RASĀYAN *J. Chem.*
Vol. 15 | No. 4 |2724-2730| October - December | 2022
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

ANTIOXIDANT ACTIVITIES, TOTAL PHENOL, FLAVONOID, AND MINERAL CONTENT IN THE RHIZOME OF VARIOUS INDONESIAN HERBAL PLANTS

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ABSTRACT

In this study, we reported the medicinal and therapeutic potential of 10 Indonesian herbal plants, including their antioxidant activities, metabolite, and mineral content. In all samples, metabolites were extracted from the rhizome of the plants using the maceration method with methanol as solvent. Antioxidant activity of the dried and powdered extract was assessed using the DPPH method, the total phenolic content (TPC) was analyzed using the Folin-Ciocalteu method, the total flavonoid content (TFC) was analyzed using the aluminum chloride colorimetry method, and mineral content was analyzed using XRF Analyzer. Our results TPC and TFC are positively correlated with the antioxidant activity of the extract, for example, high TPC (990.3 mg GAE/) and TFC (104.4 mg QE/g) in *Curcuma heyneana* extract were accompanied by the high antioxidant activity of the extract. Further, we found that *Kempferia galangal* L. extract has the most diverse mineral composition, but it has the lowest TPC and antioxidant activity.

Keywords: Antioxidant, Health Care, Herbal, Mineral Content, Total Phenolic, Total Flavonoid.

RASĀYAN *J. Chem.*, Vol. 15, No.4, 2022

INTRODUCTION

Indonesia has the second biggest biodiversity in the world, there are about 40,000 species of herbal plants known throughout the world, with approximately 30,000 of them can be found in Indonesia. Herbal plants have a high potential to be developed and utilized in herbal-based medicine, they can be used as preventive medicine, health-promoting medicine, and curative medicine. These properties were attributed to various bioactive compounds contained in herbal plants. Despite having high plant diversity, herbal plants in Indonesia are still understudied and underutilized, currently, only around 1200 species of herbal plants were already characterized and utilized as traditional medicine in Indonesia. In this study, we investigated the medicinal and therapeutic potential of 10 Indonesian herbal plants, including *Zingiber officinale* cv Rubra, *Zingiber officinale* cv alba, *Zingiber aromaticum*, *Curcuma xanthorrhiza*, *Curcuma heyneana*, *Curcuma aeruginosa*, *Curcuma zedoaria*, *Kaemferia galanga* L., *Alpinia galanga* L., and *Boesenbergia rotunda.* These herbal plants have been traditionally used as medicine and spices across the Indonesian archipelago. Several publications have reported the antioxidant activities of herbal plants, these properties are attributed to phenolic compounds contained in herbal plants.[1] Gas-chromatography mass spectroscopy (GCMS) analysis showed that herbal plants contain monoterpenes and sesquiterpenes with antibacterial activities.[2] Other studies reported that *Curcuma aeruginosa* collected from 20 regions in Indonesia produced different levels of phenolic and flavonoid compounds.[3] The bioactive compound commonly found in the

http://doi.org/10.31788/RJC.2022.1548024

Zingiberaceae plant family is curcuminoid.[4] Analysis using proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy revealed that curcumin and xanthorrhizol content in *C. xanthorrhiza* is higher than in *C. aeroginosa* and *C. longa*.[5] Herbal medicines, known locally as "jamu", are highly popular in Indonesia, they are produced at high volumes both by modern pharmaceutical industries and home industries. Most Jamu concoctions are prepared from simple ingredients such as slices of rhizome, roots, barks, dried leaves, and powdered plant organs. The recipe for Jamu is traditionally passed down across generations and the efficacy is mainly supported only by empirical evidence. Further, various herbal products currently found in the Indonesian market are sold without proper efficacy tests and information regarding safety and ingredients. To support standardization and the safety of herbal medicines, a systematic study regarding the chemical composition and bioactivity of herbal plants is required. In this study, we evaluated the antioxidative potential, total phenolic content (TPC), total flavonoid content (TFC), and mineral composition of various Indonesian herbal plants. To date, there is still no report regarding the correlation between overall mineral composition with TPC, TFC, and antioxidant activity in the rhizome of herbal plants. Results from this study will provide valuable information regarding the antioxidative potential of various Indonesian herbal plants and the correlation between rhizome antioxidant activity with TPC, TFC, and mineral composition.

EXPERIMENTAL

Plants Materials

Ten species of herbal plants were evaluated, including *Zingiber oficinale* cv rubra, *Zingiber officinale* cv alba, *Curcuma xanthorrhiza*, *Zingiber aromaticum*, *Kaempferia galanga* L., *Alpinia galanga*, *Curcuma heyneana*, *Curcuma aeruginosa*, *Curcuma zedoaria*, *Boesenbergia rotunda*. The plant samples were gathered from farmers in East Java, Indonesia, and the taxonomical identity of the plants are verified by Purwodadi Botanical Garden, National Research and Innovation Agency, East Java, Indonesia.

Chemicals

DPPH (2,2-diphenyl-1-picrylhydrazyl), methanol, gallic acid, Folin-Ciocalteau reagent, sodium carbonate (Na_2CO_3), aluminum chloride ($AlCl_3$), sodium acetate (CH_3COONa), ethanol, quercetin. All chemicals are pro analysis grade (Merck).

Instruments

Microplate reader Multiskan Go–Thermo scientific, shaker (Shimadzu), freeze dryer (type L200, Buchi), X-Ray Fluorescence Analyzer (Panalytical).

Extraction

Rhizomes from herbal plants were air-dried and then homogenized to powder. Metabolites were extracted from the powdered samples using the maceration method. In brief, 200 g of powdered rhizomes were macerated with 400 mL methanol and incubated on a shaker at 100 rpm for 48 hours. Liquid extract was separated from solid debris using filter paper and evaporated at $28^0 – 30^0$ C to reduce methanol content. The extract was then dried using a freeze dryer to acquire extract in the form of powder.

Antioxidant Assay

Rhizome extract was dissolved in different concentrations of methanol: 0, 6, 12, 25, 50, 75, 100, and 200 mg/L. Next, 100 µL of each extract concentration was added with 50 µL of 100 µM DPPH in 96 plates plate. Following incubation for 30 min at dark, the decrease in the absorbance was recorded at 517 nm using a microplate reader. The antioxidant activity of the extract was calculated using the following equation.[6]

$$\%\,Scavenging\ Capacity = \frac{\text{Control Absorbance} - \text{Sample Absorbance}}{\text{Control Absorbance}}\ x\ 100\% \qquad (1)$$

Measurement of Total Phenolic Content

As much as 10 mg of the extract was dissolved with 10 mL methanol, and the 25 µL of the mixture was mixed with 25 µL of Folin-Ciocalteau reagent and 75 µL aqua dest in a microplate. The mixture was then incubated at dark for 5 minutes then 100 µL of 7% Na_2CO_3 was added. Next, the mixture was re-incubated in dark for 90 minutes while gently shaken. Mixture absorbance was measured at 753 nm using a microplate reader with gallic acid used as the standard solution to measure total phenolic content.[7]

Measurement of Total Flavonoid Content

As much as 50 µL of methanol-dissolved extract (1 mg/mL) was mixed with 100 µL of 10% $AlCl_3$, 150 µL of 96% ethanol, and 10 µL of 1 M CH_3COONa. The mixture was then incubated at dark for 40 minutes. Mixture absorbance was measured at 430 nm using a microplate reader with quercetin used as the standard solution to measure total flavonoid content.[8]

Measurement of Mineral Content

As much as 1 g of dried extract was analyzed using X-Ray Fluorescence Analyzer at 25.9°C and humidity 64%.

RESULTS AND DISCUSSION

Results from the antioxidant assay, measurement of total phenolic content (TPC), and total flavonoid content (TFC) were presented in Table-1. Among the analyzed samples, *Curcuma heyneana* rhizome extract showed the highest antioxidant activity, although its TPC was below *Zingiber officinale cv alba*. On the other hand, *Kaempferia galanga* showed the weakest antioxidant activity against DPPH, to the low TPC in this extract. These results suggest positive correlation between TPC and the antioxidative activities of the extract.

Table-1: Antioxidant Activity (IC_{50}), Total Phenolic Content (TPC), and Total Flavonoid Content (TFC) of Rhizome Methanol Extract from the Ten Herbal Plants

Plant Sample	IC_{50} (µg/mL)	TPC ± SD (mg GAE/g)	TFC ± SD (mg QE/g)
Zingiber oficinale cv rubra	50.00	923.67 ± 181.49	104.14 ± 9.76
Zingiber officinale cv alba	81.50	1250.30 ± 68.35	8.22 ± 0.64
Zingiber aromaticum	315.00	563.67 ± 68.35	97.67 ± 7.39
Curcuma xanthorrhiza	88.50	813.67 ± 21.21	141.70 ± 8.12
Curcuma heyneana	37.75	990.30 ± 87.20	104.40 ± 4.81
Curcuma aeruginosa	158.50	692.00 ± 14.14	ND
Curcuma zedoaria	247.00	795.30 ± 23.57	16.92 ± 1.92
Kaemferia galanga	>500.00	57.00 ± 7.07	ND
Alpinia galanga	412.00	112.00 ± 4.71	ND
Boesenbergia rotunda	313.00	697.00 ± 7.07	4.33 ± 1.78

ND = not determined

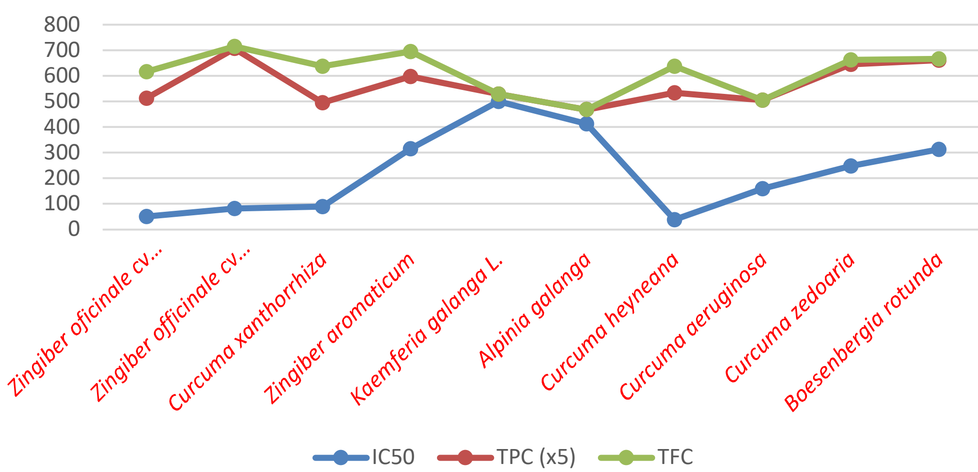

Fig.-1: Line Chart Representing Relationship Between IC_{50}, Total Phenolic Content (TPC), and Total Flavonoid Content (TFC) of Rhizome Methanol Extract from Ten Herbal Plants

From Fig.-1 we can see that in general TPC is negatively correlated with IC_{50} value. Lower IC_{50} represents stronger antioxidant activity, thus higher phenolic content correlates with higher antioxidant activity. This result was in accordance with other researcher, has been reported that a linear and significant positive correlation between antioxidant activity and TPC.[9] This result also infers that phenolic compounds are the dominant antioxidant agent in the sample. The phenolic compound can scavenge DPPH radicals through two pathways: hydrogen-atom transfer (HAT) and single-electron transfer (SET). HAT reaction occurs when antioxidant agent transfers a hydrogen atoms to reactive species, while a SET reaction occurs when the antioxidant agent is transferring an electron to free radical species.[10] Nevertheless, we also observed that the high TPC found in *Zingiber officinale* cv alba is not accompanied by strong antioxidant activities. Such a finding was also reported that high TPC is followed by the stronger antioxidant activity of the extract.[11] This suggests that different phenolic compounds could have a different rate of antioxidant activity depending on their structure and TPC does not represent all antioxidative compounds in an extract.[11] In accordance, Sulaiman *et al*. (2011) also showed that TPC is not always positively correlated with the antioxidative activities of an extract[12], indicating the minor role of phenolic compounds in the antioxidative capacity of extracts. Further, we observed that TFC is not always positively correlated with TPC and antioxidant activity. In general, the flavonoid is part of the phenolic compound family but not all flavonoid has a phenolic group in their chemical structure. Next, the mineral content of the extract was presented in Table-2. The ten studied minerals contain 9 to 14 minerals in their rhizome extract. The 9 minerals that can be found in all samples are P, S, K, Ti, Mn, Fe, Cu, Zn, and Re. The Highest P concentration was detected in *Zingiber officinale* cv. alba (4.05%), highest S concentration was found in *Curcuma aeruginosa* (1.85 %), the highest K concentration was found in *Curcuma heyneana* (91.65 %), the highest Ca concentration was found in *Kaemferia galanga* L (53.7%), the highest Ti concentration was found in *Zingiber aromaticum* (1.3 %), the highest Mn concentration was found in *Alpinia galanga* (27.9%), highest Fe concentration was found in *Curcuma zedoaria* (17.2%), the highest Zn concentration was found in *Curcuma aeruginosa* (1.35%), the highest Zr concentration was found in *Curcuma aeruginosa* and *Curcuma zedoaria* (0.2%), the highest Re concentration was found in *Zingiber officinale* cv. alba (1.2%), the highest Rb concentration was found in *Curcuma aeruginosa* (0.96%), the highest Ba concentration was found in *Zingiber aromaticum* (4.4%), the highest Si concentration was found in *Alpinia galanga* and *Curcuma aeruginosa* (8.75%), while the highest Sr concentration was found in *Kaemferia galanga* L. (0.75%).

From 15 identified minerals, K was found to be the most abundant mineral in the studied herbal plants (ranging from 23.4% - 91.65% in concentration), while Sr was detected as the least abundant mineral. Sr was only found in two plants *Zingiber oficinale* cv rubra and *Kaemferia galanga* L. Among the samples, *Zingiber oficinale* cv rubra, *Kaemferia galanga* L., *Zingiber aromaticum,* and *Curcuma aeruginosa* were detected as plants with the most diverse mineral composition (14 minerals), while *Curcuma heyneana* has the least diverse mineral composition, only 9 minerals can be detected. Interestingly, *Curcuma heyneana*, an herbal plant with the highest antioxidant activity, showed the least diverse mineral composition. On the other hand, *Kaempferia galanga,* one of the herbal plants with the most diverse mineral composition showed the weakest antioxidant activity. Previously, researcher suggested that minerals did not affect the antioxidant activity of an extract, since in their experiment no positive or negative correlations were detected between mineral composition and the antioxidative capacity of an extract.[13] However, other reports described a correlation between antioxidant activity with the presence of certain minerals. reported The increase in superoxide dismutase (SOD) activity, a well-known antioxidative enzyme, in mulberry that has Mg deficiency has been reported[14]. This finding suggests a negative correlation between Mg content and antioxidant activity. A positive correlation between Mn content and DPPH scavenging activity in bananas (*Musa* sp.) has been reported.[12] Another work reported that P deficiency can lead to increased flavonoid concentration.[15] It is suggested that a positive correlation between K concentration and antioxidant activity is probably because of the role of K in the activation of enzymes that are involved in the production of flavonoid and phenolic compounds.[16] The abundance and high concentration in the studied herbal plants suggests that the rhizomes of these plants have high medicinal and therapeutic potentials since potassium plays a pivotal role osmotic balance of cells and nerve system, especially through its role in the transmission of nerve impulse. These functions are also connected to heart muscle contraction activity.[17-21] Potassium deficiency can lead to fatigue, cramps, delayed reflexes, acne, dry skin, and heart problems.[21] Beside Potassium, Calcium (Ca) is also detected at high concentrations in the studied herbal plants (ranging from 11.45% - 53.70% in concentration). Calcium is essential for the human body due to its role in maintaining the biological role of various tissue systems (musculoskeletal, nervous, and cardiac systems, bones and teeth, and parathyroid glands). Moreover, Ca also play an important role as an enzyme cofactor in various reactions, it's also involved in maintaining body mineral balance and general physiological function.[22-25]

Table-2: Mineral composition in various herbal plants from Indonesia (n=2)

Species	Minerals (%)														
	P	S	K	Ca	Ti	Mn	Fe	Cu	Zn	Zr	Re	Rb	Ba	Si	Sr
Zingiber oficinale cv rubra	3.1[a]±0.00[b]	1.65±0.07	68.4±0.00	15.45±0.07	0.3±0.00	4.07±0.00	3.7±0.01	1.15±0.07	0.44±0.01	0.00	0.86±0.08	0.39±0.01	0.55±0.07	0.32±0.02	0.19±0.03
Zingiber officinale cv alba	4.05±0.07	1.35±0.07	63,05±0.07	19.4±0.00	0.58±0.06	1.85±0.07	6.4±0.04	1.8±0.00	0.09±0.01	0.09±0.01	1.2±0.28	0.00	0.00	0.00	0.00
Curcuma xanthorrhiza	3.75±0.07	1.4±0.00	77.5±0.00	0.00	0.2±0.00	4.93±0.04	4.90±0.02	1.2±0.00	0.67±0.01	0.09±0.01	0.85±0.07	0.34±0.02	0.95±0.07	3.2±0.28	0.00
Zingiber aromaticum	3.3±0.00	1.5±0.00	40.8±0.14	15.9±0.14	1.3±0.00	7.17±0.01	15.65±0.21	1.5±0.00	0.64±0.06	0.15±0.07	0.9±0.14	0.55±0.04	4.4±0.00	6.10±0.28	0.00
Kaemferia galanga L.	1.85±0.07	3.1±0.00	23.4±0.00	53.7±0.14	0.72±0.03	0.67±0.02	11.3±0.14	1.1±0.00	0.2±0.00	0.03±0.02	0.35±0.07	0.00	0.55±0.07	2.20±0.14	0.75±0.06
Alpinia galanga	2.35±0.07	1.7±0.00	52.3±0.00	0.00	0.2±0.00	27.9±0.14	3.63±0.05	0.95±0.01	0.52±0.01	0.08±0.01	0.5±0.00	0.59±0.04	0.55±0.07	8.75±0.21	0.00
Curcuma heyneana	2.1±0.00	0.81±0.03	91.65±0.07	0.00	0.00	1.5±0.00	2.00±0.00	1.00±0.00	0.01±0.01	0.08±0.00	0.75±0.07	0.00	0.00	0.00	0.00
Curcuma aeruginosa	3.4±0.00	1.85±0.07	42.2±0.00	11.45±0.07	1.15±0.07	13.65±0.07	14.5±0.14	1.35±0.07	1.35±0.07	0.2±0.00	0.7±0.14	0.96±0.02	0.30±0.14	8.75±0.21	0.00
Curcuma zedoaria	2.95±0.07	1.35±0.07	44.5±0.00	20.85±0.07	1.25±0.00	3.2±0.00	17.2±0.00	1.5±0.00	0.52±0.00	0.2±0.00	0.85±0.07	0.00	0.55±0.21	5.1±0.00	0.00
Boesenbergia rotunda	2.8±0.14	1.7±0.00	45.2±0.00	25.1±0.4	0.63±0.00	6.51±0.09	8.89±0.08	1.20±0.00	0.51±0.03	0.00	0.65±0.07	0.36±0.01	1.25±0.35	4.4±0.57	0.00

[a]Mean (n=2)
[b]Standart deviasi

As a preventive measure, moderate Ca consumption could decrease colon cancer risk.[26, 27] Higher Ca consumption during pregnancy is also suggested to avoid pre-eclampsia.[27] The Fe concentration in the studied herbal plants ranged between 2%-17.2%, in commonly consumed vegetables and fruits, Fe levels ranged between 0.13 – 3.01 mg/100 g.[28] The main function of Fe in the human body is in the biosynthesis of hemoglobin and myoglobin.[23,29,30] Mn was detected at low concentrations in the studied herbal plants, only between 0.67% – 7.17%. Mn is also found at low concentrations in vegetables (0.01-0.078 mg/100 g) and fruits (0.66 mg/100 g).[31] In the human body, Mn is known as a cofactor in antioxidant reactions during glucose metabolism (carbohydrate metabolism and gluconeogenesis).[23] Cuprum (Cu) and Zinc (Zn) is also detected at low concentration in the studied herbal plants, between 0.95% - 1.8% for Cu and 0.09% - 1.35% for Zn. In the human body, Cu is involved in the function of level 1 detoxification enzymes (such as the cytochrome c oxidase enzyme family).[23] Cu is also involved in the development of connective tissue and myelin sheath.[29,30] Zn plays an important structural and functional role in proteins, especially enzymes, it is involved in more than 100 different enzymatic reactions.[23,29,30] Further, Zn is also required in the biosynthesis of nucleic acid, protein, cell differentiation, and insulin secretion.[30] Ti, Zr, Re, Rb, Ba, and Sr are found at very low concentrations in the studied herbal plants, while Si is found at slightly higher concentrations. All of these minerals are rarely found in fruits and vegetables[28], however, Ti and Si are sometimes detected in medicinal plants and aromatic plants.[32] Overall, our results showed that almost all micronutrients are essential for plant growth, except Mg, which can be detected in the rhizomes of studied herbal plants. Carbon, Oxygen, and Nitrogen are not reported in this work since these chemical elements cannot be detected by the XRF analyzer used in this study. The absence of Mg in rhizomes is probably due to their main function in leaf chlorophyll synthesis, therefore Mg might not be required and deposited in the rhizome. Mg can be found in the leaf of various medicinal plants and aromatic plants.[32]

CONCLUSION

Zingiber officinale cv alba was detected as herbal plant with the highest TPC, although the highest antioxidant activity was observed in *Curcume heyneana* that has a lower TPC compared to *Z. officinale* cv alba. This result suggests that TPC and antioxidant activity is not always in linear positive correlation. Nevertheless, a certain level of TPC is required for an extract to have sufficient antioxidative properties, for example, low TPC in *Kaempferia galanga* L. is accompanied by weak antioxidant activity of the extract.

In general mineral composition and concentration are not related to the antioxidant capacity of an extract. However, we found that the plant sample with the most diverse mineral composition, *Kaempferia galanga* L, showed the weakest antioxidant activity and the lowest TPC.

ACKNOWLEDGEMENT

This research was funded by: Universitas Airlangga through the "Skema Hibah Penelitian Riset Grup", research grant no. 343/UN3.14/PT/2020.

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[RJC-8024/2022]