

Profile of Crude Drugs Quality and Chemical Contents of a *Masuk angin* Polyherbal Formulation from Baturraden

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ABSTRACT

The Baturraden people utilize a polyherbal formulation composed of red ginger rhizomes, turmeric rhizomes, aromatic ginger rhizomes, and rice starches to alleviate *masuk angin*. This study aims to evaluate the quality profile of crude drugs of red ginger, turmeric, and aromatic ginger as well as to assess the effect of the weight ratio of the crude drugs toward the total curcuminoid and ethyl p-methoxycinnamate (EPMC) contents of the formulations containing those crude drug extracts. Crude drug quality characterization followed parameters and standards specified in the Indonesian Herbal Pharmacopoeia (IHP). The crude drugs were individually extracted using ethanol, and the resulting extracts were combined into seven formulations with varying weight ratios. Total curcuminoid and EPMC contents in the formulations were determined using the UV-Vis spectrophotometry and thin-layer chromatography (TLC)-densitometry methods, respectively. The turmeric crude drugs exhibited good quality profiles. The red ginger crude drugs met quality standards except for acid-insoluble ash and water-extractable parameters, while the aromatic ginger ones did not meet the essential oil content standard. Different weight ratios of red ginger, turmeric, and aromatic ginger extracts resulted in formulations with varying total curcuminoid and EPMC contents. Formula 4 that contains an equal weight ratio of red ginger, turmeric, and aromatic ginger crude drugs and exhibits a considerably high total curcuminoid ($8.08 \pm 1.31\%$) and EPMC ($4.43 \pm 0.33\%$) content among other formulations.

INTRODUCTION

Masuk angin is an imbalanced health condition associated with general malaise in Javanese cosmology. It is accompanied by bloating, body aches, chills, fatigue, flatulence, mild fever, and nausea. *Masuk angin* is not a formally recognized diagnosis from a conventional medical perspective. However, the symptoms are overlapped with gastrointestinal disturbances, mild upper respiratory tract infections, or stress-related somatic complaints. Hence, the efficacy of traditional treatments against *masuk angin* can be evaluated toward those underlying symptoms (Prayoga & Pradipto, 2014; Triratnawati, 2011).

According to the recent *jamu* bioinformatic studies, a traditional remedy for the treatment of specific ailments or disturbances should demonstrate the main activities addressing the ailments and supporting ones to improve the overall health condition (Afendi et al., 2016). Hence, a polyherbal formulation intended for *masuk angin* treatment should exert analgesic, anti-inflammatory, or antipyretic effects as the main activities and antimicrobial, antioxidant, and immunomodulatory effects as the supporting activities. People in Baturraden, Banyumas, Indonesia, use a polyherbal formulation consisting of red ginger (*Zingiber officinale* var. *rubrum* Theilade), turmeric (*Curcuma longa* L.) rhizomes, aromatic ginger (*Kaempferia galanga* L.) rhizomes, and rice

(*Oryza sativa* L.) starches to alleviate *masuk angin*. The local community boils the mixture in the water and takes the water extract orally as needed (Utaminigrum et al., 2021).

Regarding the main and supporting activities of *masuk angin* polyherbal formulation, red ginger rhizomes contribute to analgesic, anti-inflammatory, antimicrobial, and immunomodulatory activities (Zhang et al., 2022). On the other hand, turmeric rhizomes contribute to anti-inflammatory, antimicrobial, and antioxidant activities, while those of aromatic ginger were analgesic, antimicrobial, antioxidant, and anti-inflammatory (Ahmad et al., 2020; Kumar, 2020). The Indonesia Herbal Pharmacopeia IHP set essential oil as the marker compounds for red ginger, turmeric, and aromatic ginger crude drugs. In addition, curcumin and EPMC were the second marker compounds for the two latter crude drugs, respectively (Indonesian MoH, 2017). Regarding their activities related to *masuk angin*; curcumin and the related curcuminoids are well-studied antimicrobial, antioxidant, and anti-inflammatory agents (Oglah et al., 2020). As for EPMC, the anti-inflammatory and antimicrobial effects have been evaluated (Dwita et al., 2021).

Baturraden people use medicinal plants available in their neighborhood and the commercially available crude drugs to prepare polyherbal formulations they use for medicinal purposes (Nofrianti et al., 2021; Permatasari et al., 2011; Utaminigrum et al., 2020). The quality of crude drugs, whether produced commercially or in-house, may vary significantly depending on the plant's intrinsic nature, environment, processing, and storage conditions. As the raw materials, crude drugs of good quality are expected to result in herbal medicines with satisfactory safety and efficacy profiles (Al-Harrasi et al., 2022). This study was conducted to address the lack of information on the quality profiles of red ginger, turmeric, and aromatic ginger crude drugs used in local formulations, as well as the insufficient evaluation of marker compound profiles in *masuk angin* polyherbal formulations.

METHODS

Materials

Crude drugs (red ginger rhizomes, turmeric rhizomes, and aromatic ginger rhizomes) were purchased from the Center for Research and

Development of Medicinal Plants and Traditional Medicine (Balai Besar Penelitian dan Pengembangan Tanaman Obat dan Obat Tradisional, B2P2TOOT) Tawangmangu (Karanganyar, Indonesia). A thin layer chromatography stationary phase (silica gel 60 F₂₅₄ plate), reagents (chloralhydrate, hydrochloric acid, and iodine solution), and solvents (chloroform, ethanol, ethyl acetate, toluene, and water) were in pro analytical grade (Merck, New Jersey, United States). Reference compounds (curcumin and EPMC) were from MarkHerb (Bandung, Indonesia).

Crude Drug Standardization

The powdered crude drugs were individually subjected to the purity and content aspects of quality evaluation, with the parameters of loss on drying, total ash, acid-insoluble ash, ethanol extractable, water-extractable, and chemical content. The method for each parameter analysis followed the compendial Indonesian Herbal Pharmacopeia (IHP). The quality of the crude drugs was justified by comparing the obtained values to those of standard ones specified in the IHP (Indonesian MoH, 2017).

Extraction and Preparation of Polyherbal Formulation

The red ginger, turmeric, and aromatic ginger crude drugs were extracted separately using the maceration method described in the IHP (Indonesian MoH, 2017). 150 g of powdered crude drugs were extracted with 1500 ml of 70% ethanol for 24 h.

Table 1. Weight ratio of red ginger, turmeric, and aromatic ginger extracts

Formulation	Extract weight ratio (%)		
	Red ginger	Turmeric	Aromatic ginger
Formula 1	100	0	0
Formula 2	0	100	0
Formula 3	0	0	100
Formula 4	33	33	33
Formula 5	50	25	25
Formula 6	25	50	25
Formula 7	25	25	50

After filtration, the residue was re-macerated using 750 ml of 70% ethanol for another 24 h. The macerates were combined and evaporated with a rotary evaporator until a thick mass was obtained. The extraction yield was calculated accordingly. The extract formulations were

prepared by weighing and combining red ginger, turmeric, and aromatic ginger extracts in the specified ratio (Table 1).

Total Curcuminoid Content Determination

Curcuminoids were prepared into standard solution concentrations of 8, 7, 6, 5, 4, 3, and 2 µg/ml in ethanol. The absorbance of each standard solution was measured using a UV-Vis spectrophotometer (Shimadzu, Kyoto, Japan) at a maximum wavelength of 422 nm. Sample solutions were prepared by dissolving 10 mg of each extract formula in 10,0 ml of ethanol. The absorbance of the appropriately diluted sample solutions was recorded at 422 nm. The total curcuminoid content was reported in % and calculated using Equation 1 (Indonesian MoH, 2017).

$$\text{Total Curcuminoid Content (\%)} = \frac{C \times V \times df}{W} \times 100\% \quad (1)$$

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

EPMC Content Determination

EPMC was prepared into standard solution concentrations of 1200, 1000, 800, 500, and 200 µg/ml in ethanol. Sample solutions were prepared by dissolving 150 mg of each extract formula in 10,0 ml ethanol. 1 µl of each standard and appropriately diluted sample solution was spotted onto a silica gel F₂₅₄ plate, which was further separated over the mobile phase of toluene-ethyl acetate (95:5). The plate was scanned under TLC densitometer (Camag, Basel-Landschaft, Switzerland) at 305 nm. The linear EPMC curve equation ($y = 18.98x + 3107.3$, $r = 0.9726$) was constructed from the relationship between the concentration and area of standard EPMC spots. The sample spot area was plotted in the EPMC curve equation to obtain the sample concentration. The EPMC content was reported in % and calculated using Equation 2 (Indonesian MoH, 2017).

$$\text{EPMC Content (\%)} = \frac{C \times V \times df}{W} \times 100\% \quad (2)$$

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

Data Analysis

The effect of formulation on normally distributed total curcuminoid and EPMC contents was analysed by one-way analysis of

variance (ANOVA), while their mean separation analysis was conducted using Duncan's test. The significant effect and difference were assigned at $p \leq 0.05$. All statistical analyses were performed using IBM SPSS Statistics version 26 (IBM Corp., New York, United States) licensed to Mahidol University.

RESULT AND DISCUSSION

Crude Drug Standardization

This study evaluated the purity and content aspects of the crude drugs of red ginger, turmeric, and aromatic ginger (Table 2). Turmeric and aromatic ginger crude drugs met the standards for loss on drying, total ash, and acid-insoluble ash parameters, and hence were of good quality in this aspect. On the other hand, red ginger crude drugs' acid-insoluble ash was not within the IHP's specified value. Turmeric crude drugs also met all IHP standards for content aspects, while the red ginger ones were outside the standard value range for water extraction. The aromatic ginger crude drugs did not meet the volatile content requirement. Hence, only turmeric crude drugs satisfied the content quality aspect.

The loss on drying and ash content were directly linked to the contamination risks of microorganisms and inorganic matters, which highly correlated to their use of safety (Al-Harrasi et al., 2022). Crude drug loss on drying mainly depended on the drying condition and moisture absorption during storage. Our red ginger result was higher than that of Bogor, Indonesia, which depends on the temperature used during the drying process (Nasution et al., 2023). Loss on drying of turmeric rhizomes in this study was lower than those from Antioquia, Colombia and Bangkok, Thailand (Hartanti & Theeravit, 2018; Llano et al., 2022). Similar to that in red ginger, the loss of drying of aromatic ginger was higher than that from Bandar Lampung, Indonesia (Anggraini & Saputri, 2021). Nevertheless, all studies regarding loss on drying of those crude drugs reported loss on drying within the standard of IHP (Indonesian MoH, 2017).

The ash of crude drugs is mainly derived from the environment where the plant grows. The total ash of red ginger crude drugs in this study was lower than that of Bogor-originated ones (Nasution et al., 2023). Compared to our results, turmeric crude drugs from Bogor showed lower

total ash and acid-insoluble ash (Handayani et al., 2023). However, it is also affected by the drying method, as demonstrated by aromatic

ginger from Sukabumi, Indonesia (Indradi et al., 2022).

Table 2. The quality profile of the crude drugs

Parameters	Crude drugs					
	Red ginger		Turmeric		Aromatic ginger	
	Obtained (%)	Standard	Obtained (%)	Standard	Obtained (%)	Standard
Loss on drying	8.62±0.13	≧10%	8.87±0.28	≧10%	9.15±0.43	≧10%
Total ash	5.55±0.04	≧5.6%	7.18±0.02	≧8.2%	8.44±0.05	≧8.7%
Acid-insoluble ash	0.91±0.10*	≧0.6%	0.70±0.07	≧0.9%	0.97±0.06	≧2.5%
Water-extractable	16.21±0.58*	≧17.0%	18.98±6.54	≧11.5%	20.57±0.24	≧10.6%
Ethanol extractable	10.05±0.44	≧5.8%	12.00±0.24	≧11.4%	5.24±0.15	≧4.6%
Volatile content	1.23±0.12	≧1.10%	2.02±0.15	≧1.85%	1.14±0.15*	≧2.40%
Curcuminoid content	-	-	3.95±0.03	≧3.82%	-	-
EPMC content	-	-	-	-	5.08±0.40	≧1.80%

Notes: ≧ = not more than, ≧ = not less than, * = Did not meet the standard requirement

The solvent extractable and chemical contents were directly linked to the crude drug bioactive content, which positively correlated to their use efficacy (Al-Harrasi et al., 2022). The water-extractable represented the polar compound content in a given crude drug, while ethanol-extractable defined those of the semipolar one. Red ginger crude drug from Kubu Raya, Indonesia, contained dominant polar compounds (Cahyanto, 2021). Bangkok-originated turmeric crude drugs showed water and ethanol-extractable that slightly different from our recent result. However, more water-soluble compounds in the crude drugs are similar to our study and consistent with IHP requirements (Hartanti & Theeravit, 2018). Our aromatic ginger water-extractable results were higher than Sukabumi's, while the ethanol-extractable one was in the opposite trend. Nevertheless, both studies showed that aromatic ginger rhizomes are mainly of polar compounds, as specified in the IHP (Indonesian MoH, 2017; Indradi et al., 2022).

The volatile content of a given crude drug varied according to the intrinsic, processing, and distillation factors. Bogor-originated red ginger yielded higher volatile oils, than our sample (Batubara et al., 2023). On the other hand, ginger crude drugs from *Wisata Kesehatan Jamu* Kalibakung, Tegal, Indonesia, contained much lower volatile contents (Hartanti & Hamad, 2024; Puspitasari et al., 2024). According to botanical sources, the volatile content of Brazilian turmeric crude drugs was higher than our result (Guimarães et al., 2020). However, it is higher than that of Ponorogo, Indonesia (Aziz et al., 2019). Aromatic

ginger in this study contained much lower volatile content than Sukabumi-originated one (Indradi et al., 2022). Variations in the quantity and composition of volatile compounds lead to significant differences in the pharmacological activity of crude drugs. Higher levels of essential oils are often associated with more substantial antimicrobial, anti-inflammatory, and antioxidant effects, which are the supporting activities of the *masuk angin* herbal formulation.

Total Curcuminoid and EPMC Contents

All crude drugs met the yield extraction requirement of the IHP (Table 3) (Indonesian MoH, 2017). The weight ratio of red ginger, turmeric, and aromatic ginger crude drugs significantly affected the formulation's total curcuminoid and EPMC contents. Formula 1 and Formula 3 did not contain curcuminoids, and Formula 6 (11.46±0.02 %) and Formula 4 (8.08±1.31 %) contained the highest level of curcuminoids among those consisting of all three crude drugs (Figure 1). Hence, turmeric rhizome is the main contributor to the total curcuminoid content of the formulations. Formula 1 and Formula 2 did not contain EPMC, and Formula 7 (7.55±0.30 %) and Formula 4 (6.27±0.42 %) contained the highest level of EPMC among those consisting of all three crude drugs (Figure 1-2). Like curcuminoid in turmeric, EPMC was also proposed as the pharmacological marker of the formulation, with aromatic ginger as its primary contributor. The total curcuminoid and EPMC contents were obtained from the linear standard curve equation of $y = 0.1134x + 0.0273$ ($r = 0.9979$) and $y = 18.98x + 3107.3$ ($r = 0.9726$), respectively.

Table 3. Extraction yield

Crude drugs	Yield (%)	Standard
Red ginger	19.52	≤17.0%
Turmeric	26.62	≤11.0%
Aromatic ginger	21.14	≤8.3%

Notes: ≤ = not less than

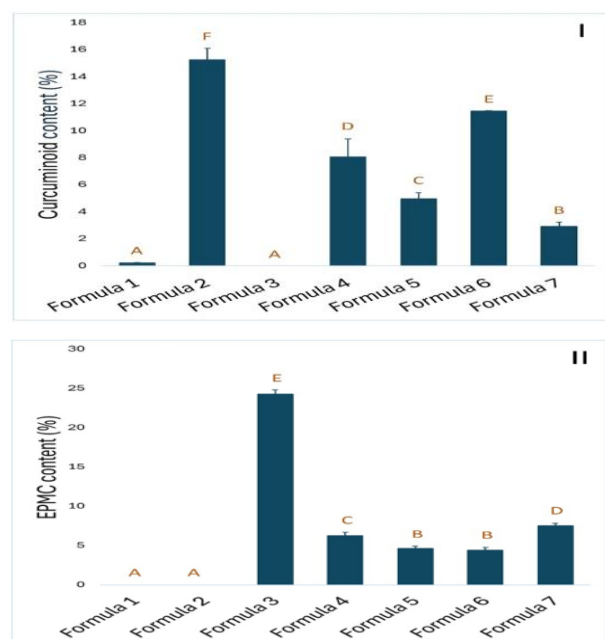


Figure 1. Profile of curcuminoid content (I) and EPMC content (II) of the polyherbal extract formulation; the different alphabets on each bar represented different values of the respective parameters, evaluated by one-way ANOVA and Duncan's test (n=3)

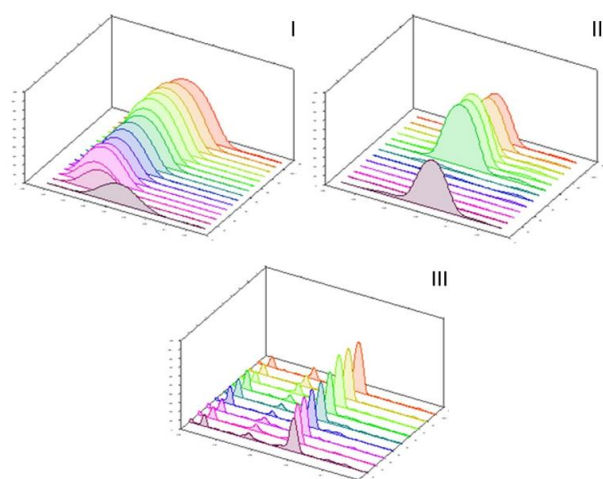


Figure 2. The densitogram of standard EPMC (I), Formula 1 – Formula 4 (II), and Formula 4 – Formula 7 (III)

The total curcuminoid content of turmeric and EPMC of aromatic ginger crude drugs depends on the plant's intrinsic and processing factors. Geographical conditions significantly affect the secondary metabolite content in a given plant. Aromatic ginger grown in various

locations in Eastern India exhibited different contents of EPMC and other related derivatives. Similarly, the curcuminoid content variation was also observed in turmeric across India (Kulyal et al., 2021; Singh et al., 2022).

Different drying methods for Colombian turmeric resulted in a curcuminoid content comparable to our results (Llano et al., 2022). The aromatic ginger from Sukabumi showed a lower EPMC content (Indradi et al., 2022). Curcuminoids and EPMC exhibit well-documented antioxidant, anti-inflammatory, and antimicrobial activities. The high levels of these compounds in polyherbal formulations are associated with enhanced pharmacological efficacy, particularly in managing pathologic conditions related to oxidative stress and inflammation.

Proposed as the formulation's pharmacological marker, curcuminoid has been proven to show a high selective sensitivity against *Streptococcus pyogenes*, methicillin-sensitive *Staphylococcus aureus*, *Acinetobacter lwoffii*, *Enterococcus faecalis*, and *Pseudomonas aeruginosa* (Adamczak et al., 2020). Curcuminoids' antioxidant and anti-inflammatory activity are closely linked and exhibit promising therapeutic effects in neurodegenerative pathologies, particularly Alzheimer's disease (Abdul-Rahman et al., 2024).

EPMC was proven to be the bioactive compound in Kheaw-Hom remedy, a Thai traditional medicine to treat fever and inflammation in children, and has been proven to be effective in the *in vitro* and *in vivo* inflammatory models (Sukkasem et al., 2024). From a standardisation perspective, curcuminoid and EPMC content are critical quality markers for turmeric and aromatic ginger crude drugs, respectively (Indonesian MoH, 2017). The consistent levels across botanical sources, postharvest processing, and production batches enhance the reliability and reproducibility of the formulation's effects. Hence, the development of a standardised product required high-quality components' crude drugs as raw materials.

Low or highly variable curcuminoid and EPMC levels suggest poor raw material quality, which may compromise both formulation efficacy and regulatory acceptance. It also leads to undermining consumer trust and

complicating the clinical validation process (Hassanzadeh et al., 2020).

Turmeric crude drugs contained the highest total curcuminoid level (15.26 ± 0.83 %), while that of EPMC in single aromatic ginger was 24.27 ± 0.54 %. Hence, curcuminoids were solely derived from turmeric, and EPMC were exclusively derived from aromatic ginger rhizomes. In the polyherbal formulation, both compound contents were significantly lower due to the smaller amount of the crude drug containing them; i.e., Formula 6 contained curcuminoids at 11.46 ± 0.02 % and an EPMC level of 7.55 ± 0.30 % was detected in Formula 7.

However, a polyherbal formulation is preferable to a single herbal. In a polyherbal formulation, the presence of multiple compounds enables the bioactive ones to interact dynamically with one another, providing some advantages colloquially identified as the intelligence of the mixture. It may be manifested as synergistic or polyvalent effects, which are hardly distinguishable. Nevertheless, those lead to a better overall efficacy or safety profile (Heinrich et al., 2023). In addition, polyherbal formulations warrant a better chance that all compounds needed to contribute to the primary and supporting activities are present (Afendi et al., 2016).

Such interactions toward antioxidant activity have been reported in science-based *jamu* development formulations for diabetes treatment (consisting of the king of bitter, Java tea, Indonesian bay leaf, Indonesian cinnamon, turmeric, Javanese turmeric, and seed-under-leaf), a novel formulation (mixture of the king of bitter, bitter vines, turmeric, Comosa turmeric, and seed-under-leaf), Sukoharjo-originated *jamu* pahitan (combination of the king of bitter, bitter vines, Java tea, papaya, and pink and blue ginger), and Malaysian TC-6 formulation (containing turmeric, Bentong ginger, black pepper, calamansi, and stingless bee honey) (Hartanti, Chatsumpun, Kitphati, et al., 2023; Hartanti, Chatsumpun, Sa-ngiamsuntorn, et al., 2023; Hartanti & Hamad, 2023; Yap et al., 2023).

This study contributes to filling the knowledge gap on the quality characteristics of red ginger, turmeric, and aromatic ginger crude drugs, which are components of a traditional polyherbal formulation for *masuk angin* treatment. By examining the volatile oil, curcuminoid, and EPMC profiles of the

formulation, the research provides information on the chemical markers present in a local *masuk angin* remedy. These findings lay the groundwork for better quality assurance and standardisation of polyherbal formulation. Future studies should explore a broader spectrum of active compounds and validate their pharmacological effects through in vitro and in vivo approaches, as well as, ultimately, clinical evaluation, to support the safe and effective use of these formulations.

CONCLUSIONS

The turmeric crude drug demonstrated satisfactory quality profiles, whereas red ginger and aromatic ginger did not meet the standards. The proportions of these crude drugs significantly influenced the total curcuminoid and EPMC contents of the formulations. The formulation with equal ratios showed the most favourable marker compound profile. This study is the first to link crude drug quality with marker content in *masuk angin* formulations, providing a foundation for standardisation. Further work should focus on validating biological activity, optimising formulation, and conducting clinical evaluations of the formulation with an equal ratio of components.

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AUTHORS' CONTRIBUTIONS

DH: Research funding acquisition, data curation, manuscript preparation, and manuscript review and editing. EMI: Experimentation and manuscript review and editing. RW: Research supervision and manuscript review and editing. AH: Research funding acquisition and manuscript review and editing.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

ETHICAL CONSIDERATION

The authors declare that ethical issues (including plagiarism, data fabrication, double

publication, etc.) have been thoroughly observed and strictly avoided during all study phases.

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