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Antioxidant activity of combined aqueous extracts of *Curcuma longa* Linn. rhizome and *Tamarindus indica* Linn. fruit pulp

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Abstract
Turmeric-tamarind herbal drink (Jamu kunyit asam) is a well-known traditional herbal drink in Indonesia. The two main ingredients of the drink are aqueous extracts of turmeric rhizome and tamarind fruit pulp. This study aimed to investigate the antioxidant activities effect of combined aqueous extracts of turmeric rhizome and tamarind fruit pulp because it has not been investigated. The aqueous extracts were mixed in various combinations. The mixtures' pH, total phenolics content, and antioxidant activity (DPPH) were measured. The mixture's pH was determined mainly by the tamarind extract dose. Nevertheless, the ratio of the extracts did not influence the total phenolic compounds. At the lower dose of tamarind extract, the antioxidative interaction of both sections was synergistic, but at the higher amount, it was additive. All mixtures' antioxidant potential (DPPH-IC50) was lower than BHT (The standard control). The antioxidative interaction is synergistic if the extracts ratio is unequal.

Keywords: Jamu, kunyit asam, herbal drink, herbal medicine

1. Introduction
Jamu kunyit asam (A turmeric-tamarind herbal drink) has long been used as a unique traditional herbal medicine in Indonesia, especially in Java. This herbal drink is traditionally used to treat or prevent various diseases. Its capacity as herbal medicine depends on its antioxidant potential. The antioxidant activity of the turmeric tamarind drink is determined by two main ingredients, turmeric, and tamarind aqueous extracts. Both ingredients have a reasonably strong antioxidant capacity. Therefore, it is necessary to know whether the mixture of the two ingredients provides added value when compared to each sole ingredient. The interaction of the mixed extracts can be antagonistic, additive, or synergistic [1].

Turmeric-tamarind herbal drink has many benefits. As a rich natural antioxidant herbal drink, it can reduce pain during menstruation [2] due to its analgesic power [3]. The drink is suitable for maintaining skin health, mainly to prevent skin wrinkles [1]. This herbal drink is estimated to reduce the possibility of Covid virus infection [4], intended as a health drink for people with diabetes when its sugar is not added or replaced with low-calorie sweeteners such as aspartame. It has an inhibitory activity on the α-glucosidase. As a drink for diabetes people, it has no adverse side effects [1]. The bioactive compounds from turmeric rhizome and tamarind fruit pulp largely determine the health benefits of turmeric tamarind. Turmeric rhizome contains many curcuminoids, while tamarind fruit pulp contains much furfural [5].

Curcuminoid compounds are potent antioxidants. Turmeric rhizome dry powder contains 3-5% curcumin and two of its derivative compounds in smaller amounts, namely demethoxycurcumin, and bisdemethoxycurcumin. Curcuminoids have antioxidant and anti-inflammatory properties, so they can be used to treat various diseases [4, 6]. The curcuminoids are antibacterial, anti-convulsant, analgesic, antidiarrheal, antiseptic, and antitumor [7].

Tamarindus indica fruit pulp contains a number of bioactive compounds, particularly furfural like 5-hydroxymethylfurfural (HMF). HMF is an organic compound that possesses several bioactivities including antioxidant, anti-allergic, antiproliferative, anti-sickling, antihypoxic and anti-hyperuricemic, antibioflm, and antimicrobial action. HMF as one of the main compounds in Tamarindus indica pulp extract with over 30% of the extract component. Another major compound in *Tamarindus indica* pulp extract is 3-O-Methyl-d-glucose (16.31%) that have been implicated in preservative activities as well as antitumor and anti-infammatory potentials. Moreover, ethanol and methanol extract of tamarind seed coat exhibits antioxidant activity [8]. It is rich with polyphenolics, especially proanthcyanidins [9].

The antioxidant activity of any herbal drink is essential for its efficacy. Its efficacy depends on the presence of its bioactive compounds since herbal formula contains a mixture of antioxidants, they can interact with each other, whether antagonistic, additive, or synergistic. Antioxidant compounds in the herbal formula do not always have added value in their interaction. Therefore, types of interactions between ingredients are essential. The total antioxidant activity of herbal formulations does not automatically express their additive effect. Their total antioxidant effect is determined by the kind and sum of antioxidants and the type of interaction of the antioxidants. The problem of the effect of antioxidants in mixtures is complicated to solve. Therefore, there is more information about the antioxidant activity of multi-component mixtures than of single components in the synergy concept [10]. Knowledge about the interaction properties of the mixture of the two main ingredients, turmeric, and tamarind, must be investigated whether they are antagonistic, additive, or synergistic. The results of this study were expected to contribute ideas about the best comparison of the two ingredients for the development of turmeric-tamarind herbal drink. Therefore, the purpose of this study was to determine the antioxidant activity of a mixture of turmeric rhizome infusion and tamarind water extract. Apart from that, the effect of adding tamarind on pH and total phenolic content will also be observed [10]. Based on the above-mentioned facts, the current study aimed to establish the antioxidant effects (additive, synergistic or antagonistic), determined as DPPH scavenging activity, in a mixture of turmeric with tamarind extracts.

2. Materials and methods

2.1 Research materials and extract preparation

Powders of turmeric rhizome and tamarind fruit pulp were bought in Jakarta. Three grams of each were added with 100 mL of distilled water and boiled at 90 ºC for 15 minutes. After cooling, the extracts were filtered and added water till 100 mL. Turmeric and tamarind extracts were combined into five ratios (Table 1). The extracts were then analyzed for their pH, total phenolic compounds, and antioxidant activities (DPPH).

2.2 UV-VIS scanning of the extracts

UV-VIS spectra of the extracts were scanned from 200 to 700 nm with Spectrophotometer (Biochrom, LIBRA S22).

2.3 Measurement of total phenolic compounds (TPC)

Standard graphs were prepared by dissolving gallic acid in methanol with various concentrations and dilutions. 10% Folin-Ciocalteu solution (wrapped in aluminum foil) was prepared by dissolving 10 mL or 10,000 µL of Folin-Ciocalteu with 100 mL of distilled water (10x dilution). Na_2CO_3 solution was prepared by dissolving 7.5 g with 100 mL of water. Gallic acid solution (wrapped in aluminum foil) was prepared by dissolving 0.05 g of the material with 100 mL of methanol. The three solutions (gallic acid solution, solvent, and 10% Folin-Ciocalteu reagent) were mixed and vortexed to be homogeneous. The mixture was left for 10 minutes at room temperature (25 °C). Then Na_2CO_3 was added and incubated at room temperature (25 °C) for 2 hours. The total phenolic content was calculated by comparing it with standard gallic acid using UV-VIS spectrophotometry at a wavelength of 765 nm [11, 12].

2.4 Measurement of antioxidant activity using the DPPH method

DPPH (1, 1-diphenyl-2-picryl-hydrazyl) solution (100 ppm, 10 mg/100 ml) was prepared and stored in a dark place. Each sample infusion to be tested was put into test tubes of eight concentrations starting from 0-500 µL, then added 1.5 mL of 100 ppm DPPH solution and 500 µL of distilled water in each test tube. The test tube was stirred with a vortex, then covered the test tube with aluminum foil and incubated at room temperature for 30 minutes. After that, transfer the mixture in the test tube into the cuvette and measure the absorbance of the sample using UV-VIS spectrophotometry at a wavelength of 517 nm, then compare it with BHT as a standard. The parameters examined were the antioxidant activity of the IC_{50} value obtained [2, 11, 12].

2.5 Evaluation of interaction: synergistic, additive, or antagonistic effects

Analysis of the effect of a mixture of antioxidants may be synergistic, additive, or antagonistic. The analysis was carried out based on the IC_{50} value of the DPPH test of turmeric rhizome infusion and tamarind aqueous extracts. Combination indexes (CI) were calculated using the following formula:

$CI = C_1/ (C_x)_1 + C_2/(C_x)_2$

Where: C_1 and C_2 are the concentrations of turmeric and tamarind extract, respectively, in the tested mixture, which produces the IC_{50} value. $(Cx)_1$ and $(Cx)_2$ are the unmixed concentrations of turmeric and tamarind extract, which produce the same effect (IC_{50}). CI values > 1, CI = 1, and CI <1 indicate the presence of antagonists, additives, or synergists, respectively [13].

2.6 Statistical analysis

Experimental data was presented as means ± SD. Statistical analysis was performed using one-way ANOVA followed by Fisher's LSD multiple comparisons test using GraphPad Prism version 8.2 (GraphPad Software Inc., San Diego, Ca, USA). $p< 0.05$ was considered significant.

3. Results and Discussion

UV-VIS scanning of the extracts revealed the presence of two peaks at 284 and 432 nm. The first peak denoted the presence of furfural of *T. indica* extract. The second peak denoted the presence of curcumin in *C. longa* extract (Figure 2).

The amount of tamarind extract determined the pH of the mixture. The higher the doses of tamarind extract, the lower the ph. In contrast, various ratios of turmeric and tamarind extracts did not influence the TPC of the mixtures. The results showed that the TPC of the mixtures was additive (Table 2).

The antioxidant activity of the mixture is influenced by the different ratios of turmeric and tamarind extracts (Table 3). The results showed that the quality of tumeric-tamarind herbal drink was accurately measured via its antioxidant activity. By analyzing the antioxidant evaluation results, it was found that the drink had intense antioxidant activity. Finally, it was determined that the CI value of an equal ratio of tumeric and tamarind aqueous extracts was in the range of 1, indicating that the interaction of the tumeric-tamarind pair was additive. But at lower concentrations or higher ratios, the antioxidant activities were stronger. The CI values were lower than 1, which means there was a synergistic interaction. This suggests the role of two different oxidative components in either tumeric or tamarind extract that can each experience synergistic interactions. The interaction between antioxidants

can be positive or negative, which can affect the activity of these antioxidants [10, 13].

Based on its antioxidant activity, the interaction between turmeric and tamarind is synergistic, depending on the dosage of turmeric and tamarind. The mixture has synergistic properties, although a CI value is close to 1.00, which is close to additive properties. The synergistic nature of the mixture will become more evident if the added tamarind is less (Table 3). The mechanism responsible for the synergistic antioxidant activity has not been elucidated due to the nature of complex mixtures, mainly plant extracts. Synergies can be explained as the presence of regeneration of more potent antioxidants by weaker antioxidants (with higher reducing potential), the formation of stable intermolecular complexes between antioxidants that exhibit higher antioxidant activity than their parent compounds, and the formation of new phenolic products with greater antioxidant power than the parent compound mixture. Differences in the solubility of antioxidants and the distribution of antioxidant phases are probably the cause [10, 13].

The synergistic effect of antioxidant mixtures depends on the type of antioxidant and its concentration, as in the case of the antioxidant lycopene, which only interacts synergistically with vitamin E at specific concentrations and ratios. Synergism does not depend linearly on lycopene concentration. The mixture containing the highest concentration did not show a synergistic effect. High or low concentrations of each antioxidant can contribute to eliminating this synergistic effect [10, 13].

Although research relevant to interactive effects among the bioactive compounds mounted up, the interaction mechanism is still unclear. The present study summarizes the progress on the synergistic and antagonistic effects of dietary herbal materials, the evaluating models for antioxidant interactions, and the possible interaction mechanisms in vitro and in vivo, emphasizing biological-related molecular mechanisms of phytochemicals. The interaction mechanism research is valuable for guiding how to take advantage of synergistic effects and avoid antagonistic effects in daily diets or phytochemical-based treatments for preventing chronic diseases [14].

This work aims to study the antioxidant interactions between turmeric and tamarind extracts through the measurement of free-radical-scavenging activity of DPPH. Further investigations based on the interaction index and isobologram analysis showed that the antioxidant activity (DPPH) of the combination of turmeric with tamarind presented an increase with the rising of their concentrations in their mixture. The best synergistic effect between turmeric and tamarind based on DPPH was observed at 64:36 and 36:64, respectively. The excellent synergic antioxidant activity of the combination of turmeric with tamarind in our study suggests the mixture has more broad and effective application prospects in the herbal formula [15].

Tamarindus indica is one of the antidiabetic herbal materials due to its ability to inhibit the activity of α-amylase and α-glucosidase [16]. It also has antioxidant and anticancer [9], antibacterial, and antifungal activities [8]. Aqueous extract of Tamarindus indica fruit pulp exhibited an intense α-amylase inhibitory activity and showed moderate α-glucosidase inhibitory activity. The extract showed glucose uptake. The glucose uptake potential proves its postprandial hypoglycemic effect. Hence, it may be considered an antidiabetic agent for the control of postprandial hyperglycemia [16], and antioxidant activities from *Tamarindus indica* pulp fruit extract [17, 18]. Polysaccharides of tamarind seed displayed a potential solubility improvement of lipophilic bioactive molecules, including curcumin. Curcumin, a bioactive pigment, has poor bioavailability because of its water insolubility. Tamarind extract has capacity to solubilize curcumin. The solubility of curcumin was improved 180-fold by the addition of extract. Our findings indicated that tamarind extract was a promising added-value agent in foods and pharmaceuticals for the oral intake of curcumin [19].

3.1 Tables and Figures

Table 1: Research design

Ratio	Tumeric extract (mL)	Tamarind extract (mL)
1.00:0.00	100	0
0.00:1.00	0	100
0.50:0.50	50	50
0.64:0.36	64	36
0.36:0.64	36	64

Table 2: Measurement results and total phenol concentration (TPC)

Ratio*	Mean pH ± SD	Mean TPC ± SD (mg GAE/ mL extract)
1.00:0.00	5.55 ± 0.35	10.07 ± 1.81
0.00:1.00	3.21 ± 0.08	11.57 ± 0.67
0.50:0.50	3.76 ± 0.06	11.53 ± 0.62
0.66:0.34	4.04 ± 0.07	11.22 ± 0.28
0.34:0.66	3.54 ± 0.06	11.36 ± 0.13

Note: *ratio between turmeric and tamarind aqueous extracts. Results with different letters are significantly different ($p < 0.05$)

Table 3: Results of the analysis of antioxidant activity using the DPPH method (IC50)

Ratio*	Repetition					Average ± SD *** (µl/mL)	CI	Interaction
1.00:0.00	40.51	40.24	40.01	40.01	40.14	40.18 ± 0.21		
0.00:1.00	40,46	40.64	40.56	40.38	40.87	40.58 ± 0.19		
0.50:0.50	40.02	40.07	40.07	40.01	40.07	40.05 ± 0.03	1.00	Additives
0.64:0.36	21.88	21.25	21.98	20.99	22.92	21.80 ± 0.75	0.54	Synergism
0.36:0.64	20.80	20.80	21.83	20.80	21.22	21.09 ± 0.45	0.52	Synergism
BHT						8.10**		

Note: *ratio between tumeric and tamarind extracts (see Table 1); **µg/mL; ***µl extract/mL. Results with different letters are significantly different ($P < 0.05$)

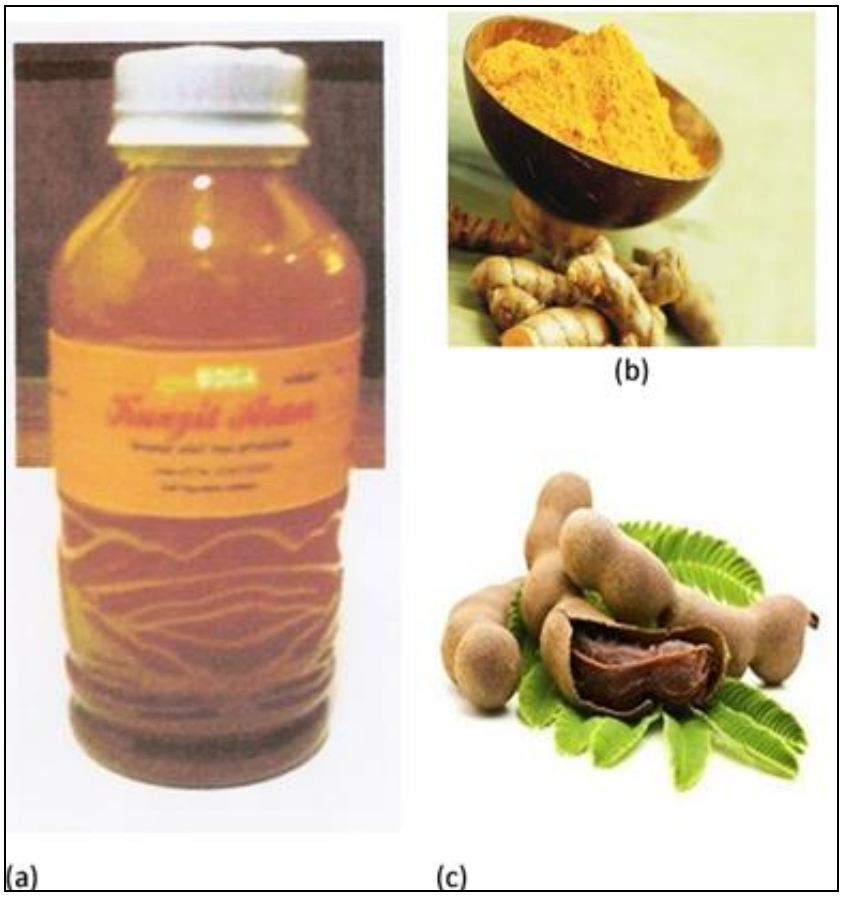

Fig 1: (a) and (b). Turmeric, (c) Tamarind fruit [10]

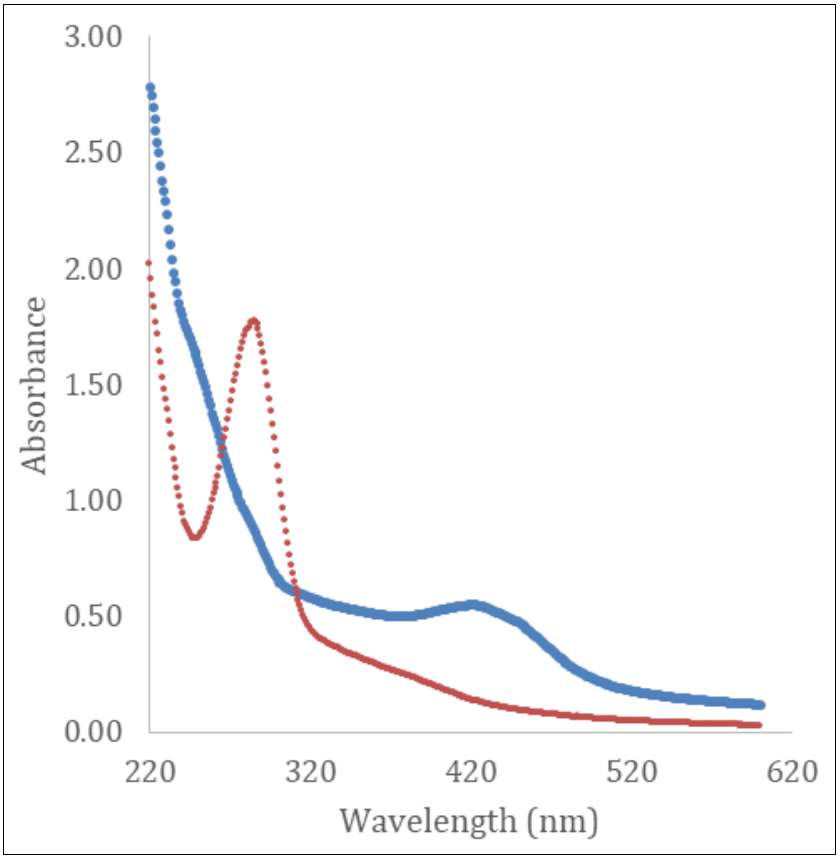

Fig 2: UV VIS spectra of turmeric (blue) and tamarind extract (red)

4. Conclusions

The best synergistic antioxidant between turmeric and tamarind extracts based on DPPH assays were observed at 0.64:0.34 and 0.34:0.64. Additionally, their antioxidant properties are the same as the tamarind extract. The ratio of turmeric and tamarind extracts in jamu kunyit asam (tumetic-tamarind formulation) determines the quality of the formulation. Turmeric infusion can increase the antioxidant

activity of the turmeric-tamarind herb, either additively or synergistically, depending on the concentration and ratio. The turmeric-tamarind herbal drink's quality and efficacy must be developed to become a better product and maintain its quality. Thus, this study provided a preferred way for monitoring the quality consistency of herbal medicine, exploring possible bioactive components of herbal medicine, and assessing the interaction between herbs.

5. Credit authorship contribution statement

Atika Minnie Fantasi: Conceptualization, Methodology, Investigation, Writing - original draft, Visualization. Susana Elya Sudradjat: Methodology, Validation, Investigation, Visualization. Kris Herawan Timotius: Methodology, Investigation, Resources, Conceptualization, Writing - review & editing, Formal analysis, Writing - review & editing, and Visualization.

6. Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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