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Antioxidant properties and interaction effects of a novel polyherbal formulation

Dwi Hartanti*, and Alwani Hamad

[1]Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Muhammadiyah Purwokerto, Jl. KH. Ahmad Dahlan, Kembaran, Banyumas 53182, Central Java, Indonesia

[2]Department of Chemical Engineering, Faculty of Engineering and Science, Universitas Muhammadiyah Purwokerto, Jl. KH. Ahmad Dahlan, Kembaran, Banyumas 53182, Central Java, Indonesia

*Corresponding author: dwihartanti@ump.ac.id

Abstract

A novel polyherbal formulation was developed from crude drugs of *Andrographis paniculata* (Burm.f.) Wall., *Tinospora crispa* (L.) Hook.f. & Thomson, *Curcuma longa* L., *Curcuma comosa* Roxb., and *Phyllanthus niruri* L. The powdered crude drugs were combined in a ratio of 5-5-3-3-3. The determination of total phenolic content (TPC), the 2,2-diphenyl,1-picrylhydrazyl (DPPH) scavenging activity, and ferric reducing antioxidant power (FRAP) of the polyherbal formulation and the individual constituents were conducted as per standard methods. The interaction effect of the constituents in the polyherbal formulation toward antioxidant activities was predicted by comparison and difference methods. *C. longa* and *P. niruri* contained the highest TPC. They showed the strongest antioxidant activities, while those of the polyherbal formulation were in the median range of the constituent's respective value. The combination of crude drugs generated interaction effects of additive and antagonist towards DPPH scavenging activity and FRAP, respectively.

Keywords: Antioxidant Activity, Crude Drugs, Interaction Effect, Phenolic Compounds, Polyherbal Formulation

Introduction

Oxidative stress is known to take part in the etiology and pathophysiology of numerous conditions, including diabetes mellitus (1). Antioxidant-rich diet has been proven to have favorable effects in preventing diabetes mellitus in Rotterdam study, in which a correlation was observed between consumption of total dietary antioxidant and a risk of type 2 diabetes (2). Natural antioxidants are also widely found in medicinal plants. *Curcuma longa* L., *Curcuma comosa* Roxb., and *Phyllanthus niruri* L. have been known for their antioxidant activity. Polyphenol compounds and flavonoids are the primary antioxidants of *C. longa* and *P. niruri*, while those of *C. comosa* are polypeptides and diarylheptanoids (3–5).

A combination of medicinal plants as a polyherbal formulation is commonly used in Indonesia. For example, an antidiabetic formulation with strong glucose uptake and insulin secretion stimulatory properties contained *Andrographis paniculata* (Burm.f.) Nees., *Tinospora crispa* (L.) Hook. f. & Thomson, *Carica papaya* L., *Curcuma aeruginosa* Roxb., and *Orthosiphon aristatus* (Blume) Miq. (6). Using a polyherbal formulation in a traditional medicine practice is favorable for its synergistic effects, in which a better overall activity or lower toxicity than the sum of its components is expected (7). However, the multiple ingredients present in the mixture of a polyherbal formulation might not constantly interact synergistically as the additive or antagonist effect may also occur. From this point of view, an optimal formulation should consist of components with synergistic interaction effects.

This study evaluated the total phenolic content (TPC) and antioxidant activities of a

novel polyherbal formulation consisting of *A. paniculata*, *T. crispa*, *C. longa*, *C. comosa*, and *P. niruri* and its constituents. The interaction effects toward antioxidant activities in this novel formulation were also evaluated.

Material and Methods

Chemicals and Reagents

The 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP) reagent, Folin-Ciocalteau reagent, Gallic acid, Trolox, acetic acid, $AlCl_3$, chloroform, deionized water, ethanol, n-hexane, sodium carbonate, and NaOH are all from Sigma (United States).

Plant Materials

Crude drugs of *A. paniculata*, *T. crispa*, *C. longa*, and *C. comosa* were purchased from Vejpong Pharmacy, Bangkok, Thailand, while that of *P. niruri* was bought from WKJ Kalibakung Science-based *Jamu* Development Clinic, Tegal, Indonesia.

Preparation of the Polyherbal Formulation

The powdered crude drugs of *A. paniculata*, *T. crispa*, *C. longa*, *C. comosa*, and *P. niruri* were homogenously mixed in a ratio of 5-5-3-3-3. The ratio used is obtained from a modified antidiabetic polyherbal formula from Hortus Medicus Science-based *Jamu* Development Clinic, Tawangmangu, Indonesia, in which *C. comosa* was used instead of *C. xanthorrhiza* (8).

Determination of TPC

The TPC in the crude drugs of the formulation and its constituents were determined by a modified Folin Ciocalteau method (9). Ethanolic extracts were individually prepared by ultrasonic-assisted extraction with ethanol in a ratio of 1:20 for 30 min. A total of 250 µl of appropriately diluted extracts or Gallic acid solutions (at 0-1200 µg/ml) was reacted with 500 µl of Folin-Ciocalteau reagent in 7.75 ml of water for 5 minutes and subsequently added with 1.5 ml of saturated sodium carbonate. Upon incubation at room temperature for two hours, the absorbance of the reaction was measured in a spectrophotometer (Thermo Scientific, USA) at 764 nm. A regression equation ($y = 2.635x + 0.0212$, $R^2 = 0.99$) was calculated from the concentrations and absorbances of Gallic acid solutions, and TPC was calculated accordingly. The TPC in the extracts was reported as mg gallic acid equivalents (GAE)/g dry weight (DW).

DPPH Radical Scavenging Assay

It was conducted according to a previous report (9). A total of 500 µl of appropriately diluted extracts or Trolox solutions (at 0-400 µM) was reacted with 5000 µl of 25 µl/ml DPPH solution in methanol. The reaction mixtures were kept in a tightly closed container in the dark condition for 30 min, and the absorbance was measured at 517 nm. A regression equation ($y = 0.205x - 1.9395$, $R^2 = 0.98$) was calculated from the concentration and % inhibition, obtained from the relative comparison of the absorbance of sample to that of blank, of Trolox solutions, and the DPPH radical scavenging assay was calculated from the equation accordingly. The DPPH radical scavenging assay of the extracts was reported as mM Trolox equivalent (TE)/g DW.

FRAP assay

The protocol followed a previously reported method (9). A total of 210 µl of appropriately diluted extracts or Trolox solutions (at 0-225 µM) was reacted with 3790 µl of FRAP reagents. The mixtures were kept at room temperature for 30 mins, and the absorbance was measured at 594 nm. The calibration curve of Trolox ($y = 0.0025x + 0.0335$, $R^2 = 0.99$) was prepared and used as one in the DPPH radical scavenging activity assay. The FRAP of the extracts was reported as mM TE/g DW.

Determination of interaction effect

The interaction effect of the mixture of crude drugs toward antioxidant activities was analyzed by comparison and difference methods following previous methods (10,11). In the comparison method, the obtained value was statistically compared to the predicted one calculated as follows:

$$Predicted = \frac{(5 \times AP) + (5 \times TC) + (3 \times CL) + (3 \times CC) + (3 \times PN)}{19}$$

The difference method utilized equation as follow:

$$Difference\ (\%) = \frac{PHF \times 100}{AP + TC + CL + CC + PN} - 100$$

PHF, AP, TC, CL, CC, and PN represented the DPPH radical scavenging activity and FRAP of polyherbal formula, *A. paniculata, T. crispa, C. longa, C. comosa,* and *T. crispa*, respectively.

Statistical analysis

The difference in TPC, DPPH radical scavenging activity, and FRAP of each extract were evaluated by one-way ANOVA and Duncan's tests. Comparison of theoretical and experimental antioxidant activities was evaluated by paired t-test. A significant value was assigned at p-value <0.05. All analysis was conducted by the general procedures of IBM SPSS Statistics ver. 26 (IBM, USA).

Results

TPC

C. longa showed the highest TPC among the tested samples. The TPC of the formulation was within the range of those of constituents, which was higher than *A. paniculata*, *T. crispa*, and *C. comosa*, but was comparable to *P. niruri* (Figure: 1).

Antioxidant Activities

C. longa and *P. niruri* had the highest antioxidant activities, while *A. paniculata* and *T. crispa* were the lowest. Both DPPH scavenging activity and FRAP of the formulation were in the median range of those of the individual constituents (Figure: 2).

Interaction Effects

Evaluation by comparison method showed that a combination of constituents generated different effects toward DPPH scavenging activity and FRAP of the polyherbal formula, with additive and antagonist effects,

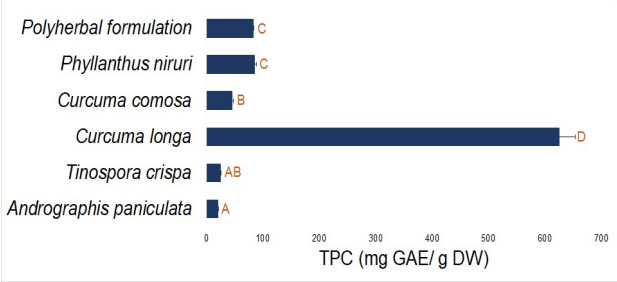

Figure: 1: TPC of the polyherbal formula and its constituents. The different alphabets on each bar showed a statistically different TPC (one-way ANOVA and Duncan's test, p= 0.000, N= 3/group)

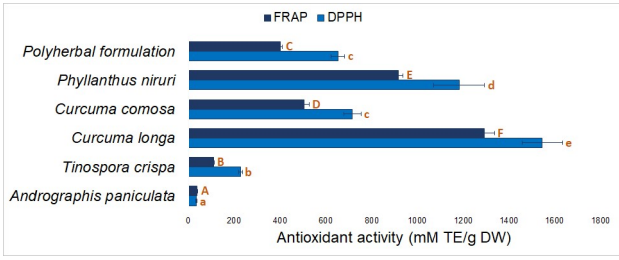

Figure: 2: DPPH scavenging activity and FRAP of the polyherbal formula and its constituents. The different lowercase and uppercase alphabets on each bar represented a statistically different DPPH scavenging activity and FRAP (one-way ANOVA and Duncan's test, p= 0.000, N= 3/group)

Table 1: Interaction effect of the plant constituents to antioxidant properties of the formulation

Antioxidant activity	Comparison method			Difference method	
	Value (mM TE/g DW)		Interaction effect	Difference (%)	Interaction effect
	Obtained	Predicted			
DPPH	652.63±28.84	613.10±19.89	Additive	-82.40	Antagonist
FRAP	401.77±9.42	468.57±7.24	Antagonist	-85.99	Antagonist

respectively. Evaluation using the difference method showed that a mixture of five crude drugs resulted in antagonist interaction effects in both antioxidant mechanisms (Table: 1).

Discussion

The TPC of each crude drug constituent in this study was per a previous report that mentioned that *P. niruri* contained much higher phenolic compounds than that in *A. paniculata* (12). The higher phenolic contents in *C. longa* than that of *A. paniculata* similar to the results of an Indian plant antioxidant screening study (13).

Interestingly, the order of TPC in each plant was similar to a Malaysian study, where *C. longa* showed the highest TPC, followed by *T. crispa* and *A. paniculata* (14). Curcumin and related compounds are the main constituents of *C. longa* and were reported to be responsible for antiinflammation, antioxidant, and antiapoptotic activities (15). Compared to those of the individual component, the TPC of a polyherbal formulation is widely varied according to the ratio of each component. For example, the TPC of a polyherbal formulation prepared from various proportions of *Centella asiatica* (L.) Urb., *Piper sarmentosum* Roxb., and *Morinda citrifolia* L. ranged from 107.15-167.15 g GAE/ml, with individual TPC of 134.33, 125.7, and 209.12 g GAE/ml, respectively (16). Our result is also similar to the study reported that the TPC of a polyherbal formulation was within the range of those of its components (17).

Antioxidant activity evaluation involves two mechanisms, i.e., single electron transfer (SET) and hydrogen atom transfer (HAT). DPPH scavenging activity quantified both SET and HAT mechanisms, while FRAP exclusively measured the HAT one (18). The significantly higher DPPH radical scavenging activity in *P. niruri* than that in *A. paniculata* observed in this study is in accordance with a previous result reported that the IC_{50} of those plant extracts was 14.5 and 30.5 µg/ml, respectively (12). Similar to the result in this study, a Malaysian report mentioned that the EC_{50} of DPPH radical scavenging activity was 12.2±0.2 µg/ml. At the same time, *T. crispa* and *A. paniculata* showed no radical scavenging activity (14). Also, the better FRAP of *C. longa* over *A. paniculata* observed in this study followed an Indian investigation (13).

In the comparison method, the interaction effects were synergist, additive, or antagonist when the theoretical DPPH radical scavenging activity and FRAP of the formulation were statistically lower, equal, or higher than their experimental counterparts, respectively. In the difference method, the interaction effects were synergist or antagonist when the difference percent was positive or negative, respectively. The interaction effect of a mixture of several plant components into a polyherbal formulation varied from antagonist to synergist. For example, a combination of *C. longa*, *Cymbopogon citratus* Stapf, *Murraya koenigii* (L.) Spreng., and *Zingiber officinale* Roscoe generated mostly synergistic antioxidant effects, while that of *Apis dorsata* honey, *Citrofortunella microcarpa* (Bunge) Wijnands, *C. longa*, *Piper nigrum* L., and *Z. officinale* var. Bentong mixture was antagonist (10,11).

Conclusion

In the present study, *C. longa* and *P. niruri* were recognized as crude drugs with the highest TPC. They showed the most potent antioxidant activities, while those of the polyherbal formulation was in the median range of the constituent's respective value. The combination of *A. paniculata*, *T. crispa*, *C. longa*, *C. comosa*, and *P. niruri* generated interaction effects of additive and antagonist towards DPPH scavenging activity and FRAP, respectively. These informations might be used to develop an optimum antioxidant polyherbal formulation with optimum antioxidant activities with synergistic interaction between its components.

Conflict of Interest

The authors declare no conflict of interest.

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