Extraction Method and Crude Drug-to-Solvent Ratio Effects on the Antioxidant Properties and Physicochemical Profile during Storage of a Polyherbal Formulation Extract

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Abstract. Java tea, seed-under-leaf, and turmeric are well-known for their antioxidant activity. The mixture with 40% Java tea leaves, 55% seed-under-leaf aerial parts, and 5% turmeric rhizomes produced a polyherbal formulation with a satisfactory antioxidant profile. This study aimed to develop the optimal extraction condition to produce water extract with good antioxidant properties that are stable during storage. The herbal mixture was extracted using Decoction A, Decoction B, and infusion methods at crude drug-to-solvent ratios of 1:10, 1:20, and 1:100. The antioxidant activity was evaluated by the standard 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity and ferric reducing antioxidant power (FRAP) assays. The total phenolic content (TPC) and total flavonoid content (TFC) were determined according to the standard methods. The extract from Decoction A at a 1:10 ratio was stored for 18 days at 7±3°C, and their physicochemical properties, i.e., color, pH, DPPH scavenging activity, TPC, and TFC, were evaluated accordingly. The extraction method and crude drug-to-solvent ratio significantly affected the extracts' DPPH scavenging activity, FRAP, TPC, and TFC. Decoction A at a 1:10 ratio yielded extracts with the highest DPPH activity, TPC, and TFC and total flavonoid content, while Decoction B at a 1:20 ratio resulted in the highest FRAP. Extracts from Decoction A at a 1:10 ratio underwent chemical changes on day 6 of refrigerated storage. In conclusion, extraction by Decoction A at a crude drug-to-solvent ratio of 1:10 produced an antioxidant-rich extract that remained stable for six days during storage.

1 Introduction

Polyherbal formulations are used as traditional remedies, dietary supplements, and functional foods. They are composed of a mixture of multiple plant-based constituents. A novel polyherbal formulation developed from Java tea (*Orthosiphon aristatus* (Blume) Miq.), seed-under-leaf (*Phyllanthus niruri* L.), and turmeric (*Curcuma longa* L.) showed a promising antioxidant properties [1]. Extraction methods and the ratio of crude drug-to-solvent significantly affected the antioxidant properties of the extracts. The optimal conditions enable high bioactive component yield while maintaining their antioxidant activity [2].

In traditional herbal medicine, two standard methods, infusion and decoction, are used to prepare the oral herbal dosage forms. Infusion involved pouring boiling water over crude drugs, sitting them at room temperature, and collecting the filtrate as the dilute extract. On the other hand, a decoction is obtained by boiling crude drugs in water using a decoct apparatus for specific durations, i.e., 15 and 30 minutes, and collecting a more concentrated extract. Hence, the extraction temperature and time differ significantly between those two methods. Crude drug-to-solvent ratio also played essential roles in the efficiency of a given extraction process. A small ratio caused solvent saturation that limited mass transfer during extraction. The Indonesian Herbal Pharmacopeia (IHP) recommends a crude drug-to-solvent ratio of 1:10for the first maceration, while the traditional herbal drink preparation "*jamu godhog*" uses a ratio of around 1:20 [3].

Traditional herbal preparations are typically prepared in small quantities for immediate use due to their susceptibility to degradation mechanisms. Temperature, light exposure, oxygen, and microbial spoilage can compromise their stability. However, storing water extracts at low temperatures may extend their shelf life by slowing down degradation processes. Changes in the physicochemical parameters during storage must be monitored to ensure the extract's quality [4]. This study investigated the influence of traditional extraction methods, i.e., infusion and decoctions, and crude drug-to-solvent ratios, i.e., 1:10, 1:20, and 1:100, on the overall antioxidant properties of polyherbal formulation. The changes in pH, color, total phenolic content (TPC), total flavonoid content (TFC), and DPPH scavenging activity of the polyherbal extract with the best antioxidant profile during storage were also evaluated.

2 Methods

2.1 Materials

Crude drugs of Java tea leaf, seed-under-leaf aerial part, and turmeric rhizome were purchased from a science-based jamu development network, Wisata Kesehatan Jamu Kalibakung, Tegal, Central Java, Indonesia. Reagents (2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tri-2-pyridinyl-1,3,5-triazine (TPTZ), acetic acid, aluminum chloride, chloral hydrate, Folin Ciocalteau, hydrochloric acid, iron (III) chloride, and sodium acetate), reference compounds (Gallic acid, Quercetin, and Trolox), and solvents (ethanol and water), all from Sigma Aldrich brand and p.a. grade, were purchased from Millipore Sigma (US).

2.2 Preparation of polyherbal formulation

Powdered crude drugs of Java tea leaf, seed-under-leaf aerial part, and turmeric rhizome were homogenously mixed in a ratio of 45:50:5.

2.3 Extraction

The polyherbal formulation was extracted using traditional methods. For infusion, freshly boiled water (95±2°C) was added to the crude drugs and allowed to sit until lukewarm temperature. In the decoction method, the crude drugs were boiled in a water bath at 100°C for 15 minutes (Decoction A) and 30 minutes (Decoction B). Three different solvent-to-crude drug ratios (1-10, 1-20, and 1-100) were employed for each extraction method [5].

2.4 Determination of antioxidant properties

The appropriately diluted extracts were reacted with aluminum chloride solution, Follin Ciocalteau reagent, DPPH solution, and FRAP reagent to determine TFC, TPC, DPPH scavenging activity, and FRAP, respectively, according to a previous report [6]. A calibration curve of Quercetin ($y = 0.0055x + 0.1342$, $R^2 = 0.99$) was utilized to calculate the TFC of each formulation, which was reported as mg Quercetin equivalent (QE)/g crude drugs. For TPC determination, extracts were reacted with the Folin Ciocalteau reagent. Gallic acid was used as the reference compound with a calibration curve of $y=0.0401x + 0.0437$ ($R^2 = 0.99$). TPC was reported as mg Gallic acid equivalent (GAE)/g. The calibration curve of Trolox for DPPH scavenging activity was $y = 0.0654x + 9.1889$ ($R^2 = 0.99$), while that for FRAP was $y = 0.0654x + 9.1889$ ($R^2 = 0.99$). DPPH scavenging activity and FRAP were reported as mmol Trolox equivalent (TE)/g.

2.5 Extract storage condition

The polyherbal formulation extracts obtained from Decoction A in a ratio of 1:10 were kept in airtight bottles and stored at a temperature of 7±3°C for 18 days.

2.6 Physicochemical property evaluations

The physicochemical property evaluations were conducted on days 0,1, 3, 6, 12, 18, and 26. The extracts' TFC, TPC, and DPPH scavenging activity were determined using the same methods utilized to assess antioxidant content and activity. The extract pH was determined accordingly. Three untrained panelists organoleptically evaluated the extracts' aroma, taste, and color. The extracts were also subjected to a La*b* colorimetry reading (Konica Minolta, Japan). The color distance was calculated accordingly (Formula 1) [7].

$$\Delta E* = \sqrt{(L2 - L1)^2 + (a*2 - a*1)^2 + (b*2 - b*1)^2} \quad (1)$$

With ΔE* = color distance, L = lightness, a = redness/greenness, b* = yellowness/blueness, 2 = value of a given color parameter of stored extract, and 1 = value of a given color parameter of the fresh extract.

2.7 Data analysis

Effects of extraction methods and crude drug-to-solvent ratios toward TFC, TPC, DPPH scavenging activity, and FRAP, and their respective mean separation, were individually analyzed by two-way ANOVA and Duncan's test. The TFC, TPC, DPPH scavenging activity, pH, and color distance of each evaluation day were compared by one-way ANOVA and Duncan's test. The significant differences were assigned at $p < 0.05$.

3 Results and discussion

Both the extraction method and crude drug-to-solvent ratio significantly affected the TFC and TPC of the extracts. Decoction A produced the highest TFC, followed by Decoction B and Infusion. Similarly, the ratio at 1:10 generated the highest TFC, with 1:20 as the lowest one. The extract of Decoction A at a 1:10 ratio contained the highest flavonoid content (36.23±0.37 mg QE/g). Decoction generated extracts with higher phenolic compounds than infusion, while the ratios of 1:10 and 1:20 produced comparable high TPC (Fig. 1).

Extraction conditions, i.e., solvents, temperature, pressure, time, and plant material-to-solvent ratio, affected the efficiency of plant antioxidant compounds, including flavonoids and phenolic compounds [8]. Decoction and infusion principally differ by extraction time and temperature, in which infusion was conducted in a shorter time and at a lower temperature. This result was similar to the previous report, mentioning that the TFC and TPC of a polyherbal drink increased with an extraction time of up to 15 minutes and started to be static afterward [1]. However, the coffee bean extraction showed the opposite trend, in which extracts from infusion contained higher amounts of catechin, caffeic acid, and Quercetin than those from decoction [9].

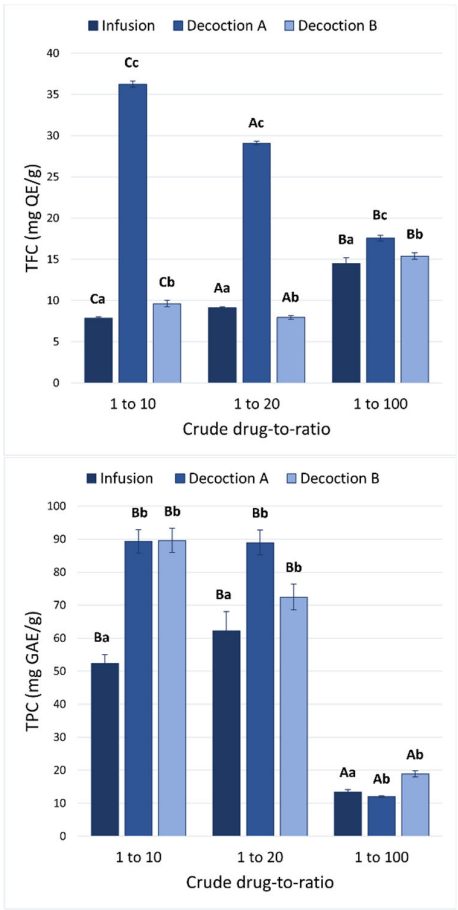

Fig. 1. Profile of TFC (Upper Panel) and TPC (Lower Panel) of Polyherbal Formulation Extracts. Different upper case and lower case on each bar's end represented the different values of TFC due to the effects of the crude drug-to-solvent and extraction method, respectively.

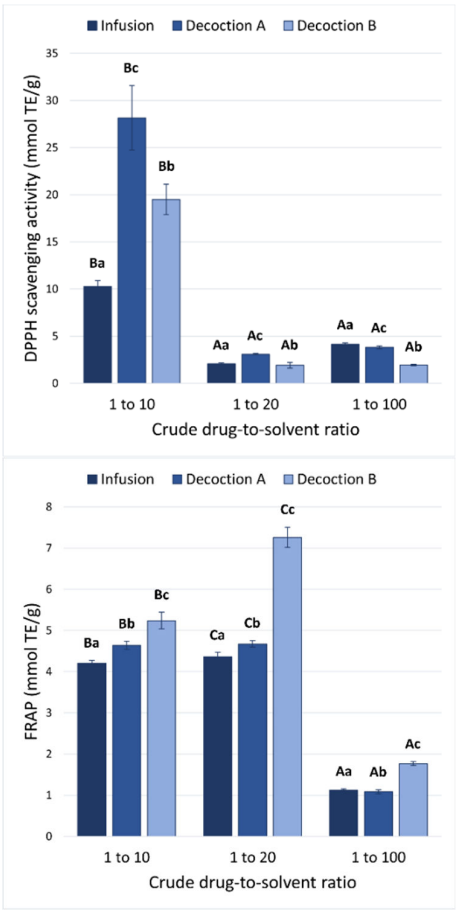

Fig. 2. Profile of DPPH scavenging Activity (Upper Panel) and FRAP (Lower Panel) of Polyherbal Formulation Extracts. Different upper case and lower case on each bar's end represented the different values of TFC due to the effects of the crude drug-to-solvent and extraction methods, respectively.

Regarding crude drug-to-solvent ratio, TPC and TFC of seed-under-leaf extracts showed the optimum ratio of 1:20 [10]. This result was similar to the maceration of dog-rose, hawthorn, and sea buckthorn, which generated the highest concentrations of phenolic compounds and flavonoids in a ratio of 1:10 [11].

All three components of the polyherbal formulation likely contributed to the extracted flavonoids and phenolic compounds. Seed-under-leaf herbs are rich with flavonoids, i.e., astragalin, kaempferols, nirurin, quercetin, and rutin. At the same time, eupatorin, hydroxy tetramethoxyflavone, hydroxy trimethoxyflavone, salvigenin, sinensetin, tetra methoxy hydroxy prenylflavone, tetramethylscutellarein, and trimethylkaempferol were isolated from Java tea leaves [12–14]. A limited number of flavonoids, mainly the glycosidic forms of isorhamnetin and Quercetin, were detected in turmeric rhizomes [15].

Phenolic compounds are also commonly identified as the bioactives of Java tea, seed-under-leaf, and turmeric. Ellagic acid, gallocathechin, hypophyllanthin, and phyllanthin were identified in seed-under-leaf aerial parts. In addition, caffeic acid and rosmarinic acid are found in Java tea leaves [16].

In contrast, bisdemethoxycurcumin, caffeic acid, casuarinin, coumaric acid, curcumin, demethoxycurcumin, and sinapic acid have been identified in turmeric rhizomes [12,15].

Similarly, the extraction method and crude drug-to-solvent ratio significantly modified the extracts' DPPH scavenging activity and FRAP. A ratio of 1:10 and Decoction A generated extracts with the most substantial radical scavenging potential. Among all conditions tested, extracts from Decoction A at a ratio of 1:10 showed the highest DPPH scavenging activity (28.14±3.41 mmol TE/g). On the other hand, the ratio of 1:20 generated the highest reducing potential, and the same phenomenon was observed in extracts from Decoction B. Hence, Decoction B, in a ratio of 1:20, produced extracts with the best FRAP (7.26±0.24 mmol TE/g) (Fig. 2). The results were similar to the optimum extraction time of green and jasmine tea, in which the longer extraction time resulted in a higher FRAP. The same trend was observed in coffee [9,17]. The DPPH scavenging activity of seed-under-leaf extracts was the highest at a crude drug-to-solvent ratio of 1:20, while those of dog-rose, hawthorn, and sea buckthorn were at 1:10 [10,11].

Phenolic compounds, including flavonoids, showed antioxidant activity through hydrogen atom transfer, single electron transfer, sequential proton loss electron transfer, and transition metal chelation [18]. DPPH scavenging activity assay evaluated the first three mechanisms [19]. It explained our result that extraction under Decoction A condition in a ratio of 1:10 produced extract with the highest TFC and TPC and the best DPP scavenging activity. On the other hand, single electron transfer and metal chelation are the antioxidant mechanisms detected by FRAP assay. Besides phenolic compounds, carotenoids, isothiocyanates, terpenes, and terpenoids showed antioxidant activity through these mechanisms [20]. Hence, the best FRAP shown in extracts from Decoction B at a ratio of 1:20 might have originated from other plant-based antioxidants.

The best overall antioxidant properties was Decoction A in a ratio of 1:10. At this extraction condition, TFC was significantly and strongly correlated positively to FRAP ($R = 0.999$, $p = 0.028$). On the other hand, an insignificant correlation was observed between TPC and DPPH scavenging activity and FRAP. It indicated that flavonoids in the polyherbal formulation were mainly responsible for FRAP but were not attributable to the radical scavenging activity. Such correlation has been previously reported in Indonesian Java tea and Bangladeshi turmeric, the two of three components of the studied polyherbal formulation [21,22].

During storage, the TFC and TPC of the extracts decreased on days 18 and 6, respectively. However, the DPPH scavenging activity of the extract rapidly decreased on day 3. The pH of the fresh extract was 7.14±0.09, which decreased on day 12. On day 3, TFC, TPC, DPPH scavenging activity, and pH were 97.95±1.56, 91.54±6.37, 89.72±3.01, and 92.90±2.25% of those of fresh one, respectively (Fig. 3).

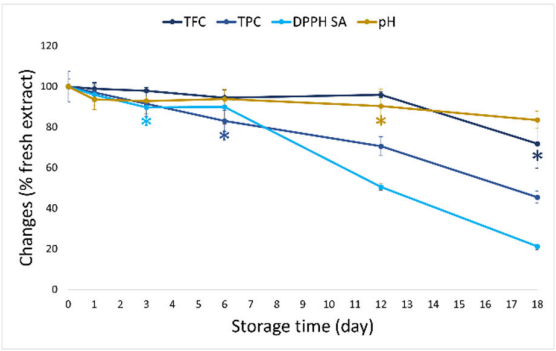

Fig. 3. The Physicochemical Profile of Extract during Storage. The asterisks represented the first day when a given parameter decreased statistically.

The decreased pH, TFC, and TPC of water extract during storage were caused by oxidation, hydrolysis, or enzymatic reactions. As reported in Malaysian ginger, the oxidation process produces oxidated compounds, which generally lose their antioxidant properties [23]. Free flavonoids in Caracara citrus fruit juice were stable during 16 weeks of storage at 4°C, while their glycosidic forms underwent a slight decrease [24]. It might explain our results that the TFC of the extracts remained stable until the final day of storage. Our results regarding TPC were similar to a previous report that a more rapid decrease in TPC (by 53%) during storage was observed in a nutraceutical supplement, with notable changes in the free phenolic fractions [4].

The fresh extract odor was aromatic turmeric with a grassy hint from Java tea, with decreasing aroma intensity from day six onward. The extract's pleasant, slightly bitter taste started to be more bitter on day 3. The fresh extract color was visually brown and remained the same until the final day of storage. However, La*b* colorimetry showed that extract's lightness was fair ($L = 33.14±0.80$), tend to be reddish ($a^* = 1.72±0.18$) and bluish ($b^* = -0.76±0.02$). During storage, the color rapidly changes into lighter, less red, and less yellow, resulting in significant color distance on day 1. However, this change in color distance was not visually noticeable (Table 1). The decreasing herbal scent intensity of the extracts might be related to the evaporation of the volatile compounds. The oxidation and hydrolysis processes are responsible for the changes in color during extract storage.

Table 1. The organoleptic profile of extracts during storage

Storage	Character or value			
	Aroma	Bitterness	Color	ΔE*
Day 0	***	*	Brown	0
Day 1	***	*	Brown	1.08±0.11
Day 3	***	**	Brown	1.54±0.12
Day 6	**	**	Brown	1.14±0.09
Day 12	**	***	Brown	0.57±0.04
Day 18	**	***	Brown	1.44±0.11

4 Conclusion

Extraction of polyherbal formulation with Decoction A at a crude drug-to-solvent ratio of 1:10 produced an antioxidant-rich extract with the antioxidant contents remaining unchanged for six days during storage.

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