Evaluation of polyphenol content, antioxidant activity, tyrosinase inhibitory of ethanol/water extracts from rhizomes of Vietnamese *Zingiber officinale*, *Curcuma longa*, *Alpinia officinarum,* and *Boesenbergia pandurata*

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Supplementary Information

3.3. Total polyphenol content (TPC)

In this study, the polyphenol content (TPC) of ethanolic extracts was determined using the Folin-Ciocalteu method which was described by Sankhalkar S et al. (2016)[1] and Tan X et al. (2020)[2]. The wavelength used to measure the absorbance of samples was 650nm which agreed with the reports of Sankhalkar S et al. (2016)[1] and Tan X et al. (2020)[2]. Before applying the modified essay to determine the TPC of all extracts, it was evaluated LOD (Limit of Detection), LOQ (Limit of Quantification), linear range, calibration curve, and repeatability according to the ICH guideline Q2 and a report by Thompson M et al. (2002)[3].

Eight blank samples were used for the determination of the LOD and LOQ. The results showed that LOD and LOQ were 0.099 µg/mL and 0.246 µg/mL, respectively. The linear range was examined with gallic acid concentration in a range of 0.25 µg/mL to 8.75 µg/mL; the result is shown in Figure 1a. When the concentration of gallic acid was higher than 7.50 µg/mL, its absorbance and concentration had not good linearity with a correlation coefficient of $R^2 < 0.99$. Therefore, the linear range was made out in the range of 0.25 µg/mL to 7.50 µg/mL gallic acid. The calibration curve then, was determined and displayed in Figure 1b. The calibration curve equation was determined, Absorbance = (0.0881×Concentration) - 0.0123. This equation had a correlation coefficient of R^2 = 0.9999, showing that the absorbance and concentration of gallic acid had good linearity and could be used to evaluate the TPC of all extracts. Acid gallic 1.00 µg/mL was used to evaluate the repeatability and the result showed that RSD (%) was 4.55%, less than 11%. This value satisfied the requirement of method validation.

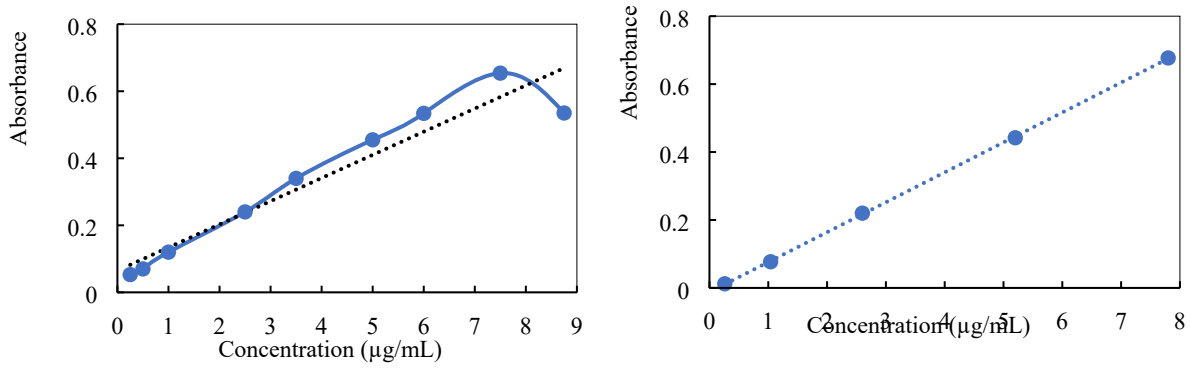

Figure 1. The calibration curve of gallic acid

3.4. Antioxidant ability

In this study, the antioxidant ability of ethanolic extracts was evaluated through their DPPH free radical inhibition ability. The DPPH free radical scavenging ability was evaluated using inhibitory percentage (I%). Table 1 shows the I% values of the obtained extracts and ascorbic acid in different tested concentrations. Each concentration was triplicated.

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Table 1. Inhibitory percentage (I%) of extracts in different tested concentrations in antioxidant ability experiments

Z. officinale	Concentration (µg/mL)	20.2	50.5	101	202
	I%	43.77	57.90	69.91	74.79
		37.70	58.29	71.49	74.00
		39.29	64.10	72.42	72.55
C. longa	Concentration (µg/mL)	10	50	100	200
	I%	4.78	58.16	84.62	87.43
		5.37	60.67	85.51	88.17
		5.67	57.42	86.40	89.21
A. officinarum	Concentration (µg/mL)	5150	7210	9270	11330
	I%	20.54	88.81	90.71	90.33
		22.82	89.57	90.90	90.14
		22.25	89.19	91.85	90.52
B. pandurata	Concentration (µg/mL)	50.03	100.06	200.13	250.16
	I%	27.56	36.82	62.82	68.35
		23.83	36.22	64.14	72.20
		24.43	38.03	66.43	72.32
Ascorbic acid	Concentration (µg/mL)	0.11	0.27	0.50	1.35
	I%	3.75	11.89	23.14	74.14
		3.99	12.61	20.51	72.47
		5.91	13.81	21.95	73.42

3.5. Tyrosinase enzyme inhibition ability

In this study, the tyrosinase enzyme inhibition ability of ethanolic extracts was evaluated. The tyrosinase inhibition ability was evaluated using inhibitory percentage (I%). Table 2 shows the I% values of the obtained extracts and kojic acid in different tested concentrations. Each concentration was triplicated.

Table 2. Inhibitory percentage (I%) of extracts in different tested concentrations in tyrosinase inhibition ability experiments

Z. officinale	Concentration (µg/mL)	50	100	200	300
	I%	30.56	52.41	91.24	95.81
		33.33	52.60	90.67	95.43
		35.19	58.50	90.10	92.58
C. longa	Concentration (µg/mL)	25	50	100	-
	I%	46.73	90.19	-	-
		49.53	88.79	-	-
		48.13	92.99	-	-
A. officinarum	Concentration (µg/mL)	100	200	300	400
	I%	40.75	50.16	52.04	49.22
		43.57	56.74	49.22	61.44
		41.69	52.98	51.10	54.86
B. pandurata	Concentration (µg/mL)	250	300	400	-
	I%	19.62865	29.97347	67.65217	-

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		14.85411	29.17772	67.65217	-
		-	31.56499	68.17391	-
Kojic acid	Concentration (µg/mL)	1.36	6.80	13.60	-
	I%	32.39	54.93	64.79	-
		29.58	57.75	74.65	-
		33.80	52.11	67.61	-

"-": be not tested or determined

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