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PHYTOCHEMICAL PROFILING, ANTIOXIDANT AND CYTOTOXIC ACTIVITY OF TURMERIC-GINGER COMBINATION EXTRACT AGAINST CANCER CELL LINE

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ABSTRACT

Turmeric is recognized for various health benefits, commonly used in nutraceuticals and complementary medicine to alleviate pain and inflammation. It boosts the immune system and is also used for cancer treatment. The challenge in developing of turmeric formulation is the low bioavailability of curcumin as main compound. Literature reviews indicate that ginger is capable of improving blood circulation and enhancing the bioavailability of some compounds, including curcumin. This study aimed to examine the phytochemical profile, cytotoxic activity and antioxidant potential of the turmeric-ginger combination. The extraction was carried out by maceration 3x24 hours using 70% ethanol, evaporated, and dried in the oven. The thin layer chromatography (TLC) and high-performance liquid chromatography (HPLC) were used to obtain phytochemical profiles. Cytotoxic effect was carried out by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (MTT assay). Antioxidant assay was evaluated through H_2O_2-induced intracellular reactive oxygen species (ROS) quantification using flow cytometer. The HPLC profile revealed that curcumin levels in the turmeric-ginger combination ranged from 6-8%, with gingerol 4-11%. In T47D, HeLa, and WiDr cells, turmeric alone exhibited strong cytotoxicity, while ginger was less effective, and the combination actually reduced cytotoxic effect. However, in HepG2 cells, both turmeric and ginger alone exhibited weak cytotoxicity, surprisingly turmeric-ginger combination 50:50 showed synergistic effect. In Vero cells, ginger 25 and 50 µg/mL demonstrated a more significant capacity to decrease intracellular ROS levels induced by H_2O_2 when compared to turmeric. The combinations of turmeric ginger 70:30, 60:40, and 50:50 revealed a synergistic effect in reducing ROS levels. This research provides scientific evidence with a synergistic approach supporting the combination of turmeric-ginger to alleviate cancer growth and inhibit ROS generation.

Key words: turmeric, ginger, formulation, ROS generation, cytotoxic

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INTRODUCTION

Cancer is one of the leading causes of death and a significant barrier to improving the life expectancy of the global population. According to data from the World Health Organization (WHO) in 2019, the increase in cancer-related deaths is relatively higher as compares to strokes and coronary heart disease (Sung *et al.*, 2021). The Global Cancer Observatory (Globocan) reported that there were 396,914 new cancer cases and 234,511 cancer-related deaths in 2020. Lung, colon, and liver cancers are the most common cancers in men, with percentages of 14.1%, 11.9%, and 9.0%, respectively. The highest incidence of cancer in women is breast, cervical, and ovarian cancer, with percentages of 30.8%, 17.2%, and 7.0%, respectively (World Health Organization, 2021).

Current cancer treatments do not fully address the severity of the disease, leading to increased mortality and reduced quality of life for cancer patients. Conventional treatments are limited by side effects, such as organ damage and chemotherapy resistance. This emphasizes the need for alternative therapies that are both safer and more effective (Shankar *et al.*, 2022).Bioactive compounds from medicinal plants, can effectively influence cellular growth and proliferation of cancer cells due to their ability to target multiple pathways (Kanwal *et al.*, 2020). These compounds have shown chemo-preventive effects by reducing the risk of early-stage cancer and preventing metastasis. Chemo-preventive compounds inhibit the formation and activation of carcinogens, enhance the detoxification process by trapping reactive oxygen species (ROS), and halt the proliferation and differentiation of cancer cells. The

excessive generation of free radicals in metabolic processes poses a threat to living systems. Antioxidants, which neutralize free radicals, can prevent these chain reactions and safeguard biological systems from the damaging effects of oxidative stress. Phytochemicals like phenolic compounds inhibit ROS/RNS and reduce free radical production (Tahir *et al.*, 2023). Chemopreventive compounds have also been shown to enhance the effectiveness of conventional chemotherapy drugs (George *et al.*, 2021).

Turmeric (*Curcuma longa* L.) is one of the medicinal plants that has been extensively studied for its potential in combating cancer. Turmeric rhizomes are often used as a food additive, spice, or medicine. Curcumin is the active compound in turmeric rhizomes and contributes to various pharmacological functions, including anti-inflammatory, chemo-preventive, hepatoprotective, antioxidant, and immunomodulatory effects. Numerous studies have shown the effect of curcumin in inhibiting proliferation, angiogenesis, and metastasis, as well as inducing apoptosis in cancer cells (Shankar *et al.*, 2022). Drug delivery and bioavailability are challenges in achieving the pharmacological effects of curcumin. Poor absorption and rapid metabolism and systemic clearance contribute to low levels of curcumin in plasma and tissues. Adding adjuvants to curcumin formulations is one strategy to improve drug delivery systems and bioavailability (Tabanelli *et al.*, 2021).

Numerous approaches have been undertaken to improve the bioavailability of curcumin. Several adjuvants (bioavailability enhancers) are reported to enhance the bioavailability of curcumin. Ginger (*Zingiber officinale* Rosc.) could be added as a potential adjuvant. A concoction of ginger-turmeric tea has been studied for its anti-inflammatory activity, efficiently combating inflammation in the body. Ginger-turmeric tea also helps prevent cancer, fight diabetes, and serve as a natural remedy for high cholesterol. A clinical study using ginger and turmeric formulations has been reported to improve the quality of life related to body composition, bone mineral density, osteoporosis biomarkers, and micro-RNA in postmenopausal women with osteoporosis. The formulations used in the study were still separate and have not been combined into a single combination formula (Aghamohammadi *et al.*, 2021). Further development of more practical formulations will involve the development of a combined turmeric-ginger formulation to maximize their synergistic therapeutic effects. Therefore, this study was conducted to examine the phytochemical profile, cytotoxic effect and antioxidant potential of the turmeric-ginger combination.

MATERIALS AND METHODS

Sample preparation: Plant samples were taken from the collection gardens of the Center for Research and Development of Medicinal Plants and Traditional Medicines that were more than 10 months old. The sample was identified and the voucher specimen was deposited at Herbarium Tawangmanguense. The rhizomes are cleaned from the soil and other adhering dirt, then washed and it was sliced with a thickness of 2-3 mm. Furthermore, the rhizome was dried in an oven at 40°C for 3x24 hours until the moisture content was around 12%.

Extraction and Formulation: The rhizomes of turmeric and ginger were cleaned of impurities and washed, cut or chopped and dried, then each was ground or powdered with a size of 40 mesh. Each powder was weighed with the composition of the formula according to the treatment, then extracted by maceration 3x24 hours using 70% ethanol, and the extract collection was evaporated with a rotary evaporator until thick. The extract is dried until the solvent has completely evaporated. Ethanol 70% was obtained from a local supplier (General Labora, Yogyakarta, Indonesia).

TLC phytochemical profiling: The phytochemical profile was carried out on turmeric-ginger combination by TLC using silica gel GF 254 as stationary phase and chloroform: ethanol: ethyl acetate (80:19.5:0.51) as mobile phase. Visualization of the spot was done by boric acid in methanol reaction. The chromatogram profile was observed under UV light with λ 254 nm and 366 nm, and visible light. and gingerol standards.

Curcumin and gingerol quantification: The amount of 100.0 mg extract was carefully weighed in a sample bottle, dissolved in10.0 ml methanol, and sonicate for 15 minutes. The solution was filtered with a 0.45 nm membrane filter. Determination of 6-gingerol used Waters HPLC system, Reliant C-18 5 µm column (4.6 x 150 mm) with mobile phase aquadest:methanol (20:80), flow rate 1.0 mL/min, at a wavelength of 280 nm, and standard 6-gingerol (Sigma Aldrich USA) Determination of curcumin used Waters HPLC system, X-bridge C-18 column (3.0 x 250 mm) with mobile phase aquadest: acetic acid 2%: acetonitrile (49: 1: 50), flow rate 0.5 ml/min, at a wavelength of 425 nm, and standard curcumin (98% HPLC Sigma Aldrich).

Cytotoxicity assay on cancer cell lines: The cytotoxic test was carried out by observing the viability of breast cancer cells using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (MTT assay). The cancer cell lines used in the study were T47D (breast), WiDr (colon), HeLa (cervical), and HepG2 (liver). The confluent cell cultures were harvested and then distributed into 96-well microplate wells with a total of 10^4 cells/well. The cells were incubated for 24 hours in a CO_2 incubator so cells could adapt so they were ready for treatment. The test extract was stocked in Dimethyl sulfoxide (DMSO) solvent and then diluted using culture

media according to the specified concentration series. The cells were washed with PBS then the test extracts, either single or a series of combinations, were put into the wells (triplo). The cells were incubated again for 24 hours in the CO_2 incubator. After incubation, the test solution was removed and MTT reagent was added in the amount of 100 µl/well. Stopper reagent was added after 3 hours of incubation with MTT. Cells were incubated overnight at room temperature and protected from light. At the end of the incubation, the plate was shaken with a horizontal shaker for 10 minutes and then read with an ELISA reader at a wavelength of 595 nm. Combination Index (CI) was evaluated using Loewe formula as follows:

$$CI = \frac{(D)1}{(Dx)1} + \frac{(D)2}{(Dx)2} \quad (1)$$

(Dx) 1, (Dx) 2 = concentration of test substance 1 and test substance 2 used in a single treatment required to reduce the number of cells by x%, and (D)1, (D)2 = concentration of test substance 1 in combination with the concentration of test substance 2 which together reduce the number of cells by x%. The CI value quantitatively defines synergism (CI<1), additive effect (CI=1), and antagonism (CI>1).

Antioxidant assay: Antioxidant assay was carried out through H_2O_2-induced intracellular ROS quantification. A total of 5x104 cells/wells of Vero cells were grown in Dulbecco's modified Eagle's medium (DMEM) culture medium on 24 well plates, incubated in a 5% CO_2 incubator, 37°C for 24 hours. Cells were harvested using 300 µL trypsin-EDTA for 3-5 minutes, then added 500 µL supplemented buffer (10% FBS in PBS) for trypsin inactivation. Cells were then collected in microtubes. A total of 25 µM DCFDA solution was given to each microtube. Cells were incubated in a CO_2 incubator, for 30 minutes at 37°C. After that, cells were treated with single and combination test extracts and ROS inductor (500 µM H_2O_2), incubated for 120 minutes. ROS analysis was performed using a BD Accuri C-6 flow cytometer at 458 nm/535 nm. The average fluorescence intensity was used to determine intracellular ROS levels (Hadi *et al.*, 2020). Combination Index (CI) value was evaluated by the Chou-Talalay method using the CompuSyn software.

RESULTS

Extract Formulation: The yield of turmeric-ginger combination showed that the addition of ginger up to 20% significantly increased the yield of the extract. The highest extract yields up to 19.25% was obtained from the turmeric-ginger ratio of 90:10. However, the addition of ginger above 20% decreased the extract yield compared to single turmeric extract even though it was not significantly different, as shown in Table 1.

Standardization of turmeric ginger combination extract: In this study, the standardization of the combination of ginger and turmeric extract was carried out using TLC and HPLC. The chromatogram profile of the combination of turmeric and ginger extract resulting from thin layer chromatography can be seen in Figure 1 below.

Qualitative analysis of the chromatogram profile of ginger turmeric extract showed similarities in the number and color of spots (Figure 1). The presence of curcumin compounds in turmeric ginger extract with the addition of ginger in various combinations did not change, with indications that there was no change in the color and Rf value of the spots that appeared compared to standard curcumin. Quantitative standardization of ginger-turmeric combination extract was carried out by measuring curcumin and gingerol levels using the HPLC method. The linearity of the two standards was determined by constructing a calibration curve for the relationship between the concentrations of curcumin and gingerol and the area of the two compounds. Fig. 2. showed the correlation coefficient value (R^2) for curcumin and gingerol was 0.994 and 0.997. respectively. This value indicates that the HPLC technique to measure curcumin and gingerol levels was specific and linear for measurement curcumin compounds (Ghozali, 2018).

Table 1. Determination of yield for turmeric ginger extract formula.

No	Composition (%)		Material weight (g)	Weight of extract (g)	Extract yield (%)
	Turmeric	Ginger			
1	100	0	200	32.0	16.00[b]
2	90	10	200	38.5	19.25[a]
3	80	20	200	35.5	17.75[a]
4	70	30	200	31.5	15.75[b]
5	60	40	200	31.5	15.75[b]
6	50	50	200	30.0	15.00[b]

Note: number follows by the same alphabet shows non-significant different (P> 0.01)

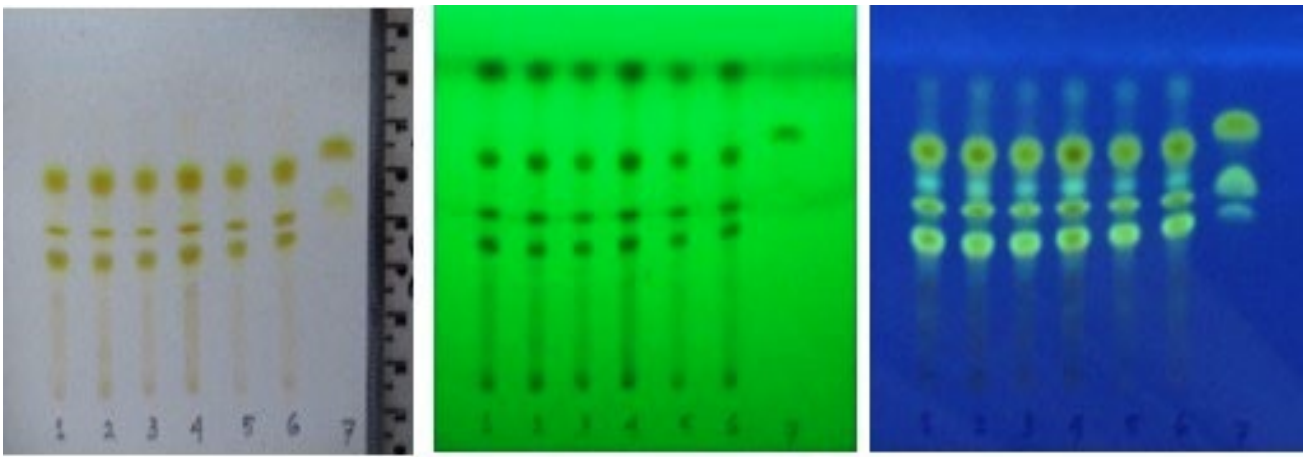

Figure 1. TLC profile of the turmeric ginger combination. Notes: 1. T (100%); 2. TG (90:10); 3. TG (80:20); 4. TG (70:30); 5. TG (60:40; 6. TG (50:50); 7. Curcumin standard

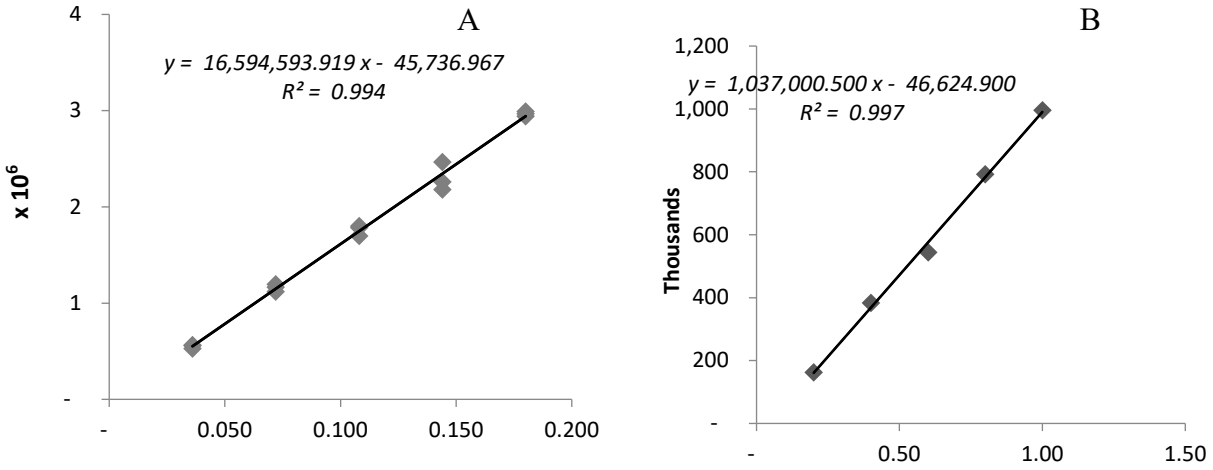

Figure 2. Determination of the linearity curve of curcumin and gingerol from turmeric and ginger simplicia; A. curcumin standard, B. gingerol standard

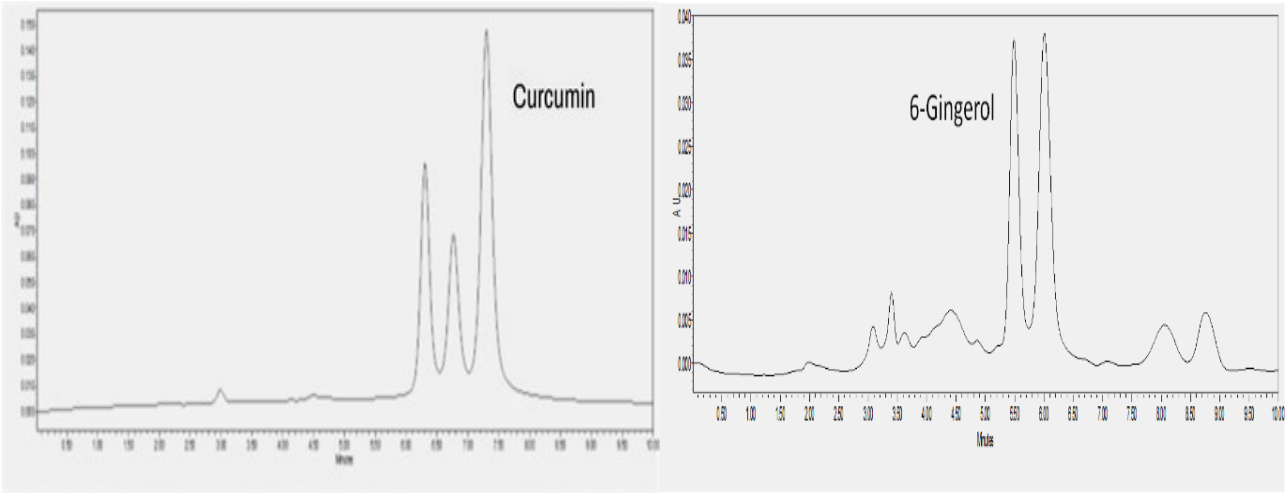

Figure 3. HPLC chromatograms of standard curcumin (a) and standard 6-gingerol (b) to calculate the curcumin and 6-gingerol content within the extract.

Table 1. Determination of curcumin and gingerol in the turmeric-ginger extract combination

No	Composition (%)		Curcumin (%)	Gingerol (%)
	Turmeric	Ginger		
1	100	0	8.09±0.15	N/A
2	90	10	8.29±1.34	N/A
3	80	20	7.45±0.33	5.07±0.67
4	70	30	7.01±0.50	4.58±0.48
5	60	40	6.51±0.31	5.71±0.19
6	50	50	6.38±0.19	6.33±0.82
7	0	100	N/A	11.63±1.15

Cytotoxic evaluation of turmeric ginger extract: The cytotoxic effects of turmeric-ginger extracts were conducted by MTT assay on T47D, WiDr, Hela and HepG2 cancer cell line, also Vero cells as a control of normal cellular function. The results on single turmeric extract indicated cytotoxic effects on all cell types, with the highest activity in T47D cells, followed by HeLa, WiDr, and HepG2 cells. Ginger alone exhibited a very weak cytotoxic effect compared to turmeric. The addition of ginger to the extract formulation led to reduction in the effects of turmeric on T47D, HeLa, and WiDr cells. The enhanced cytotoxic effects were only observed in the formulation of turmeric-ginger 50:50 on HepG2 cells. Figure 4 illustrates the correlation between the extract concentration and cells viability.

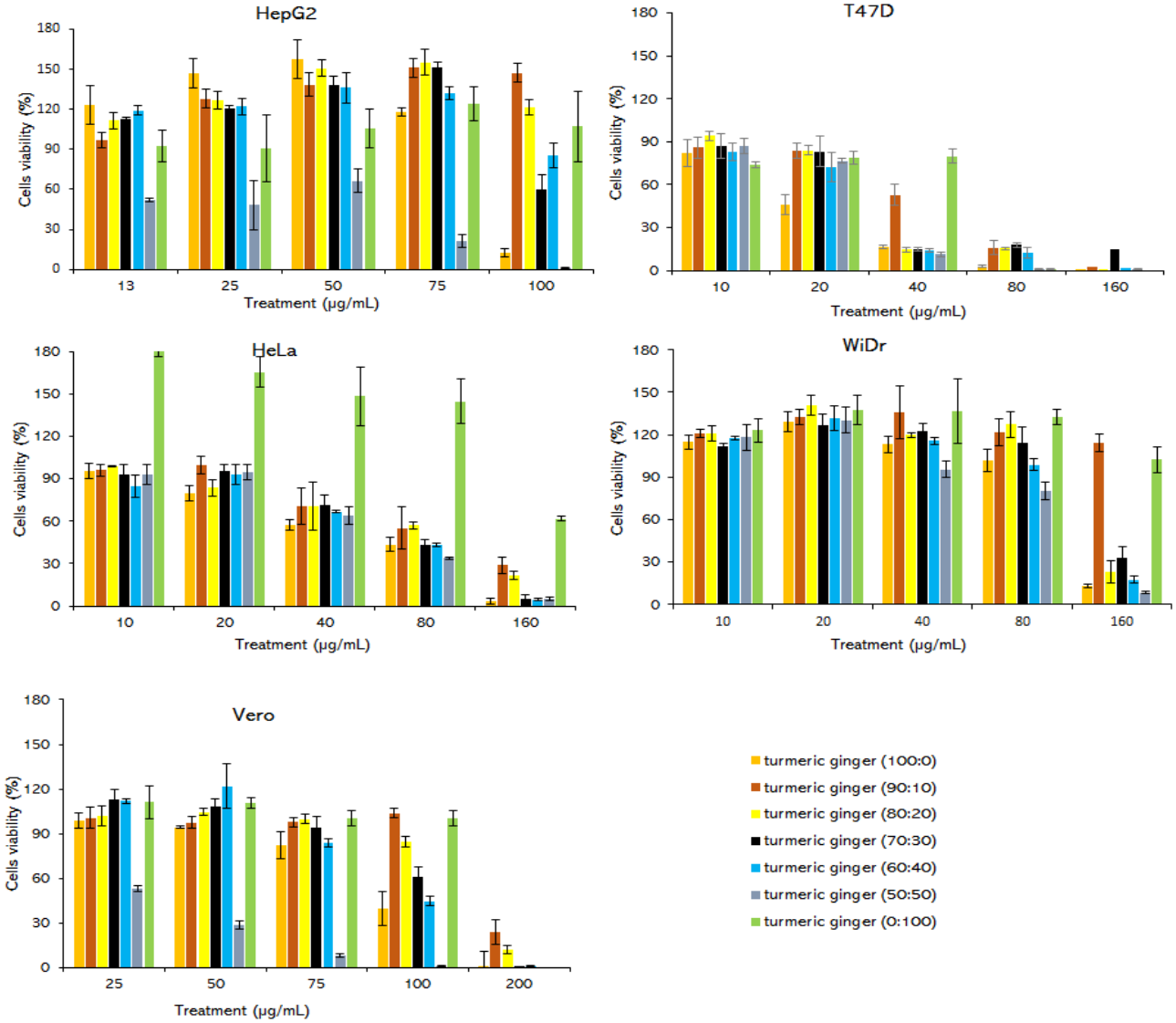

Figure 4. Cytotoxicity analysis using MTT assay on HepG2, T47D, HeLa WiDr, and Vero cells. Cells were treated with varying concentrations of turmeric-ginger extract for 24 h. Cells viability was determined in triplicate from three independent experiments. Data represented as mean ± SD

The IC_{50} value was derived by examining the linear regression of extract concentration and cell viability (Table 2). The analysis revealed that the IC_{50} value was comparatively higher in Vero cells when compared to cancer cells. These findings suggested that the extracts were more toxic or selective on cancer compared to normal cells. In T47D, HeLa and WiDr cells, turmeric alone exhibited strong cytotoxic activity with an IC_{50} value of 17.2, 54.4 and 81.3 µg/mL respectively, while ginger alone showed weak effects with the IC_{50} up to 100 µg/mL to all cells. In HepG2 cells, turmeric and ginger alone demonstrated a relatively weak cytotoxic effect, with an IC_{50} value of 105.6 and 297.3 µg/mL respectively. The addition of 10-40% ginger resulted to an elevation of the IC_{50} value. However, the addition of 50% ginger was surprisingly enhanced the cytotoxic effect, resulting in a significantly lower IC_{50} value compared to single turmeric and ginger. We investigated the impact of the extracts on the normal kidney Vero cell line. The extract displayed low cytotoxic effects on Vero cells, with an IC_{50} value of more than 100 µg/mL, except for turmeric ginger 50:50 of 73 µg/mL.

Furthermore, to determine the interaction of ginger and turmeric extracts, we calculated the combination index using the Loewe additivity model, often referred to as the Loewe formula. It is a mathematical approach used in pharmacology and toxicology to assess the combined effect of two or more compounds. It is a way of quantifying the interaction between these substances when they are used together. This model is particularly relevant in the study of drug combinations, where it helps determine whether the combined effect of drugs is additive, synergistic, or antagonistic. The interpretation of CI = 1, indicates additivity (the observed effect matches the expected effect); CI < 1, suggests synergy (the compounds work better together); CI > 1, suggests antagonism (the compounds work together less effectively) (Hemaiswarya *et al.*, 2022). As seen in Table 3, synergistic interaction was only produced in the combination of turmeric and ginger (50:50) in HepG2 cells. The other combination ratios in all cell lines resulted in CI values indicating antagonistic interactions.

Antioxidant evaluation of turmeric ginger extract: We conducted a DCFDA staining assay and found that ginger alone at a concentration of 25 and 50 µg/mL exhibited a greater ability to decrease intracellular ROS levels compared to turmeric. However, the combination turmeric ginger 70:30, 60:40 and 50:50 showed significant reduction in intracellular ROS levels induced by H_2O_2.

In order to evaluate the characteristics of drug interactions in the previous combinations, the Chou-Talalay method was employed using the CompuSyn software to determine the combination index (CI). The combination of turmeric ginger 70:30, 60:40, and 50:50 exhibited synergistic interaction with CI values < 1 (Figure 6). This implies that the synergistic outcome between turmeric and ginger were observed when the amount of ginger added exceeds 30%.

Table 2. The IC_{50} of turmeric ginger extract on cell cancer lines.

No	Composition (%)		IC_{50} value (µg/mL)				
	Turmeric	Ginger	Vero	T47D	HepG2	HeLa	WiDr
1	100	0	113.0±5,2	17.2±3.2	105.6±8.6	54.4±1.5	81.3±2.4
2	90	10	170.5±2.3	36.8±2.7	500.0±2.5	151.1±4.9	136.3±6.1
3	80	20	145.9±1.7	28.1±1.6	264.4±3.4	124.3±6.5	114.7±2.8
4	70	30	126.6±3.0	24.3±4.9	151.9±3.5	98.2±2.1	90.6±3.5
5	60	40	121.4±5.3	22.7±1.5	152.6±7.3	86.9±3.5	81.7±3.1
6	50	50	73.2±14.9	20.9±3.9	52.3±9.0*	94.0±2.1	106.0±3.5
7	0	100	388.2±3.5	111.5±8.4	297.3±10.6	153.5±7.6	226.5±6.6

Table 3. The Combination Index (CI) of turmeric ginger combinations on cytotoxic activity

No	Composition (%)		CI value				
	Turmeric	Ginger	Vero	T47D	HepG2	HeLa	WiDr
1	90	10	3.6	2.5	6.4	3.8	2.3
2	80	20	1.1	1.9	3.4	3.1	1.9
3	70	30	1.1	1.6	1.9	2.4	1.5
4	60	40	0.6	1.5	2.0	2.2	1.4
5	50	50	1.4	1.4	0.7	2.3	1.8

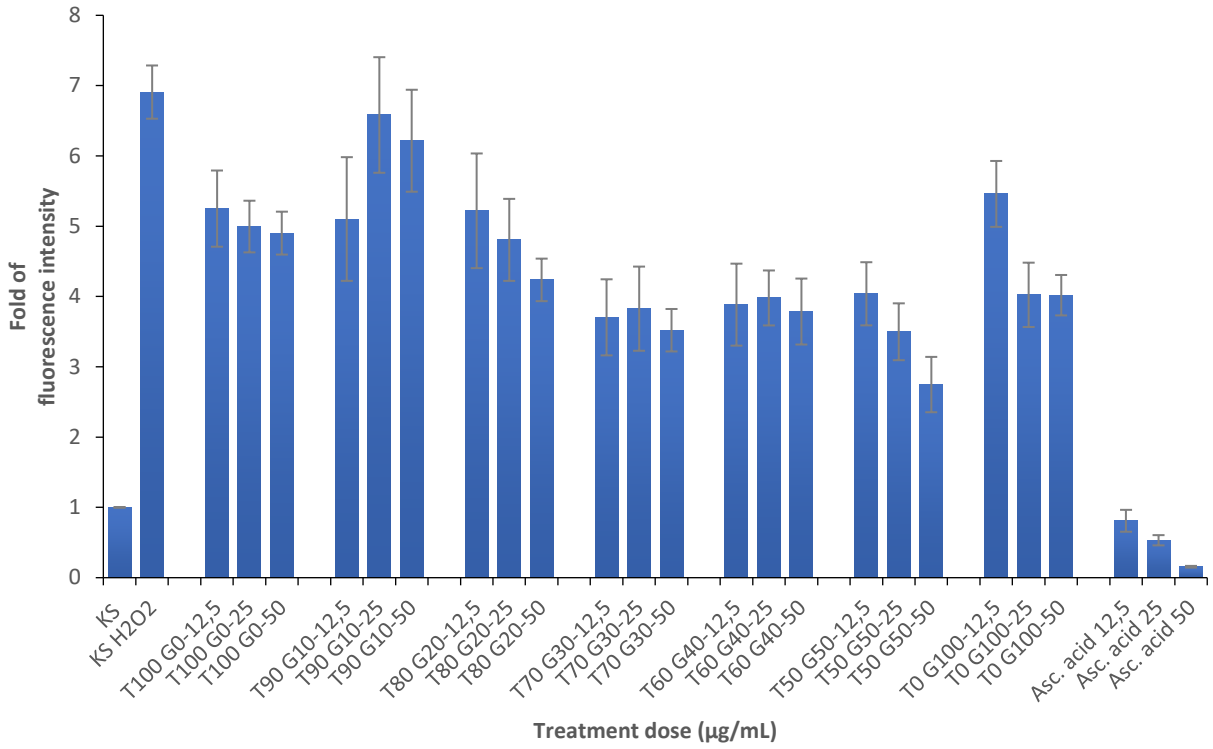

Figure 5. The ROS intracellular level of Vero cells induced by H_2O_2 following turmeric ginger treatment. The mean fluorescence was determined by flow cytometer. The mean of three independent experiments was used to calculate the results.

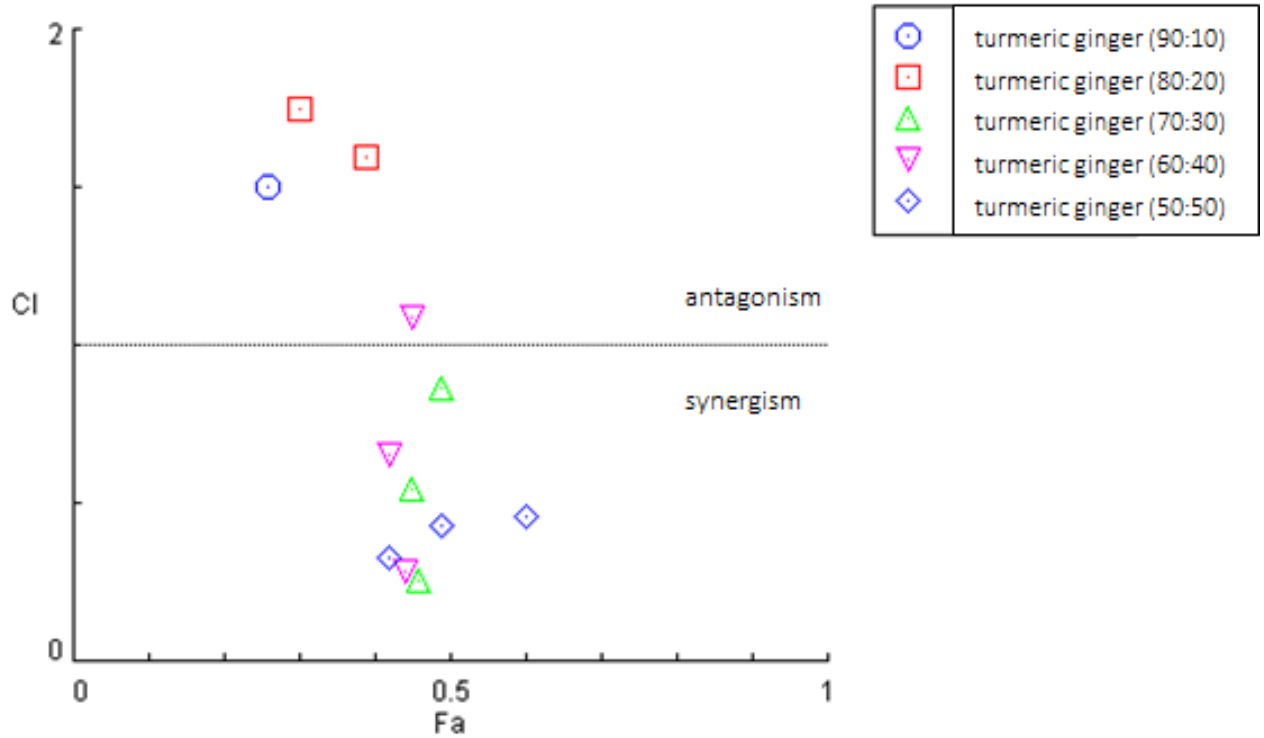

Figure 6. The combination index vs. fraction affected (CI vs. Fa plots) for ROS intracellular using CompuSyn software. CI values less than 1 are recognized as having a synergistic effect.

DISCUSSION

Ginger and turmeric are widely used in nutraceuticals and complementary medicines for their various health benefits. These include anti-inflammatory effects, immune system enhancement, pain relief, and antitumor. The individual anticancer properties of ginger and turmeric have been extensively researched in preclinical studies (Ahmad and Damayanti, 2018; Mbese *et al.*, 2019; Jalali *et al.*, 2020). This study revealed that the co-administration of turmeric with ginger in certain composition synergistically inhibited cancer cell growth and ROS generation. Thus, this could be utilized to improve the formulation techniques of turmeric.

In the present study, the turmeric-ginger combination was extracted using the maceration technique with 70% ethanol. Previous research showed that soxhlet method was the best method to extract curcumin from turmeric rhizome compared to microwave, ultrasound and enzymatic assisted extraction method (Sahne *et al.*, 2017). Sabir *et al.* (2021) revealed that ethanolic extract seem to be the most appropriate solvent to increase and to prevent the curcumin content within turmeric extract. Other research showed that the acetone provided better extraction results than other solvents to produce the highest levels of curcumin (Joshi *et al.*, 2021). However, choosing the appropriate solvent and extraction method should consider several factors, such as selectivity, solubility, cost, and safety. With this various consideration, ethanol is commonly employed as universal solvents for extraction process in phytochemical investigations and industrial-scale extraction (Zhang *et al.*, 2018).

The TLC chromatogram indicated that all turmeric-ginger combinations had a similar phytochemical profiles. Following the initial TLC, our research proceeded to quantitatively assess the curcumin and gingerol in each extract combination by HPLC method. The findings were expressed in relation to their corresponding standard curcumin and gingerol. The analysis of HPLC chromatogram showed that curcumin and gingerol at varying levels were detected in all extracts according to their composition ratios. Curcumin levels obtained through HPLC analysis in the turmeric-ginger combination ranged from 6-8%, with gingerol levels ranging from 4-11%.

In summary, the cytotoxic effects of turmeric-ginger combination varied in different cell lines. Turmeric alone showed strong cytotoxic potential compared to ginger. Many references indicated that single turmeric had a strong cytotoxic effect on various cell lines (Cozmin *et al.*, 2024). Thus, turmeric provided a more dominant cytotoxic activity in combination with ginger. The addition of 10-20% ginger, actually decreased the cytotoxic effect, although not significantly, in T47D cells. However, the combination of turmeric and ginger reduced the cytotoxic effect in HeLa, and WiDr cells significantly. On the other hand, in HepG2 cells, the combination showed a synergistic effect. These findings suggest that the response of turmeric and ginger may depend on the specific cell type and the interaction between these compounds. The quantification results using HPLC showed the combination of turmeric ginger 50:50 ratio contains 6.38±0.19% curcumin and 6.33±0.82% gingerol.

Interestingly, single ginger treatment showed a stronger dominance in inhibiting the increase of ROS in Vero cells. The combination of turmeric-ginger 70:30, 60:40, and 50:50 exhibited synergistic interaction with CI values < 1. ROS played significant roles in various cellular processes such as proliferation, differentiation, migration, apoptosis, and necrosis. Maintaining low to moderate levels of ROS and reactive nitrogen species (RNS) are essential for preserving many critical physiological functions, redox balance, and the regulation of key transcription factors (Jomova *et al.*, 2023). The excessive ROS production occurred in pathological conditions and exposure to stressful environmental factors. These pathological effects typically involve the opening of ion channels, lipid peroxidation, modifications to proteins, and oxidation of DNA. The pro-inflammatory molecules generated as a result of ROS in stressful situations contribute to inflammation, which plays a significant role in the aging process and the development of various diseases. These diseases encompass vascular disorders, autoimmune conditions such as rheumatoid arthritis and inflammatory bowel disease, neurodegenerative disorders and age-related macular degeneration, as well as respiratory ailments, acute lung injury, and cystic fibrosis (Checa and Aran, 2020).

Our findings indicated that turmeric-ginger have the ability to modulate ROS levels in Vero cells, which could be beneficial for nephro-protection. Kidney damage exacerbates the quality of life and raises morbidity rates in high-complexity patients, such as those with both cancer and kidney injury (García-Carro *et al.*, 2022). In the recent study conducted by Akinyemi *et al.* (2018), it was observed that the oral administration of essential oils extracted from ginger and turmeric rhizomes exhibited nephroprotective effects in rats treated with cadmium (Cd). The possible mechanism of nephroprotective activity might be linked to their ability to inhibit adenosine deaminase activity and modulate inflammatory cytokines (Akinyemi *et al.*, 2018).

The results of most studies suggest that phenolic compounds play a crucial role in the antioxidant activity of medicinal plants. These compounds have potential applications in food supplements and the pharmaceutical industry (Salehi *et al.*, 2020). Curcumin is the main polyphenolic compound of turmeric rhizomes which used as an antioxidant, arthritis, anxiety, anti-inflammatory, and hypocholesterolemia agent (Hewlings and Kalman,

2017). Curcumin is also widely used for the treatment of breast, prostate, bone and lung cancer (Allegra *et al.*, 2016). However, the low solubility and bioavailability of turmeric limited its potential as a pharmaceutical oral dosage form (Hegde *et al.*, 2023). The effectiveness of orally ingested curcumin is limited due to low absorption, rapid metabolism, and elimination in the body. Curcumin, being a highly hydrophobic molecule, is practically insoluble in water. It reveals a relatively short half-life due to alkaline instability at physiological pH. The combination of poor aqueous solubility and alkaline instability lead to very low plasma levels of curcumin, severely restricting the therapeutic potential of curcumin (Kothaplly *et al.*, 2022).

Studies have shown that ginger enhances blood circulation and the bioavailability of certain drugs when administered together. Ginger is also known to modulate drug metabolism enzymes and P-glycoprotein, which affect absorption. In Ayurvedic practice, ginger is used to improve blood flow, indirectly enhancing the absorption of drugs and other compounds (Nduka *et al.*, 2013). Gingerol, shogaol, and paradol represent the primary phenolic compounds found in ginger, which play a significant role in its biological effects (Subositi *et al.*, 2022). Gingerol has also been reported to enhance the bioavailability of curcumin (Jacob, 2012). Literature reviews indicate the benefits of ginger as a nutraceutical source for preventing chronic diseases, antioxidant properties, neuroprotection, and cardiovascular protection (Mao *et al.*, 2019). In a patent work by Qazi *et al.* (2003), it was shown that gingerol enhanced the bioavailability of several nutraceutical and drugs such as vitamins A, E, C, folic acid, b-carotene, silymarin, isoleucine, zinc and potassium. The patent showed a detailed study of gingerol as a bioavailability enhancer. Unfortunately, our study has not been able to demonstrate the exact effect of ginger in enhancing the bioavailability of curcumin. Further preclinical studies using animal models and clinical trials are needed to establish the reinforcement of the turmeric-ginger combination's activity.

Conclusions: The ratio of ginger 10-20% to the turmeric-ginger combination significantly increase the extract yield, curcumin level, and cytotoxic effects compared to the single turmeric extract. The impact of turmeric and ginger on cytotoxicity varies according to the specific cell type. Turmeric alone displays strong cytotoxic effects on T47D, HeLa, and WiDr cells. However, the combination with ginger revealed cytotoxic reduction effect. Meanwhile, the turmeric-ginger combination 50:50 demonstrates a synergistic effect in HepG2 cells. The synergistic impact in suppressing ROS production is attained with turmeric-ginger ratios of 60:40, 70:30, and 50:50. This research provides scientific evidence supporting the use of a combination of turmeric and ginger to reduce cancer cell growth. The combination also working as an antioxidant by suppression of ROS, potentially beneficial in reducing the nephrotoxicity of chemotherapy treatment.

Author contributions: All authors contributed equally to the concept idea and design of the study. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest: All of the authors declares that there is no conflict of interest regarding the publication of this paper.

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REFERENCES

Aghamohammadi, D., N. Dolatkhah, S.K. Shakouri, P. Hermann and F. Eslamian. (2021). Ginger (Zingiber officinale) and turmeric (Curcuma longa L.) supplementation effects on quality of life, body composition, bone mineral density and osteoporosis related biomarkers and micro-RNAs in women with postmenopausal osteoporosis: A study protocol for a randomized controlled clinical trial. J. Complement. Integr. Med. 18(1): 131–137. https://doi.org/10.1515/JCIM-2020-0017/PDF

Ahmad, Z. and Damayanti (2018). Skin aging: pathophysiology and clinical manifestation. Periodical of Dermatology and Venereology. 3(3): 208–215. https://doi.org/10.20473/bikk.V30.3.2018.208-215

Akinyemi, A. J., O.L. Faboya, A.A. Paul, I. Olayide, O.A. Faboya and T.A. Oluwasola (2018). Nephroprotective effect of essential oils from ginger (Zingiber officinale) and turmeric (Curcuma longa) rhizomes against cadmium-induced nephrotoxicity in rats. J. Oleo Sci. 67(10): 1339–1345. https://doi.org/10.5650/jos.ess18115

Allegra, A., V. Innao, S. Russo, D. Gerace, A. Alonci and C. Musolino (2016). Anticancer activity of curcumin and its analogues: preclinical and clinical studies. Cancer Invest. 35(1): 1–22. https://doi.org/10.1080/07357907.2016.1247166

Cozmin, M., I. I. Lungu, C. Gutu, A. Stefanache, L.D. Duceac, B.D. Șoltuzu, D. Damir, G. Calin, E.R. Bogdan Goroftei, C. Grierosu and M. Boev (2024). Turmeric: from spice to cure: a review

of the anti-cancer, radioprotective and anti-inflammatory effects of turmeric sourced compounds, Frontiers in Nutrition, 11: 1399888. https://doi.org/10.3389/fnut.2024.1399888

Checa, J. and J.M. Aran (2020). Reactive oxygen species: drivers of physiological and pathological processes. J. Inflamm. Res. 13: 1057–1073. https://doi.org/10.2147/JIR.S275595

García-Carro, C., J. Draibe and M.J. Soler (2022). Onconephrology: update in anticancer drug-related nephrotoxicity. Nephron. 147(2): 65–77. https://doi.org/10.1159/000525029

George, B.P., R. Chandran and H. Abrahamse (2021). Role of phytochemicals in cancer chemoprevention: insights. Antioxidants. 10(9): 1455. https://doi.org/10.3390/antiox10091455

Ghozali, I. (2018). Application of multivariate analysis with IBM SPSS 25 program, 9th ed. Diponegoro University Press, Semarang, Central Java.

Hadi, I., R.I. Jenie and E. Meiyanto (2020). Pentagamavunon-0 (PGV-0) enhance cytotoxic effect of doxorubicin through increasing of apoptosis, senescence and ROS level on Triple Negative Breast Cancer 4T1. Indonesian J. Cancer Chemoprevention. 11(1), 7–15. https://doi.org/10.14499/indonesianjcanchemoprev11iss1pp7-15

Hegde, M., S. Girisa, B. BharathwajChetty, R. Vishwa and A.B. Kunnumakkara (2023). Curcumin formulations for better bioavailability: what we learned from clinical trials thus far? ACS Omega. 8(12): 10713–10746. https://doi.org/10.1021/acsomega.2C07326

Hemaiswarya, S., P.K. Prabhakar and M. Doble (2022). Measuring herb-drug interaction and synergy; pp. 5–14. In herb-drug combinations: a new complementary therapeutic strategy. Springer Nature Singapore, Singapore. https://doi.org/10.1007/978-981-19-5125-1_2

Hewlings, S.J. and D.S. Kalman. (2017). Curcumin: a review of its effects on human health. Foods (Basel, Switzerland). 6(10): 92. https://doi.org/10.3390/foods6100092

Jacob, C.V. (2012). Synergistic composition for enhancing bioavailability of curcumin. United States Patent Appl. Publ. within the TVPP US201113083388. 08 Apr 2011. https://www.surechembl.org/document/US-20120058208-A1

Jalali, M., M. Mahmoodi, S.P. Moosavian, R. Jalali, G. Ferns, A. Mosallanezhad, M. H. Imanieh and Z. Mosallanezhad (2020). The effects of ginger supplementation on markers of inflammatory and oxidative stress: a systematic review and meta-analysis of clinical trials. Phytotherapy Research. 34(8): 1723–1733. https://doi.org/10.1002/ptr.6638

Jomova, K., R. Raptova, S.Y. Alomar, S.H. Alwasel, E. Nepovimova, K. Kuca and M. Valko (2023). Reactive oxygen species, toxicity, oxidative stress, and antioxidants: chronic diseases and aging. Arch of Toxicol. 97(10): 2499–2574. https://doi.org/10.1002/ptr.6638

Joshi, N.D., A.A. Kulkarni and M.N. Cherekar (2021). Extraction and purification of curcumin from turmeric. Pelagia Research Library Asian J. Plant Science and Research. 2021(8): 256–259. https://doi.org/10.36648/2249-7412.21.S2.256-259

Kanwal, N., A. Rasul, G. Hussain, H. Anwar, M.A. Shah, I. Sarfraz, A. Riaz, R. Batool, M. Shahbaz, A. Hussain and Z. Selamoglu (2020). Oleandrin: abioactive phytochemical and potential cancer killer via multiple cellular signaling pathways. Food Chem. Toxicol. 143: 111570. https://doi.org/10.1016/J.FCT.2020.111570

Kothaplly, S., S. Alukapally, N. Nagula and R. Maddela. (2022). Superior bioavailability of a novel curcumin formulation in healthy humans under fasting conditions. Adv Ther. 39(5): 2128–2138. https://doi.org/10.1007/s12325-022-02081-w

Mao, Q. Q., X.Y. Xu, S.Y. Cao, R.Y. Gan, H. Corke, T. Beta and H. Bin Li (2019). Bioactive compounds and bioactivities of ginger (Zingiber officinale Roscoe). Foods. 8(6). https://doi.org/10.3390/FOODS8060185.

Mateen Tahir, M., A. Rasul, A. Riaz, R. Batool, B. Nageen, M. Adnan, I. Sarfraz, A. Sadiqa and Z. Selamoglu. (2023). Ficus benghalensis: a plant with potential pharmacological properties, from tradition to pharmacy. Tropical J. Pharmaceutical Research. 22(11): 2407–2413. https://doi.org/10.4314/tjpr.v22i11.22

Mbese, Z., V. Khwaza and B.A. Aderibigbe (2019). Curcumin and its derivatives as potential therapeutic agents in prostate, colon and breast cancers. Molecules (Basel, Switzerland). 24(23): 4386. https://doi.org/10.3390/molecules24234386

Shankar, M.G., M. Swetha, C.K. Keerthana, T.P. Rayginia and R.J. Anto (2022). Cancer chemoprevention: a strategic approach using phytochemicals. Front. Pharmacol. 12: 4044. https://doi.org/ 10.3389/fphar.2021.809308

Nduka, S.O., M.J. Okonta and C.O. Esimone (2013). Effects of Zingiber officinale on the plasma pharmacokinetics and lung penetrations of ciprofloxacin and isoniazid. American J. Therapeutics. 20(5): 507–513. https://doi.org/10.1097/mjt.0b013e31820544be

Qazi, G., K. Bedi, R. Johri, M. Tikoo, A. Tikoo, S. Sharma, S. Abdullah, O. Suri, B. Gupta, K. Suri, N. Satti, R. Khajuria, S. Singh, A. Khajuria and B. Kapahi. (2003). Bioavailability enchancing activity of Zingiber officinale Linn and its extracts/fractions thereof. (US20030170326A1).Braz.J.Biol.

Sabir, S.M., A. Zeb, M. Mahmood, S.R. Abbas, Z. Ahmad and N. Iqbal (2021). Phytochemical analysis and biological activities of ethanolic extract of Curcuma longa rhizome. Brazilian J. Biology. 81(3): 737–740. https://doi.org/10.1590/1519-6984.230628

Sahne, F., M. Mohammadi, G.D. Najafpour and A.A. Moghadamnia (2017). Enzyme-assisted ionic liquid extraction of bioactive compound from turmeric (Curcuma longa L.): Isolation, purification and analysis of curcumin. Ind Crops Prod. 95: 686–694. https://doi.org/10.1016/j.indcrop.2016.11.037

Salehi, B., Z. Selamoglu, M. Sevindik, N.M. Fahmy, E. Al-Sayed, M. El-Shazly, B. Csupor-Löffler, D. Csupor, S.E. Yazdi, J. Sharifi-Rad, D.K. Arserim-Uçar, E.H. Arserim, N. Karazhan, A. Jahani, A. Dey, H. Azadi, S.A. Vakili, F. Sharopov, N. Martins and D. Büsselberg (2020). Achillea spp.: a comprehensive review on its ethnobotany, phytochemistry, phytopharmacology and industrial applications. Cell Mol Biol. 66(4): 78–103. https://doi.org/10.14715/CMB/2020.66.4.13.

Subositi, D., H. Widodo, R. Mujahid, N. Rahmawati, F.I. Mustofa, S. Haryanti, I.Y.M. Sholikhah, A. Maruzy and Y. Widiyastuti. (2022). Genetic diversity and chemicals profile of ginger (Zingiber officinale Roscoe) in Indonesia. Int J Adv Sci Eng Inf Technol. 12(3): 929–936. https://doi.org/10.18517/ijaseit.12.3.15660

Sung, H., J. Ferlay, R.L. Siegel, M. Laversanne, I. Soerjomataram, A. Jemal and F. Bray (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 71(3): 209–249. https://doi.org/10.3322/CAAC.21660

Tabanelli, R., S. Brogi and V. Calderone (2021). Improving Curcumin bioavailability: current strategies and future perspectives. Pharmaceutics. 13(10). https://doi.org/10.3390/pharmaceutics13101715

World Health Organization. (2021). Indonesia (Source: Globocan 2020). 1–2 pp.

Zhang, Q.W., L.G. Lin and W.C. Ye (2018). Techniques for extraction and isolation of natural products: a comprehensive review. Chin Med. 13: 20. https://doi.org/10.1186/s13020-018-0177-x.