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ANTIOXIDANT AND CYTOTOXIC ACTIVITIES OF *KAEMPFERIA PARVIFLORA* WALL. ex. BAKER EXTRACTS AND ITS ISOLATED COMPOUNDS

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Summary

Antioxidant and Cytotoxic Activities of *Kaempferia parviflora* Wall. ex. Baker Extracts and its Isolated Compounds

Kaempferia parviflora Wall. ex. Baker, a well-known black ginger, is widely cultivated in Vietnam. Four extracts and two most abundant compounds isolated from this plant were tested for their antioxidant capacity and cytotoxic effect against several human cancer cell lines. The results demonstrated that the water fraction expressed the most potent scavenging activity on DPPH (IC_{50} = 136.58 µg/mL) and ABTS radical (IC_{50} = 41.73 µg/mL) and also possessed a higher total phenolic (152.60 mg GAE/g dried extract) and flavonoid content (154.92 mg QE/g dried extract) than other fractions from *K. parviflora* ($p < 0.05$). In contrast, the ethyl acetate fraction displayed the most potent inhibitory effect with the IC_{50} values ranging from 8.36 to 38.64 µg/mL against 4 cancer cell lines including A549, Du145, HepG2 and H1299. Two compounds, 5-hydroxy-3,7- dimethoxyflavone (DMF) and 5,7,4′-trimethoxyflavone (TMF) exhibited a moderate cytotoxic effect (IC_{50}: 47.53 to 77.09 µM) against HepG2 and H1299 but no cytotoxic effect against A549 and Du145 (IC_{50} > 100 µM).

Keywords: *Antioxidant, Cytotoxicity, Polymethoxyflavones, Kaempferia parviflora.*

1. Introduction

Kaempferia parviflora Wall. ex. Baker, a well-known black ginger originating from Thailand, is widely cultivated in Vietnam. Various extracts of *K. parviflora* and its isolated methoxyflavones were reported to express many biological effects such as anti-diabetic activity [1], the anti-inflammatory effect [2],[3], the improvement of sexual health [4],[5] or the antiplasmodial and antifungal activity [6]. A few studies showing the antioxidant effect of *K. parviflora* were previously documented by Korean scientists [7],[8]. Besides Korea, the anticancer activity of this plant primarily attracted the attention of many research groups in Asian countries. In 2021, Thai scientists proved that the ethanol extract from *K. parviflora* exhibited high cytotoxic activity against human urinary bladder cancer cells *via* p53 gene expression [9] or inhibited the proliferation of ovarian cancer cells by suppressing NF-κB signaling [10]. Recently, the potential effect of *K. parviflora* extracts to inhibit breast cancer cell growth was performed by Indonesian researchers [11]. In Vietnam, our previous study showed that the ethyl acetate extract of *K. parviflora* displayed a promising antiplatelet aggregatory and anticoagulant effect in human blood samples [12]. Among six bioactive polymethoxyflavone isolated from that fraction, 5-hydroxy-3,7-dimethoxyflavone (DMF, 0.45% of the dried sample) and 5,7,4′-trimethoxyflavone (TMF, 1.6% of the dried sample) were shown to be the most abundant constituents in *K. parviflora* [12].

The present study evaluated the antioxidant capacity of different extracts from *K. parviflora* cultivated in Vietnam. In addition, the cytotoxic effect of these extracts and two of their components DMF and TMF, against several human cancer cell lines, including lung carcinoma cell line A549, prostate carcinoma cell line Du145, hepatocellular carcinoma cell line HepG2 and non-small cell lung carcinoma cell line H1299 were determined for the first time.

2. Materials and methods

2.1. Chemicals

Folin-Ciocalteu (FC) reagent, gallic acid, quercetin, ascorbic acid, trolox, 1,1-diphenyl-2-picryhydrazyl (DPPH), 2, 2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were purchased from Sigma-Aldrich, Merck, Germany. $NaNO_2$, AlCl3, NaOH, Na_2CO_3, dimethyl sulfoxide (DMSO), methanol, and ethanol were provided by Xilong Scientific Co., Ltd, China.

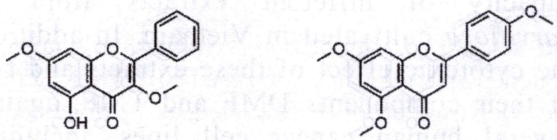
Dulbecco's modified eagle's medium (DMEM), Roswell Park Memorial Institute 1640 medium (RPMI), Fetal bovine serum (FBS), and camptothecin antibiotic were provided from Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA.

2.2. Plant material

Kaempferia parviflora rhizomes were collected at Hung Yen province in 2020. The plant was identified by Dr. Nguyen Quoc Binh, Vietnam National Museum of Nature, Vietnam Academy of Science and Technology. A voucher specimen number SH 1193 was deposited at the Department of Life Sciences, University of Science and Technology of Hanoi, Vietnam Academy of Science and Technology.

One kilogram of dried rhizome of this plant was soaked with absolute methanol at 40°C using sonication (10 liters x 2 times). The crude extract (ME) was obtained after evaporating the supernatant under reduced pressure. ME was then suspended with distilled water before being extracted gradually with *n*-hexane (HX) and ethyl acetate (EA). The leftover aqueous extract was dried under reduced pressure to obtain the water extract (WA). The extraction yields of ME, HX, EA, and WA extracts were respectively 92.5 g, 8.2 g, 52.3 g, and 42.1g.

Two pure compounds were isolated from the EA extract, including DMF and TMF, according to our previous article [12]. The structure of these compounds is demonstrated in Fig. 1.



5-hydroxy-3,7-dimethoxyflavone 5,7,4'-trimethoxyflavone

Fig. 1. Structure of 5-hydroxy-3,7- dimethoxyflavone and 4', 5, 7-trimethoxyflavone

2.3. Antioxidant capacity

2.3.1. Radical scavenging assay:

DPPH assay

This experiment followed the previous method with slight modifications [13]. The extracts were diluted into different concentrations ranging from 1 to 10 mg/mL in DMSO, except for the HX extract (10-50 mg/mL). Ascorbic acid from 0.025 to 1 mg/mL was used as a positive control. DMSO and distilled water were blanks.

In a 96-well plate, 10 µL of each extract was mixed with 190 µL of 0.1 mM DPPH and incubated for 20 minutes in the dark at room temperature. The absorption was measured at the wavelength of 517 nm using a Microplate spectrophometer (xMark, Bio-Rad). Each sample was repeated 3 times.

The percentage of inhibition was calculated through the equation:

$$\% \text{ Inhibition} = (1-A_S/A_B) \times 100\%$$

A_S: the average absorbance of the sample and A_B: the average absorbance of the blank

ABTS assay

The working solution $ABTS^{\bullet+}$ (0.1 mM) was obtained by the reaction between 7 mM ABTS solution and 2.45 mM potassium persulfate solution (1:1 v/v) and incubated for 14-16 hours in the dark at room temperature. The absorbance of the mixture was adjusted to 0.7 ± 0.05 at 734 nm [14]. Trolox (0.05-0.5 mg/mL) was used as a positive control.

The test was taken out, similar to the DPPH assay. IC_{50} was calculated as the concentration of the extracts at which 50% of DPPH or ABTS radicals were scavenged.

2.3.2. Total phenolic and flavonoid contents:

The total phenolic content (TPC) was analyzed using the Folin-ciocalteu method in 96-well plates [15]. The 10 mg/mL plant extracts were prepared in DMSO 100% as a stock solution.

10 µL of each plant extract at varying concentrations was incubated with 25 µL reagent at room temperature for 5 minutes. 50 µL of Na_2CO_3 10% was added with 115 µL distilled water. After incubation for 30 minutes, the absorption of the solution was recorded at 760 nm using a Microplate spectrophotometer (xMark, Bio-Rad). Gallic acid (0.025-1 mg/mL) was used to build the standard curve. The TPC value was calculated as milligrams of gallic acid equivalents per one gram of each plant extract (mg GAE/g extract).

$$TPC = C_{GAE} / C_0$$

C_{GAE}: the concentration of gallic acid equivalent (mg GAE/mL), C_0: the concentration of the extract (g extract/mL)

The colorimetric method investigated the total flavonoid content (TFC) in 96-well plates described by Mishra *et al.* [16] with modifications. 48 µL plant extracts (10 mg/mL in DMSO) were incubated with 8 µL of 5% $NaNO_2$ for 6 minutes at room temperature. 8 µL of 10% $AlCl_3$ was added and repeated in the identical incubation. Finally, 80 µL of 1M NaOH and 56 µL of 30% ethanol were mixed and left for 15 minutes. The absorption of the solution was measured at 510 nm. Quercetin was used to build the standard curve. DMSO was used as a negative control. The TFC of samples was calculated as milligrams of quercetin equivalents

per one gram of each plant extract (mg QE/g extract).

2.4. Cytotoxicity assay

2.4.1. Cell lines and cell culture

Lung carcinoma cell line A549, prostate carcinoma cell line Du145, hepatocellular carcinoma cell line HepG2, and non-small cell lung carcinoma cell line H1299 were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). These cell lines were cultured in DMEM medium, supplemented with 10% bovine placental serum (FBS), 100 U/ml penicillin, and 100 µg/ml streptomycin in a 5% CO_2 incubator at 37°C. The medium was changed every 2 days and checked daily.

2.4.2. Cell viability assay:

The experiment was carried out according to the previous method with slight modifications [17]. The cells's culture was performed in well 96 well-plates with a volume of 200 µL, and a density of 30 x 10^4 cells/well. After 24h, cells were treated with the sample mixed in DMSO at different concentrations. Negative control was the medium only. Camptothecin (CPT) (1 µM) was used as a positive control. After treatment for 48 hours with cancer cells, cell viability was evaluated after adding 20 µL of MTT solution into each well and wrapping the plate with foil, then incubating in a CO_2 incubator for 3-4 hours. After that, the medium was discarded, and 200 µL of DMSO was added to dissolve the formazan crystals in a shaker for 10 minutes. The absorbance of the sample was recorded at 570 nm using a spectrophotometer (xMark, Bio-Rad).

Cell viability (%) was determined using the formula: % Cell viability = A_S/A_C x 100

A_S: the absorbance of the sample, A_C: the absorbance of the negative control

2.5. Statistical analysis

All tests were repeated three times. The result was presented as mean value ± SD. The data was analyzed by one-way ANOVA and independent sample *t*-test using GraphPad Prism software, version 8.0.0 for Windows, San Diego, California, USA. Significant differences were considered at $p < 0.05$.

3. Results and discussion

3.1. Antioxidant capacity of KP extracts

3.1.1. Radical scavenging ability:

The scavenging ability of the plant extracts against DPPH and ABTS radicals is shown in Table 1. WA had a more robust antioxidant activity, followed by ME, EA, and HX. Particularly, WA (IC_{50}: 41.73 µg/mL) and ME (IC_{50}: 86.98 µg/mL) expressed a potent antioxidant activity against ABTS with low IC_{50} values. However, none of the extracts could reach the same IC_{50} as standards in both tests.

Table 1. Antioxidant activity of KP extracts

Samples	DPPH (IC_{50}, µg/mL)	ABTS (IC_{50}, µg/mL)
ME	448.34 ± 24.10^a	86.98 ± 2.23^a
HX	2544.65 ± 155.42^b	491.36 ± 3.52^b
EA	425.39 ± 27.61^a	103.91 ± 2.78^c
WA	136.58 ± 17.07^c	41.73 ± 1.06^d
Ascorbic acid	6.82 ± 0.41^d	
Trolox		8.75 ± 0.51^e

ME: methanol extract, HX: hexane extract, EA: ethyl acetate extract, WA: water extract. Various superscript letters in one column refer to significant differences ($p<0.05$).

Among different antioxidant tests, DPPH and ABTS assay are the most commonly used because the measurement is simple with stable and colored product at room temperature, helping to evaluate antioxidants rapidly by spectrophotometry [18]. A few studies on the antioxidant activity of *K. parviflora* extracts and their isolated components were investigated in the literature. The work led by Chaisuwan *et al.* showed that *K. parviflora* rhizomes extracted with 25 to 95% ethanol expressed a weaker DPPH scavenging ability (IC_{50}: 5.37 to 20.0 mg/mL) [19] than the results obtained in our study (IC_{50}: 0.136 to 2.544 mg/mL). The difference might be due to several factors such as methods of extraction, place and season of sample collection, etc.

3.1.2. Total phenolic and flavonoid contents:

TPC and TFC values of four extracts including ME, HX, EA, and WA, were presented in Fig. 2. For TPC, WA possessed the highest value (152.60 ± 10.37 mg GAE/g), while HX had the smallest amount of phenolic compounds (13.66 ± 0.43 mg GAE/ g dried extract). The ME and EA fractions had comparable TPC values (54.91 ± 6.28 and

44.18 ± 4.18 mg GAE/g, $p > 0.05$). Similarly, WA and HX were the fractions with the highest and lowest content of flavonoids (TFC: 154.92 ± 5.80 and 9.07 ± 0.74 mg QE/g dried extract, respectively). However, the total flavonoid amount in the EA sample (106.45 ± 12.73 mg QE/g) was significantly higher than that in the ME extract (28.05 ± 3.01 mg QE/g) ($p < 0.01$).

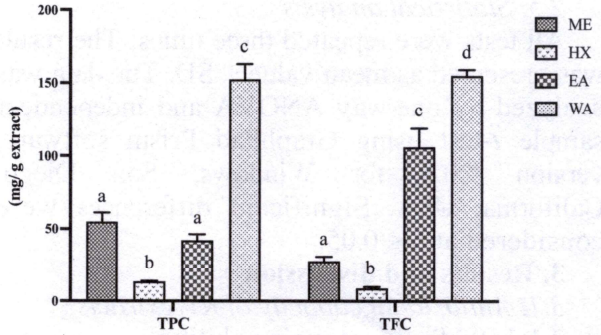


Fig. 2. Total phenolic content and total flavonoid contents of KP extracts. Different letters on columns refer to significant differences among extracts ($p < 0.05$).

In a study by Sitthichai *et al.*, the methanol extract contained the highest content of phenolic compounds (TPC = 58.45 ± 2.85 mg GAE/ g extract). In comparison, the hexane extract significantly had a more considerable amount of flavonoid components (TFC = 127.09 ± 1.00 mg QE/ g extract) than other extracts [20]. The TPC value of the methanol extract is similar, but the one of the hexane extract in that study is much higher than that obtained in our experiment. In general, hexane is a nonpolar solvent, which might be unfavorable for flavonoid extraction from the plant resource. Therefore, our results seem more reasonable when the hexane fraction contained the smallest amount of flavonoid compounds compared to other extracts using more polar solvents such as ethyl acetate, methanol, or water. Particularly, the WA fraction contained the highest content of phenolic and flavonoid compounds in the present study. This finding is correlated with the results of radical scavenging activity where water fraction also displayed the most potent effect. Many previous works also approved the positive correlation between antioxidant activity, total phenolic and flavonoid contents [21],[22].

3.2. Cytotoxic activity on human cancer cell lines

The results demonstrated that the EA fraction exhibited the most robust cytotoxicity, inhibiting more than 80% of cell viability in all four cell types at 100 µg/mL (Table 2). In particular, Du145 cells are more sensitive to the EA extract than other cell lines since it showed low cell viability at both concentrations tested. It can be predicted that the cytotoxic activity on Du145 cells of the black ginger's EA extract in the present study might be much more potent than that of the whole extract of a common ginger species, *Zingiber officinale* (the IC_{50} was 95 µg/mL) [23].

Table 2. Cytotoxic effect of *K. parviflora* on human cancer cell lines

Samples	Cell viability (%)							
	A549		Du145		HepG2		H1299	
	50 µg/mL	100 µg/mL	50 µg/mL	100 µg/mL	50 µg/mL	100 µg/mL	50 µg/mL	100 µg/mL
ME	74.35 ± 2.14	60.54 ± 1.55	85.26 ± 2.71	77.56 ± 1.18	66.83 ± 6.87	28.26 ± 1.46	91.50 ± 4.11	50.59 ± 1.56
HX	92.72 ± 1.71	69.66 ± 3.24	97.62 ± 1.05	75.28 ± 0.86	75.07 ± 7.19	44.46 ± 1.49	84.98 ± 5.43	68.98 ± 3.03
EA	**29.46 ± 0.76**	**17.05 ± 0.40**	**19.71 ± 0.33**	**12.39 ± 0.47**	**23.85 ± 8.80**	**14.87 ± 5.84**	**38.64 ± 1.44**	**8.36 ± 1.04**
WA	70.96 ± 1.39	50.06 ± 0.80	99.73 ± 0.57	99.22 ± 3.58	70.29 ± 1.77	30.32 ± 4.58	73.29 ± 2.62	63.93 ± 1.07
Control	100 ± 6.10		100 ± 0.50		100 ± 8.83		100 ± 3.29	
Campto*	55.19 ± 3.17		64.51 ± 2.47		38.08 ± 2.39		57.03 ± 3.03	

* Camptothecin (Campto) at 1µM was used as a positive anti-cancer reference.

Because the EA exhibited the highest cytotoxicity against 4 cell lines, our subsequent investigation concentrated on the two pure compounds (DMF and TMF) isolated from this extract. According to Figures 3 and 4, the percentage of cell viability in the case of HepG2 and H1299 cells gradually decreased when the concentration of DMF and TMF increased from

12.5 to 100 μM. At all concentrations tested, the cell viability was significantly lower than the control, which means that two compounds expressed their inhibitory effect on HepG2 and H1299 cells. For the other cells (A549 and Du145), DMF and TMF did not display a significant cytotoxic effect.

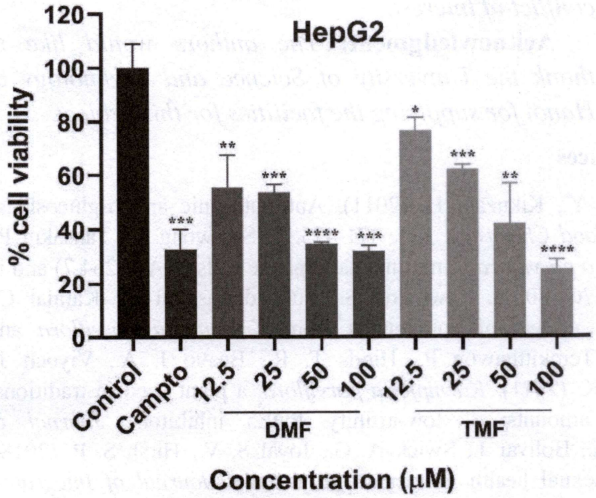


Fig. 3. Cell viability of two compounds tested on HepG2 cells
*$p < 0.05$, **$p<0.01$; ***$p<0.001$; ****$p<0.0001$ compared to the control. Camptothecin (Campo) at 1μM was used as a positive anti-cancer reference.

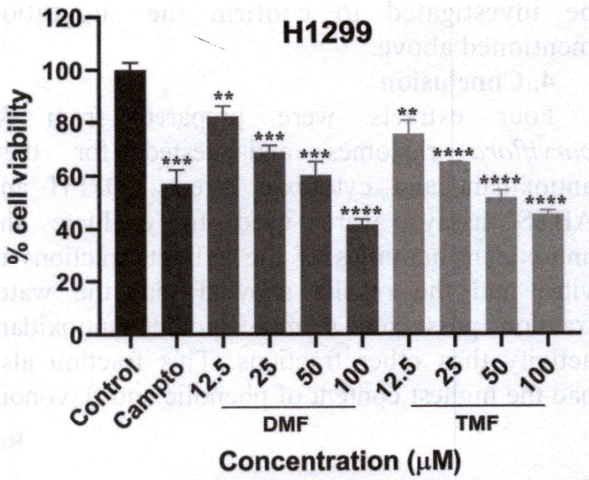


Fig. 4. Cell viability of two compounds tested on H1299 cells
*$p < 0.05$, **$p<0.01$; ***$p<0.001$; ****$p<0.0001$ compared to the control. Camptothecin (Campo) at 1μM was used as a positive anti-cancer reference.

The results in Table 3 demonstrated that DMF (IC_{50}: 44.42 μM) and TMF (IC_{50}: 33.11 μM) exhibited moderate cytotoxicity against HepG2 cells. TMF also expressed a mild cytotoxicity effect on H1299 (IC_{50} value of 53.17 μM), but only a slight inhibitory effect was recorded with DMF (IC_{50} value of 77.35 μM). No cytotoxicity effect on A549 and Du145 cells was displayed for both tested compounds (IC_{50} > 100 μM).

Table 3. IC_{50} values (μM) of two compounds on different human cancer cells

Compounds	IC_{50} (μM)			
	A549	Du145	HepG2	H1299
DMF	> 100	> 100	44.42 ± 13.10	77.35 ± 2.32
TMF	> 100	> 100	33.11 ± 2.60	53.17 ± 6.44

In the literature, extracts and their methoxyflavones were reported to exhibit potent anticancer action against many cancer cell lines such as HL-60 cells, cervical cancer HeLa cells, B16 melanoma 4A5 cells, or human cholangiocarcinoma cell lines [24]. In our study, the ethyl acetate fraction displayed a potent cytotoxic effect on 4 tested cell lines, while two methoxyflavones isolated from this fraction only expressed a moderate cytotoxic effect on HepG2 and H1299. It is suggested *K. parviflora* that more flavonoid compounds isolated from the EA extract should be tested with the expectation of good tumor-selective cytotoxicity.

In general, the water fraction exhibited the strongest antioxidant activity and also possessed the highest amount of phenolic and flavonoid compounds. In contrast, the ethyl acetate fraction expressed the most effective cytotoxicity on all tested cancer cell lines. This fact suggests that water might be the suitable solvent for extracting antioxidant agents from *K. parviflora* while ethyl acetate seems to be the best choice for extracting anticancer agents. In our previous study, the contents of DMF and TMF in the water fraction were neglectable compared to those in the ethyl acetate fraction [12]. These two compounds might be responsible for the anticancer activity of the ethyl acetate extract but not for the antioxidant activity of the water extract. However, more compounds isolated from two fractions should

be investigated to confirm the suggestion mentioned above.

4. Conclusion

Four extracts were prepared from *K. parviflora* rhizomes and tested for their antioxidant and cytotoxic effects. DPPH and ABTS assays were used to evaluate the antioxidant activities of the extracts/fractions in vitro and the results showed that the water fraction possessed a more potent antioxidant activity than other fractions. This fraction also had the highest content of phenolic and flavonoid compounds. However, for the cytotoxicity test, the ethyl acetate fraction displayed the most potent inhibitory effect on all cancer cell lines, such as A549, Du145, HepG2, and H1299. Two compounds isolated from the ethyl acetate extract expressed a moderate cytotoxic effect on HepG2 and H1299 but no effect on other cancer cells.

Conflict of interest: *The authors declare no conflict of interest.*

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References

1. Azuma T., Kayano S., Matsumura Y., Konishi Y., Tanaka Y., Kikuzaki H. (2011), Antimutagenic and α-glucosidase inhibitory effects of constituents from *Kaempferia parviflora*, *Food Chemistry,* 125, 471-475. **2**. Sae-wong C., Tansakul P., Tewtrakul S. (2009), Anti-inflammatory mechanism of *Kaempferia parviflora* in murine macrophage cells (RAW 264.7) and in experimental animals, *Journal of Ethnopharmacology,* 124, 576-580. **3**. Tewtrakul S., Subhadhirasakul S., Karalai C., Ponglimanont C., Cheenpracha S. (2009), Anti-inflammatory effects of compounds from *Kaempferia parviflora* and *Boesenbergia pandurata*, *Food Chemistry,* 115, 534-538. **4**. Temkitthawon P., Hinds T. R., Beavo J. A., Viyoch J., Suwanborirux K., Pongamornkul W., Sawasdee P., Ingkaninan K. (2011), *Kaempferia parviflora*, a plant used in traditional medicine to enhance sexual performance contains large amounts of low-affinity PDE5 inhibitors, *Journal of Ethnopharmacology*, 137, 1437-1441. **5**. Stein R. A., Schmid K., Bolivar J., Swick A. G., Joyal S. V., Hirsh S. P. (2018), *Kaempferia parviflora* ethanol extract improves self-assessed sexual health in men: a pilot study, *Journal of Integrative Medicine*, 16, 249-254. **6**. Yenjai C., Prasanphen K., Daodee S., Wongpanich V., Kittakoop P. (2004), Bioactive flavonoids from *Kaempferia parviflora*, *Fitoterapia,* 75, 89-92. **7**. Thao N. P., Luyen B. T. T., Lee S. H., Jang H. D., Kim Y. H. (2016), Anti-osteoporotic and antioxidant activities by rhizomes of *Kaempferia parviflora* Wall. ex Baker, *Nature Products Science,* 22, 13-19. **8**. Lee M., Han A. R., Jang M., Choi H. K., Lee S. Y., Kim K. T., Lim T. G. (2018), Antiskin inflammatory activity of black ginger *(Kaempferia parviflora)* through antioxidative activity, *Oxidative Medicine and Cellular Longevity*, e5967150. **9**. Tangjitjaroenkun J., Yahayo W., Supabphol S., Supabphol R. (2021), Selective cytotoxicity of *Kaempferia parviflora* extracts in human cell lines, *Asian Pacific Journal of Cancer Prevention,* 22, 73-79. **10**. Thaklaewphan P., Ruttanapattanakul J., Monkaew S., Buatoom M., Sookkhee S., Nimlamool W., Potikanond S. (2021), *Kaempferia parviflora* extract inhibits TNF-α-induced release of MCP-1 in ovarian cancer cells through the suppression of NF-κB signaling, *Biomedicine & Pharmacotherapy,* 141, 111911. **11**. Hairunisa I., Bakar M. F. A., Da'i M., Bakar F. I. A., Syamsul E. S. (2023), Cytotoxic activity, anti-migration and in silico study of black ginger (*Kaempferia parviflora*) extract against breast cancer cell, *Cancers,* 15, 2785. **12**. Le H. L., Nguyen V. H., Nguyen T. D., Nguyen T. V. A., Le D. H. (2023), Potential antiaggregatory and anticoagulant activity of *Kaempferia parviflora* extract and its methoxyflavones, *Industrial Crops and Products,* 192, 116030. **13**. Sridhar K., Charles A. L. (2019), *In vitro* antioxidant activity of Kyoho grape extracts in DPPH and ABTS assays: Estimation methods for EC50 using advanced statistical programs, *Food Chemistry*, 275, 41-49. **14**. Thaipong K., Boonprakob U., Crosby K., Cisneros-Zevallos L., Hawkins Byrne D. (2006) Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts, *Journal of Food Composition and Analysis,* 19, 669-675. **15**. Luyen L., Thom V., Huong L. T., Huong D. L., Anh N. V. (2020), Inhibitory effect on human platelet aggregation, antioxidant activity, and phytochemicals of *Canna warszewiczii* (A. Dietr) Nb. tanaka, *Phcog Res,* 12, 47. **16**. Mishra T., Goyal A. K., Middha S. K., Sen A. (2011), Antioxidative properties of *Canna edulis* Ker-Gawl, 7. **17**. Liu Y., Peterson D. A., Kimura H., Schubert D. (1997), Mechanism of cellular 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction, *Journal of Neurochemistry,* 69, 581-593. **18**. Floegel A., Kim D. O., Chung S. J., Koo S. I., Chun O. K. (2011) Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods, *Journal of Food Composition and Analysis,* 24, 1043-1048. **19**. Chaisuwan V., Dajanta K., Srikaeo K. (2022) Effects of extraction methods on antioxidants and methoxyflavones of *Kaempferia parviflora*, *Food Research,* 6, 374-381. **20**. Sitthichai P., Chanpirom S., Maneerat T., Charoensup R., Tree-Udom T., Pintathong P., Laphookhieo S., Sripisut T. (2022), *Kaempferia parviflora* rhizome extract as potential anti-acne ingredient, *Molecules*, 27(14), 4401. **21**. Muflihah, Y. M., Gollavelli G., Ling Y. C. (2021), Correlation study of antioxidant activity with phenolic and flavonoid compounds in 12 Indonesian indigenous herbs, *Antioxidants*, *10,* 1530. **22**. Ghasemzadeh A., Jaafar H. Z. E., Rahmat A. (2010), Antioxidant activities, total phenolics and flavonoids content in two varieties of Malaysia young ginger (*Zingiber officinale* Roscoe), *Molecules*, *15,* 4324-4333. **23**. Karna P., Chagani S., Gundala S. R., Rida P. C. G., Asif G., Sharma V., Gupta M. V., Aneja R. (2012), Benefits of whole ginger extract in prostate cancer, *Bristish Journal of Nutrition,* 107, 473-484. **24**. Chen D., Li H., Li W., Feng S., Deng D. (2018) *Kaempferia parviflora* and its methoxyflavones: Chemistry and biological activities, *Evidence-Based Complementary and Alternative Medicine*, e4057456.