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Crude Drug Standardization and Antioxidant Properties of an Indonesian Antidiabetic Polyherbal Formulation and Plant Constituents

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ABSTRACT

Jamu pahitan, traditionally used for diabetes treatment in Indonesia, is prepared as a mixture of *Andrographis paniculata* (Burm.f.) Wall. ex Nees, *Carica papaya* L., *Curcuma aeruginosa* Roxb., *Orthosiphon aristatus* (Blume) Miq., and *Tinospora crispa* (L.) Hook.f. & Thomson. This study evaluated the quality profile and antioxidant activity of *Jamu pahitan*'s crude drug components and assessed antioxidant properties and content of andrographolide and sinensetin of ethanolic extracts of the polyherbal formulation. The quality of the crude drugs was analyzed and standardized according to the official monographs. The antioxidant activity was evaluated by standard total phenolic content (TPC), diphenyl-1-picrylhydrazyl (DPPH) scavenging activity, and ferric-reducing antioxidant power (FRAP) assays. The andrographolide and sinensetin contents of *Jamu pahitan* were determined using the thin layer chromatography (TLC) densitometry method. The crude drugs met quality parameters set in their respective official monograph. *Orthosiphon aristatus* showed the strongest antioxidant activity and contained the highest TPC among other tested crude drugs. *Jamu pahitan*'s antioxidant activity and TPC were in the median range of those of plant components, with additive or antagonistic interaction effects toward antioxidant activity. *Jamu pahitan* 2 consisted of standardized crude drug components showing DPPH scavenging activity and FRAP of 14.97±0,47 and 2.94±0,05 mmol Trolox equivalent (TE)/g dry weight (DW), with andrographolide (0.96±0.02%), sinensetin (0.97±0.09%), and phenolic compounds (8.16±0.21 mg Gallic acid equivalent (GAE)/g DW) as the antioxidant compounds. It can be concluded that all crude drugs were of good quality, with andrographolide, sinensetin, and phenolic compounds are responsible for the antioxidant activity.

***Keywords*:** Andrographolide, Antioxidant, Crude drugs, Quality profile, Polyherbal formulations, Sinensetin, Traditional antidiabetic treatment.

Introduction

Diabetes mellitus (DM) is a serious health issue in Indonesia, where the prevalence of diabetes in adults in 2021 was 10.8% and is predicted to increase significantly in the coming years. It causes significant complications, i.e., neuropathy, nephropathy, coronary artery disease, and cerebrovascular disease, so the quality of life of individuals with DM is highly affected. In addition, it also profoundly impacts the healthcare system, as the total diabetes-related health expenditure in 2021 is around 6.3 million USD.[1] DM treatment in Indonesia mainly relies on conventional medical approaches using oral antidiabetic drugs or insulin and lifestyle changes.[2] However, the use of traditional medicines by people with DM was also commonly reported. About 22% of diabetic respondents used traditional medicines, while 6% utilized a combination of traditional and conventional ones.[3,4] Traditional herbal medicines for DM treatment are in single-plant preparation or polyherbal formulation forms.

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The most popular herbal materials in both categories are the king of bitter leaf, mangosteen pericarp, insulin leaf, God's crown pericarp, bitter melon fruit, soursop leaf, turmeric rhizome, betel leaf, moringa leaf, and bitter vine stem; which majorly characterized by bitter taste.[4] The polyherbal formulations combine two or more plant materials and are commonly named *Jamu pahitan*. Formulations from Sukoharjo, Central Java, consisted of *Andrographis paniculata* (Burm.f.) Wall. ex Nees (Acanthaceae), *Carica papaya* L. (Caricaceae), *Curcuma aeruginosa* Roxb. (Zingiberaceae), *Orthosiphon aristatus* (Blume) Miq. (Lamiaceae), and *Tinospora crispa* (L.) Hook.f. & Thomson (Menispermaceae). All of the formulation's plant components are used for the traditional management of DM in multiple places nationwide.[5] The extracts of this formulation were safe for the hepatic, pancreatic, and skeletal muscle cells and showed considerably strong insulin secretion and glucose uptake stimulatory activities.[6]

An effective polyherbal formulation should show the main activity addressing the major symptoms intended to be treated and the supporting ones, to help with the recovery and maintaining overall health.[7] Regarding DM treatment, this holistic approach uses antihyperglycemic, glucose uptake inhibitory, insulin secretion inhibitory, or glucose-metabolizing enzyme inhibitory activities as the main ones. The supporting activities of polyherbal formulation in this condition include analgesic, anti-inflammatory, antimicrobial, immunomodulatory, and antioxidant activities. In addition to antidiabetic supporting effects, antioxidants are essential in delaying angiopathy-related diabetic complications.[8] The antioxidant activities of the plant components of *Jamu pahitan* have been evaluated with *Orthosiphon aristatus* and *Carica papaya* showed promising results.[9–14] The antioxidant activity of those plants are likely attributable to andrographolide, sinensetin, and phenolic compounds.[11,15]

A synergistic interaction effect is expected from the utilization of polyherbal formulations. This effect manifests in a better therapeutic effect or lower toxicity of the polyherbal than that of individual components. In addition to the synergistic effect, antagonistic and additive ones toward antioxidant activity are observed in polyherbal formulations.[16–18]

The quality of crude drugs as herbal medicine raw materials widely varied depending on plant intrinsic genes, plant development, growing environment, harvesting and collection process, post-harvest processing, and storage conditions. Crude drug quality is attributable to identity, purity, and quality aspects. The crude drug purity aspect directly affects the safety of herbal medicine during use, while the content aspect is linked to the efficacy.[19] In addition, crude drug standardization is mandatory for raw materials intended for the production of Indonesian modern phytopharmaceutical, i.e., *obat herbal terstandar* (standardized herbal medicines) and *fitofarmaka* (phytomedicines).[20] Hence, it is essential to standardize the quality of crude drugs containing plant components of *Jamu pahitan* to ensure the formulation's safety and efficacy profile and compliance with official requirements. This study evaluated the quality profile and antioxidant activity of *Jamu pahitan*'s crude drug components. It also determined the interaction effects toward antioxidant activities and assessed the andrographolide and sinensetin contents in *Jamu pahitan* extracts.

Materials and Methods

Materials

Crude drugs of *Andrographis paniculata, Carica papaya, Orthosiphon aristatus,* and *Tinospora crispa* were purchased from Vejphong Pharmacy (13.74402 latitude, 100.50438 longitude; Bangkok, Thailand), while *Curcuma aeruginosa* was from Wisata Kesehatan Jamu (-7.10372 latitude, 109.13161 longitude; Tegal, Indonesia). Since commercial *Curcuma aeruginosa* crude drugs were not available in Thailand, they were bought from Tegal, Indonesia.

Reagents

The 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, Solvents (chloroform, dichloromethane, ethanol, ethyl acetate, formic acid, glacial acetic acid, methanol, n-hexane, and water), and standards (andrographolide, sinensetin, and gallic acid), were from Sigma-Aldrich (St. Louis, United States).

Crude drug standardization

All crude drugs were authenticated based on macroscopic and microscopic morphology and thin layer chromatography (TLC) profile comparison to their respective compendial standard. The microscopic morphology analysis was subjected to the powdered crude drugs using a camera (Leica, Wetzlar, Germany)-connected light microscope (Olympus, Tokyo, Japan). They were subjected to the physicochemical pharmacopeial characters, i.e., loss on drying, total ash, acid-insoluble ash, ethanol extractable, water extractable, and volatile content evaluations according to the compendial method.[21] The obtained values for *Andrographis paniculata, Curcuma aeruginosa, Orthosiphon aristatus,* and *Tinospora crispa* were compared to their respective standard in the Indonesian Herbal Pharmacopeia (IHP), while those of *Carica papaya* were standardized to Malaysian Herbal Monograph (MHM).[22] The mobile phase used for TLC profiling of *Andrographis paniculata, Carica papaya, Curcuma aeruginosa, Orthosiphon aristatus,* and *Tinospora crispa* were chloroform - ethanol (85:15); ethyl acetate - water - formic acid - acetic acid (50:13:5.5:5.5); dichloromethane - methanol (25:1); n-hexane: ethyl acetate (1:4); and n-hexane-ethyl acetate-glacial acetic acid (75:25:1); respectively. The Rf of obtained spots were compared to their standard ones, i.e., 0.45 for andrographolide of *Andrographis paniculata* and 0.50 for sinensetin of *Orthosiphon aristatus.* [21]

Jamu pahitan preparation

The powdered crude drugs of *Andrographis paniculata, Carica papaya, Curcuma aeruginosa, Orthosiphon aristatus,* and *Tinospora crispa* were mixed in a weight ratio of 3:2:2:3:3 and 1:1:1:1:1 to prepare *Jamu pahitan* 1 and *Jamu pahitan* 2, respectively. The mixtures were homogenized and kept in an airtight container until further analysis.

Extraction

The powdered individual plant component and *Jamu pahitan* crude drugs (100 mg) were separately extracted by maceration method for 24 h using ethanol (10 mL) as the solvent. The obtained extracts were freshly used for further analysis. Determination of TPC, DPPH scavenging activity, and FRAP followed a previously reported method with a sight modification.[23]

Total phenolic content (TPC) determination

The appropriately diluted extracts (0.1 mL) were mixed with water (7.9 mL) and Folin-Ciocalteau reagent (0.5 mL). The saturated sodium carbonate (1.5 mL) was added to each reaction mixture and stood at room temperature for 2 h. The absorbance of the mixtures was measured at the wavelength of 764 nm using a UV-visible spectrophotometer (Thermo Scientific, Waltham, United States). The obtained absorbances were plotted onto a Gallic acid linear curve equation (y = 5.994x + 0.0334), and TPC was reported as mg Gallic acid equivalent (GAE)/g dry weight (DW) crude drugs.

DPPH scavenging activity assay

For DPPH scavenging activity evaluation, appropriately diluted extracts (0.5 mL) were added to 0.025 mg/mL DPPH methanolic solution (5.0 mL). The mixtures were protected from light and kept at room temperature for 30 min, and their absorbance was determined at a wavelength of 517 nm. The obtained absorbances were compared to those of DPPH for their percent inhibition calculation, which was plotted onto a Trolox linear curve equation (y = 0.205x - 1.9395). DPPH scavenging activity was reported as mmol Trolox equivalent (TE)/g DW crude drugs.

Ferric reducing antioxidant power (FRAP) assay

Similarly, the appropriately diluted extracts (210 µL) were reacted with a freshly prepared FRAP reagent (mixture of 300 mM sodium acetate buffer (10 parts), 10 mM TPTZ in 40 mM HCl (1 part), and 20 mM ferric chloride (1 part); 3.99 mL). The reaction mixture was kept at room temperature for 30 min, and their absorbance was measured at 594 nm. The obtained absorbances were plotted onto a Trolox linear curve equation (y = 0.0025x + 0.0335), and FRAP was reported as mmol TE/g DW crude drugs.

Correlation and interaction effect analysis

The correlation between TPC, antioxidant activity, and interaction effects in the polyherbal formulation was calculated accordingly. The interaction effects calculation utilized a comparison method, as previously reported.[18] The predicted DPPH scavenging activity and FRAP of *Jamu pahitan* 1 and *Jamu pahitan* 2 were calculated using Equation 1-2.

$$Predicted\ JP\ 1 = \frac{((3\times AP)+(2\times CP)+(2\times CA)+(3\times OA)+(3\times TC))}{13} \quad (1)$$

$$Predicted\ JP\ 2 = \frac{(AP+CP+CA+OA+TC)}{5} \quad (2)$$

JP = *Jamu pahitan*, AP = *Andrographis paniculata*, CP = *Carica papaya*, CA = *Curcuma aeruginosa*, OA = *Orthosiphon aristatus*, and TC = *Tinospora crispa*

Andrographolide content determination

The ethanolic extract of *Jamu pahitan* 2 prepared in the same way for the antioxidant activity analysis was evaporated using a rotary evaporator (Ika, Staufen, Germany). The appropriately diluted extract solution in ethanol was spotted on a silica gel F254 plate. For andrographolide determination, the plate was eluted with chloroform-ethanol (85:15), and the andrographolide spot was further scanned under a Camag densitometer (Basel-Landschaft, Switzerland) at 225 nm. The concentration and area of the standard andrographolide were used to construct a curve equation. The andrographolide spot area was plotted on the curve equation ($y = 20.663x + 8599.4$) to obtain the

sample concentration. The andrographolide content was reported in % dry weight extract and calculated using Equation 3.[24]

$$Content\ (\%) = \frac{C \times V \times df}{W} \times 100\% \quad (3)$$

C = sample concentration (µg/mL), V = volume of sample solution, df = dilution factor, and W = extract weight

Sinensetin content determination

For sinensetin determination, the plate was eluted with n-hexane-ethyl acetate (1:4), and the separated spot of interest was scanned under a TLC densitometer at 334 nm. The spot area was plotted on the curve equation (y = 4.770x + 1618.346) to obtain the sample concentration. The sinensetin content was reported in % dry weight extract and calculated using Equation 3.[25]

Statistical analysis

The crude drug effect toward TPC, DPPH scavenging activity, and FRAP, as well as their mean difference, were evaluated by one-way ANOVA and Duncan's test. The correlation between TPC and antioxidant activities was assessed using Pearson's correlation analysis. The predicted values of DPPH scavenging activity and FRAP of the *Jamu pahitan* were compared to their obtained counterparts to predict the interaction effect toward antioxidant activity by paired T-test. Effects, differences, and correlations were statistically significant when p-value ≤0.05. All analyses were conducted using standard procedures of SPSS ver. 26 (IBM, New York, United States).

Results and Discussions

The identity of plant materials used in this study was confirmed by comparing each crude drug's macroscopic and microscopic morphology and chromatographic pattern to those of standard ones in the IHP or MHM.[21,22] (20,21). As shown in Figure 1, the macroscopic and microscopic morphology appearances and TLC profile of *Andrographis paniculata, Carica papaya, Curcuma aeruginosa, Orthosiphon aristatus,* and *Tinospora crispa* were similar to their respective descriptions in the official monograph. Hence, their identity was confirmed.

All crude drugs were of good quality, with all parameter values within the standard ones in their respective monograph (Table 1).

Table 1: The purity and content quality aspects of the crude drugs (n=3)

Crude drug	Value (%)				
	Loss on drying	**Total ash**	**Acid-insoluble ash**	**Ethanol-extractable**	**Water-extractable**
Andrographis paniculata	5.96±0.11	7.10±0.06	1.43±0.01	17.62±0.22	20.79±0.59
Carica papaya	7.87±0.10	8.76±0.08	0.40±0.01	15.07±0.02	26.59±0.46
Curcuma aeruginosa	9.71±0.44	6.32±0.09	1.00±0.02	4.87±0.13	13.13±0.36
Orthosiphon aristatus	8.56±0.12	6.95±0.01	1.29±0.02	10.79±0.14	23.05±0.03
Tinospora crispa	5.83±0.05	6.63±0.01	0.48±0.01	6.42±0.06	13.60±0.16

As all crude drugs met requirements for loss on drying, total ash, and acid-insoluble ash, they satisfied the purity aspects of quality. They were expected to be safe for preparation use. Similarly, the within-standard range values of ethanol-extractable and water-extractable were expected to guarantee their efficacy during preparation use.[26] A previous study reported that diterpene lactone-standardized *Andrographis paniculata* extracts exerted a better pharmacological activity profile.[11] An Indian study reported that the physicochemically standardized *Tinospora crispa* crude drugs showed good antioxidant activity.[12] Similarly, the morphologically-characterized *Orthosiphon aristatus* demonstrated good antioxidant properties.[9]

The crude drugs significantly affected the TPC of the extracts (p=0.000). *Orthosiphon aristatus* contained the highest phenolic compounds among other crude drugs and was subsequently followed by *Carica papaya*, *Tinospora crispa*, and *Jamu pahitan* (Figure 2). This was consistent with previous studies from Indonesia and Malaysia reporting the high TPC in the plant.[15,27] The phenolic compounds and flavonoids with free hydroxyl groups in the plant include 2-hydroxyphenylalanine, 5,7-dihydroxychromone, caffeic acid, caftaric acid, eupatorin, naringenin, norfenefrine, protocatechualdehyde, rosmarinic acid, and salvianolic acid A.[28,29] *Carica papaya* leaves showed a considerably high TPC as well. Previous reports mentioned that the leaves of some cultivars processed by specific drying methods were rich in phenolic compounds.[30] Ferulic acid, isorhamnetin, kaempferol, quercetin, salicylic acid, sinapic acid, syringic acid, and vanillin, among other phenolic compounds and hydroxyl-free flavonoids, have been identified from the plant.[31,32]

The low TPC in *Tinospora crispa, Curcuma aeruginosa,* and *Andrographis paniculata* indicated that phenolic compounds were not their main compounds. The most abundance phytochemicals of *Tinospora crispa* stems are clerodane-type furanoditerpenoids, i.e., free crispenes and glycosidic borapetosides.[33] The main compounds of *Curcuma aeruginosa* rhizomes were terpenoids, particularly the volatile monoterpenes and sesquiterpenes, while those of *Andrographis paniculata* aerial parts were andrographolide and the related diterpene lactones.[34,35] The higher TPC of *Orthosiphon aristatus* than that of *Andrographis paniculata* is consistent with a Malaysian report, while the superiority of *Andrographis paniculata* over *Curcuma aeruginosa* was also previously reported from Bogor-originated plants.[36,37] Also, the higher TPC in *Carica papaya* than that of *Andrographis paniculata* was consistent with a previous report.[38]

The crude drug weight ratio did not affect *Jamu pahitan*'s TPC, as both formulations showed comparable values. The TPC of the formulation was in the middle range of those of the components. Our result was different from a Malaysian study that demonstrated that the equal ratio of *Strobilanthes crispus, Phyllanthus niruri, Orthosiphon aristatus,* and *Stevia rebudiana* generated polyherbal formulation with the highest TPC, which was even higher than those of the individual component.[39] Hence, the different components and their respective ratio likely determined the TPC of a polyherbal formulation. Phenolic compounds might be the constituents responsible for the bioactivity of the formulation. Phenolic compounds have previously been reported to be responsible for the antioxidant activity of Thai polyherbal formulations.[40] Both the study's water and ethanolic extracts of *Jamu pahitan* evaluated showed a positive correlation between their TPC and insulin secretion and glucose uptake stimulatory activities.[6] A previous study reported that the TPC of the science-based antihyperglycemic polyherbal formulation from Wisata Kesehatan Jamu Kalibakung was significantly correlated to their low cytotoxicity on L6 skeletal muscle and HepG2 hepatic carcinoma cells.[23]

Similarly, different crude drug components and polyherbal formulations exerted different DPPH scavenging activity and FRAP (p=0.000). The order of both antioxidant activities was *Orthosiphon aristatus*, *Tinospora crispa*, *Carica papaya*, and *Jamu pahitan* (Figure 2).

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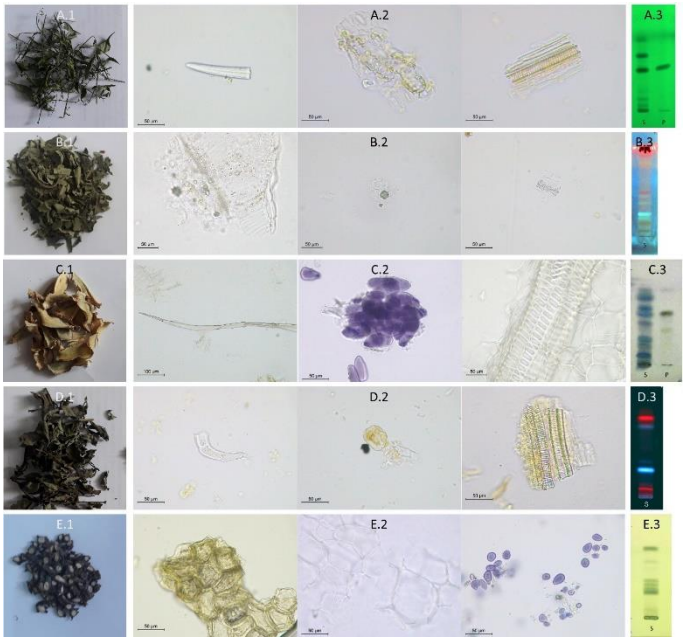

Figure 1: The identity aspects of quality of *Andrographis paniculata* (A), *Carica papaya* (B), *Curcuma aeruginosa* (C), *Orthosiphon aristatus* (D), and *Tinospora crispa* (E) crude drugs, showing macroscopic characters (1), selected microscopic characters (2), and TLC profile (3)

This is similar to previous reports on the high DPPH scavenging activity and FRAP of the plants grown in different places in Indonesia.[9,27] The antioxidant properties of *Orthosiphon aristatus* have been linked to their highly potent in-vivo anti-inflammatory activity.[41] Albeit strong, the TPC of the plant was not significantly correlated to its DPPH scavenging activity, while it did not correlate to FRAP (Table 2). This makes a difference in our study from a previous one.[9] The antioxidant activity of *Tinospora crispa* was linked with the flavonoid and phenolic contents. In addition, terpenoids and the related glycosides might contribute to the antioxidative properties.[33,42] Although statistically insignificant, TPC was weakly correlated to DPPH scavenging activity while showing a moderate correlation to FRAP (Table 2). This might indicate that compounds other than phenolic ones also contributed to the antioxidant activity of *Tinospora crispa*. *Carica papaya* leaf showed moderate antioxidant activity, in line with the previous study, which mentioned that ethanol extracted antioxidant compounds better than water in different varieties.[43]

The strong positive correlation between TPC and DPPH radical scavenging activity in this study was similar to that of Thai *Carica papaya* cultivars.[31] The weak antioxidant activities observed in *Curcuma aeruginosa* and *Andrographis paniculata* are likely related to their relatively low phenolic compound and flavonoid contents.[33,34] The much weaker antioxidant activity of *Andrographis paniculata* than that of *Orthosiphon aristatus,* as well as the better antioxidant effects of *Carica papaya* than that of *Andrographis paniculata,* have been reported previously in Malaysian reports.[36,38] The higher antioxidant activity of *Tinospora crispa* over *Andrographis paniculata* was similar to a previous report. However, our result was opposite to an earlier study reported that the antioxidant activity of *Andrographis paniculata* was higher than *Curcuma aeruginosa*.[18,37]

The crude drug weight ratio affected the formulation's DPPH scavenging activity but did not affect their FRAP, which was in the middle range of their components. *Jamu pahitan* 1 contained more *Orthosiphon aristatus* than *Jamu pahitan* 2, which might explain its higher radical scavenging activity. Nevertheless, both formulations showed comparable glucose uptake and insulin secretion stimulatory activities.[6] Both formulations showed a weak correlation between TPC and DPPH scavenging activity, while a significant, strong one was observed between TPC and FRAP (Table 2).

Table 2: Correlation between TPC and antioxidant activity of the crude drugs

Crude drugs	R-value between	
	TPC-DPPH	TPC-FRAP
Andrographis paniculata	-0.993	-0.950
Carica papaya	0.875	-0.076
Curcuma aeruginosa	0.897	0.094
Orthosiphon aristatus	0.867	0.072
Tinospora crispa	-0.390	0.687
Jamu pahitan	0.247	0.934*

* showed a significant correlation, evaluated by Pearson's correlation test, n = 3, p-value ≤0.05

The strong correlation between TPC and FRAP was also observed in a seven-constituent polyherbal formulation from Tegal, Indonesia.[23] The mixture of crude drugs *Andrographis paniculata, Carica papaya, Curcuma aeruginosa, Orthosiphon aristatus,* and *Tinospora crispa* in an equal ratio resulted in a formulation predicted to have antagonistic effects toward both DPPH scavenging activity and FRAP. In *Jamu pahitan* prepared from component ratio 3:2:2:3:3, it was predicted to have an additive effect toward radical scavenging activity and an antagonistic one toward FRAP (Table 3). The number and the ratio of each component factored the interaction effect toward the antioxidant activity of a polyherbal formulation. For example, an equal ratio of *Curcuma longa* and *Zingiber officinale* produces a formulation with a synergistic effect toward DPPH scavenging activity and an antagonistic one toward FRAP. In contrast, *Cymbopogon citratus* and *Curcuma longa* were antagonistic toward both antioxidant parameters.

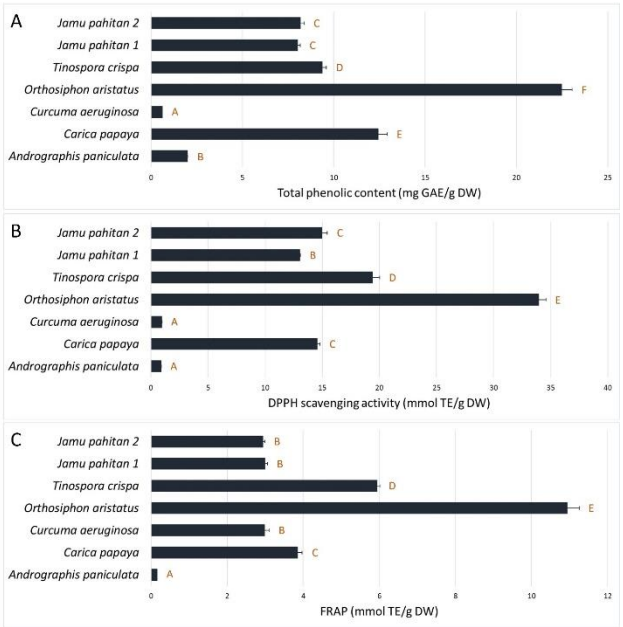

Figure 2: Profile of TPC (A), DPPH scavenging activity (B), and FRAP (C) of *Jamu pahitan* and the component crude drugs. Different alphabets on bars of each panel showed statistically different values for each parameter, evaluated by Duncan's test, n=3, p-value≤0.05

Table 3: Interaction effects of crude drugs toward antioxidant activity

Polyherbal formulation	DPPH scavenging activity (mmol TE/g DW)			FRAP (mmol TE/g DW)		
	Obtained	Predicted	Interaction prediction	Obtained	Predicted	Interaction prediction
Jamu pahitan 1	14.97±0.47	14.89±0.05	Additive	2.99±0.08	4.98±0.08*	Antagonistic
Jamu pahitan 2	13.02±0.06	13.94±0.04*	Antagonistic	2.94±0.05	4.77±0.06*	Antagonistic

* Indicated significantly higher values, evaluated by paired T-test, n = 3, p-value≤0.05.

The combination of *Cymbopogon citratus, Murraya koenigii, Curcuma longa,* and *Zingiber officinale* at a ratio of 1:1:1:5 generated a synergistic effect toward both DPPH scavenging activity and FRAP.[44] Andrographolide and sinensetin are likely responsible for *Jamu pahitan*'s antidiabetic activity. *Jamu pahitan* 2 ethanolic extract showed an andrographolide content of 0.96±0.02%. The antidiabetic activity of andrographolide was through upregulating peripheral tissue glucose uptake, improving insulin receptor signaling, protecting pancreatic β-cells from oxidative stress, and promoting insulin secretion mechanisms.[45] Sinensetin content in *Jamu pahitan* 2 ethanolic extract was 0.97±0.09%. Sinensetin's antidiabetic activity is mediated by α-glucosidase and α-amylase inhibition, glycation-induced protein oxidation suppression, and renal function improvements.[46] This explained our previous results, where an ethanolic extract of *Jamu pahitan* 2 stimulated insulin secretion and glucose uptake in the in-vitro model.[6]

Conclusion

The crude drug components of *Jamu pahitan* were of good quality, with *Orthosiphon aristatus* showing the highest antioxidant activity. The formulation exhibited moderate antioxidant capacity and TPC, with additive and antagonistic interaction effects observed. Jamu Pahitan 2 ethanol extract contained notable levels of andrographolide and sinensetin, contributing to its potential pharmacological benefits. Future research on *Jamu pahitan* should focus on optimizing its formulation to enhance synergistic antioxidant effects while minimizing antagonistic interactions among its plant components. Further studies on its bioactive compounds, particularly andrographolide and sinensetin, could explore their pharmacokinetic properties, bioavailability, and potential therapeutic applications. Additionally, clinical investigations are needed to validate its efficacy and safety, supporting its development as a standardized herbal medicine for antioxidant-related health benefits.

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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