extracted using 600 ml 95% (volume/volume) methanol solvent for 2 days at room temperature. After that, star fruits suspension was filtered using Whatman paper to obtain the filtrate. The residue was then redissolved using 95% (v/v) methanol (1: 3) for 2 days in the same temperature and the suspension was filtered as same as the previous step. Finally, the first and second filtrates were mixed together. T. acuelata leaves were macerated using a modification of Rasamison et al., method [12]. A total of 100 g T. acuelata powder was dissolved in 500ml 70% (v/v) ethanol 1: 5 for 2 days at the same temperature and then was filtered with the same paper.

The filtrate was collected and the remaining residue was redissolved using 70% (v/v) ethanol (1: 3) for 2 days in the same temperature and the suspension was filtered as same as the previous step. All collected filtrates were mixed together. The last step was to concentrate both filtrates using a rotary vacuum evaporator merk IKA at temperature 600C until thick. To ensure both extracts became paste, the extracts were shortly dried in the oven [6], [12].

Phytochemical screening

To evaluate phytochemical compounds in star fruits and T. acuelata leaves extracts, we used a screening Harborne ‘s method [13]. The Dragendorff reagent was used to test alkaloids content in both extracts while flavonoids compounds in the both extracts were tested using Mg

powder and 36% HCl solution. For detection of steroids and triterpenoids, we used Lieberman-Burchard reagent containing acetic acid and sulphate acid to generate green or blue colour for positive steroids and red or purple for positive triterpenoids. Chloric acid and 1% FeCl3 solutions were used to identify saponins and phenolic acid respectively in the star fruits and T. acuelata leaves extracts.

Identification ECG using LC-MS

The chemical analysis in this study was performed using the Waters HPLC-MS (Acquity HPLC-SQD Mass Lynx 4.1 SCN805 USA) system associated with the Electrospray Ionization (ESI) source. We adopted the chemical analysis protocol from the previous study [14]. The compounds separation was carried out at a 156 × 4.6 mm i.d.C18 DV10-2634 reverse phase column with 3 μm particle size and 130 Å pore size. The mobile phase consisted of 0.5% (v/v) formic acid and acetonitrile. Twenty μL of 100 ppm ECG standard and extract samples were injected into the column with flow rate 0.6 mL/min. The use of gradient eluent concentrations adopted from Zaiter study [14]. To determine ECG concentration in the SFE, we compared peak area and retention time between the ECG standard and the SFE samples.

Antioxidant activity

Antioxidant activity testing was carried out using the free radical 1,1-diphenyl-2-picrylhydrazil (DPPH) scavenging method. Individual extract of star fruits and T. acuelata leaves and combination of both extracts were dissolved with ethanol to reach 25 ppm final concentration. Diluted samples were mixed with 0.1mM DPPH solution and incubated in dark room for 30 minutes. Color changes of samples were spectrophotometrically measured at 517 nm [15]. The percentage of antioxidant activity was calculated using the Molyneux's formula [15], [16].

3 Result and Discussion

3.1 Efficiency of Star Fruits and T. acuelata Leaves Extraction

We have already extracted dried star fruits and T. acuelata leaves using the maceration method with methanol and ethanol solutions respectively. As can be seen from Table 1, the efficiency and the final product of maceration extractions of star fruits and T. acuelata leaves were different. The SFE had higher rendement (61.09%) than the TLE with 34.08% rendement. From morphology, the extract of T. acuelata leaves was more solid compared to the extract of star fruits (gel versus paste). The higher rendement of SFE was due to the higher water level derived from ripened star fruits, which were yellowish-orange color. The result of this study was in line with the Imaduddin and Susanto study that the total water content in ripened star fruits was 18,21% higher than half-ripened star fruit (16,51%) and unripened (13,89%) [17].

In general, ripened fruits have high water level because of breakdown process of insoluble prospection into soluble pectin which is then degraded to polygalacturonic acid [18]. In addition, water content in the star fruits would condense in the night after directly exposure to the sun in the day of drying and would resume water content in the star fruits [19]. In comparison with Maravirnadita study, the final result of SFE in our study is higher 15.61% (61.09% vs 15.61%) but she does not mention the SFE morphology whether is solid or gel.

Moreover, they dried ripened star fruits under the sun exposure, which was covered by black cloth [20]. While the yield of TLE in our study was higher than the yield of TLE in the previous study (26.10%) using 99.9% (v/v) methanol [21]. The different result of TLE is probably caused by the length of maceration process. We soaked simplicial of T. accuelata leaves 48 hours and were then filtered it. After that, we re-dissolved the residue with the same solution and time. By this process, plant cell walls and membranes would completely breakdown so that its phytochemicals in the cytoplasm were released. In addition, the ethanol solvent used in our study has a higher polarity than the methanol solvent used in the previous study so that it can attract more phytochemicals which causes the TLE yield obtained in our study to be higher. [22].

Table 1. Rendement of Star Fruits and T. acuelata Leaves Extracted Using the Maceration Method

Sample	Simplicia (g)	Extract (g)	Yield (%)	Consistency
Star fruits	920	562	61.09	Gel*
T. acuelata leaves	1,033	352	34.08	Paste

3.2 Phytochemical screening

Each plant has primary and secondary metabolites for which its growth and development. However, different parts of plants such as flower, fruit, and leaf have different compositions of both phytochemicals. Table 2 indicated that the SFE had less phytochemicals than the TLE. The earlier extract contained alkaloids, phenolics, and flavonoids whereas the later extract had two more phytochemicals (steroids and triterpenoids). However, both extracts had no saponins because they could not generate foam formation during 15 minutes with 1-3cm height. Our findings were slightly different from previous studies which SFE had saponins and steroids [23], whereas TLE had saponin [24].

This discrepancy is caused by using the different techniques, which have different sensitivity. Therefore, small number of phytochemicals can be undetectable [25]. Based on the previous study, steroids and saponins are also recognized to have antioxidant activity [23], [26].

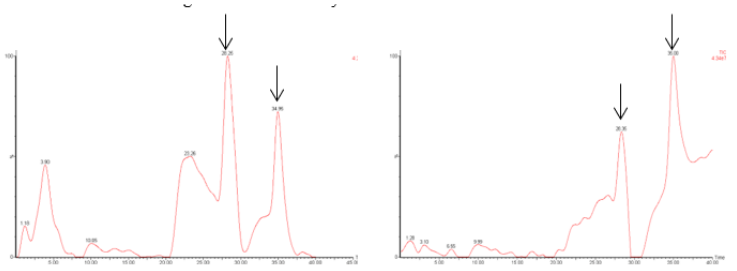
Table 2. Phytochemical's content of star fruits and T. acuelata leaves extracts

Chemical compound	SFE	TLE
Alkaloids	+	+
Phenolic	+	+
Flavonoids	+	+
Saponins	-	-
Steroids	-	+
Triterpenoids	-	+

Note: (+) contained phytochemical compounds while (-) did not contain phytochemical compounds

3.3 Epicatechin Gallate Compound in StarFruits Extract

From the researcher knowledge, we have firstly documented the ECG compound in SFE using the LC-MS. Figure 1 showed that the ECG compound in SFE was detected in two peaks with 28.35- and 35-min retention times respectively. Unfortunately, we do not get the ECG concentration using this chemical analysis.



(A) (B)

Fig 1. Chemical analysis of ECG in SFE using LC-MS. (A) indicated chromatogram of 100 ppm ECG standard injected into 18 columns whereas (B) indicated chromatogram of 100 ppm SFE sample. Black arrows designated peak and retention time of ECG compound.

3.4 Antioxidant activity

To determine antioxidant activity in the SFE, TLE, and various combinations of both extracts, we used the DPPH (2,2-diphenyl-1-picrylhydrazyl) method because of simple, fast, efficient, and cheap [27]. The DPPH purple color will change to become yellow color due to release of free electrons from DPPH molecules, binding to the hydrogen atoms from phytochemicals [15], [16]. Table 3 showed that TLE has the highest antioxidant activity (58.4%), followed by combination of 25% SFE: 75% TLE (46.81%) and 50% SFE: 50% TLE (31.37%). The percentage of antioxidant activity shows its ability as an antioxidant in inhibiting DPPH, if the percentage is large then its ability as an antioxidant is high [28] so it is better to resolve oxidative stress in obesity [5].

The result in our study was different from Annegowda et al. studies which showed that the percentage of antioxidant activity was higher in the methanol extract of star fruit (1:10) was 68.6% [29]. As well as the percentage of antioxidant activity of TLE in this study was lower than the Ceballos et al. study using methanol solvent, which was 58.5% at a concentration of 20 µg/ml [30]. All together with data in table 2, TLE alone which contains alkaloids, phenolics, flavonoids, steroids, and triterpenoids has stronger antioxidant activity than SFE alone or combination of TLE and SFE. Theoretically, flavonoids, alkaloids, and their derivatives are the main compound of antioxidant [31], [32] but we did not quantify these compounds in our extracts. The ECG compound belongs to flavonoids, which is able to inhibit cholesterol synthesis, are also good reducing compounds and to protect lipid membranes against from free radical ions [31], [33].

Furthermore, dictamine is a family member of alkaloids, which is found in TLE. This compound has antioxidant activity by providing hydrogen atoms to the free radical ions and increases cholesterol secretion [21], [32]. Therefore, TLE and combination of 25% SFE and 75% TLE potentially become a natural supplement for obesity treatment. Our limitation is no supporting data for quantification of ECG and dictamine and inhibition concentration of antioxidant activity.

Table 3. Antioxidant Activity of SFE, TLE, and Combination of SFE and TLE

Sample	% Antioxidant Activity
SFE	6.78%
TLE	58.4%
25% SFE : 75% TLE	46.81%
50% SFE : 50% TLE	31.37%
75% SFE : 25% TLE	22.56%

5 Conclusion

Alkaloids, flavonoids, and phenolics are found in methanol extract of star fruits and ethanol extract of T. acuelata leaves while steroids and triterpenoids are only found in TLE. In addition, the SFE contains the ECG compound of flavonoid derivative. TLE and the combination of 25% SFE:75% TLE have stronger antioxidant activity and become the potential candidate for obesity treatment. Other drying methods are requiring to make simplicial of ripened star fruits yielding more phytochemicals. Further research is required for concentration ECG dan dictamine, IC antioxidant activity, dan the investigation of pharmacological properties of those extracts so that it can know if it can inhibit oxidative stress.

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