Journal of
Food and Pharmaceutical Sciences

Review Article

Kaempferia galanga (L.): An Updated Overview of In Vitro and In Vivo Antioxidant Properties

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Received: 24 September 2023; Revised: 4 April 2024; Accepted: 17 April 2024; Published: 30 April 2024

Abstract: *Kaempferia galanga* L. (*K. galanga*) locally called aromatic ginger or "*kencur*" in Bahasa is traditionally used for the treatment of various diseases related to antioxidants such as anti-inflammatory, anti-cancer, and immunomodulator. This study aimed to provide a critical review of the current antioxidant activity of *K. galanga* using *in vitro* and *in vivo* assays. The information and data on *K. galanga* were collected from various sources such as Scopus, PubMed, Science Direct, and Google Scholar. This review is reported according to PRISMA, 21 articles were included in this review. The results showed that *K. galanga* plays a role in the defense systems against oxidative stress. Several *in vitro* assays have been used to measure the antioxidant activity of *K. galanga*, namely, 1-diphenyl-2-picrylhydrazyl (DPPH), ferric reduction of antioxidant power (FRAP), and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS). The *in vivo* antioxidant activity of *K galanga* included lifespan, survival life, intracellular reactive oxygen species (ROS) levels, malondialdehyde (MDA) levels, and antioxidant enzyme activities, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px). Based on these findings, it can be concluded that *K. galanga* has powerful antioxidant activities with IC_{50} of 7.93 μg/mL-3.09 ± 0.34 mg/mL, and therefore, could have potential as a natural antioxidant.

Keywords: antioxidant, *Kaempferia galanga* (L.), *in vitro*, *in vivo*, oxidative stress

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1. INTRODUCTION

A Global Burden of Disease study, in Indonesia showed a decrease in the prevalence of infectious diseases between 1990 and 2019, despite communicable diseases such as lower respiratory infections, diarrheal illnesses, and tuberculosis. Otherwise, non-communicable diseases, especially stroke, ischaemic heart disease, and diabetes become prioritized by policymakers [1]. Numerous neurological conditions (such as Alzheimer's disease, Parkinson's disease, multiple sclerosis, amyotrophic lateral sclerosis, memory loss, and depression), pulmonary conditions (such as asthma and chronic obstructive pulmonary disease), rheumatoid arthritis, nephropathy, ocular diseases, cancer, diabetes, ageing, infection, inflammation, and the foetus are all linked to oxidative stress [2–4]. In the human body, oxidative stress is caused by an imbalance between reactive oxygen species (ROS) and antioxidants [5].

Reactive oxygen species (ROS) are produced during biological processes in living organisms [6]. ROS plays a role in the immune system against pathogens such as fungi and bacteria [7]. However, the overproduction of ROS leads to biomolecular damage, including DNA, lipids, and proteins [8]. In addition, ROS induces the breakdown of the peptide chains, cross-linking proteins,

oxidizing amino acids, and lipid peroxidation, thus causing cell death [9]. Free radicals, commonly known as ROS, contain one or more unpaired electrons. Non-free radicals, such as hydrogen peroxide and singlet oxygen, can be reactive free radicals [10]. The odd number of electrons in free radicals causes instability and is a highly reactive molecule. Radical ions are notated with the • symbol [11]. ROS include hydroxyl (HO•), superoxide ($O_2•^-$), nitrogen dioxide ($NO_2•$), and nitric oxide (NO•). The ROS and non-free radical species are summarized in Table 1 [10].

Table 1. The reactive oxygen species (ROS) and non-free radical species

Non-free radical species		Reactive oxygen species	
Hydrogen peroxide	H_2O_2	Hydroxyl radical	HO•
Singlet oxygen	$^1O_2^-$	Superoxide radical	$O_2•^-$
Nitrous acid	HNO_2	Nitrogen dioxide radical	$NO_2•$
Nitryl chloride	NO_2Cl	Nitric oxide radical	NO•
Nitroxyl anion	NO^-	Nitrosyl cation	NO^+
Ozone	O_3	Hydroperoxyl radical	HOO•
Lipid hydroperoxide	LOOH	Lipid radical	L•
Peroxynitrite	$ONOO^-$	Peroxyl radical	ROO•
Hypochlorite	HOCl	Lipid peroxyl radical	LOO•
Dinitrogen trioxide	H_2O_3	Lipid alkoxyl radical	LO•
Nitrous oxide	N_2O	Protein radical	P•
Peroxynitrous acid	ONOOH	Thiyl radical	RS•

Antioxidants are compounds that directly or indirectly inhibit oxidation and reduce the overexpress of free radicals [12]. Normally, the human body produces antioxidants to protect against the harmful effects of ROS, which are called endogenous antioxidants. Endogenous antioxidants include superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px). SOD can be converted the superoxide anion to H_2O_2, CAT metabolizes H_2O_2 to water and oxygen, and GSH-Px reduces H_2O_2. Otherwise, exogenous antioxidants are produced from diet or food supplements as non-enzymatic antioxidants, such as vitamin C, vitamin E, carotenoids, and flavonoids. Vitamin C is a polar antioxidant and vitamin E is a non-polar antioxidant that protects against lipid peroxidation. In addition, the popular synthetic antioxidants include butylated hydroxyanisol (BHA), butylated hydroxytoluene (BHT), and tert-butylhydroquinone (TBHQ) [13].

The use of herbal plants with high antioxidant activity has been explored to prevent disease severity. *K. galanga* is one of the traditional medicines used for the treatment of various diseases, including anti-inflammatory [14], anti-cancer [15], antimicrobial [16], anti-sedative [17], and immunomodulator [18]. Several diseases are associated with oxidative stress and antioxidant activity. Measurement of antioxidant activity is important in studying the efficiency of antioxidants in preventing and treating diseases related to oxidative stress. Therefore, studies on natural antioxidants and food supplements that act against oxidative stress will exist in the future. This review highlights the antioxidant activities of *K. galanga in vitro* and *in vivo* using several assays. In addition, it can provide strong evidence that antioxidants can contribute to the improvement of some infectious conditions and chronic degenerative diseases to improve quality of life.

2. MATERIALS AND METHODS

The literature search was performed using the plant name using search engines including Scopus, PubMed, ScienceDirect, and Google Scholar. The following keywords were used "*Kaempferia*

galanga" AND "antioxidant or oxidative stress". The inclusion criteria in this review were only *in vitro* and *in vivo* published data from 2018-2023 and references without English-language titles were excluded. The databases were identified, analyzed, and chosen based on their relevance to the topic. The name of the plant was cross-checked with http://www.worldfloraonline.org website on August 3, 2023.

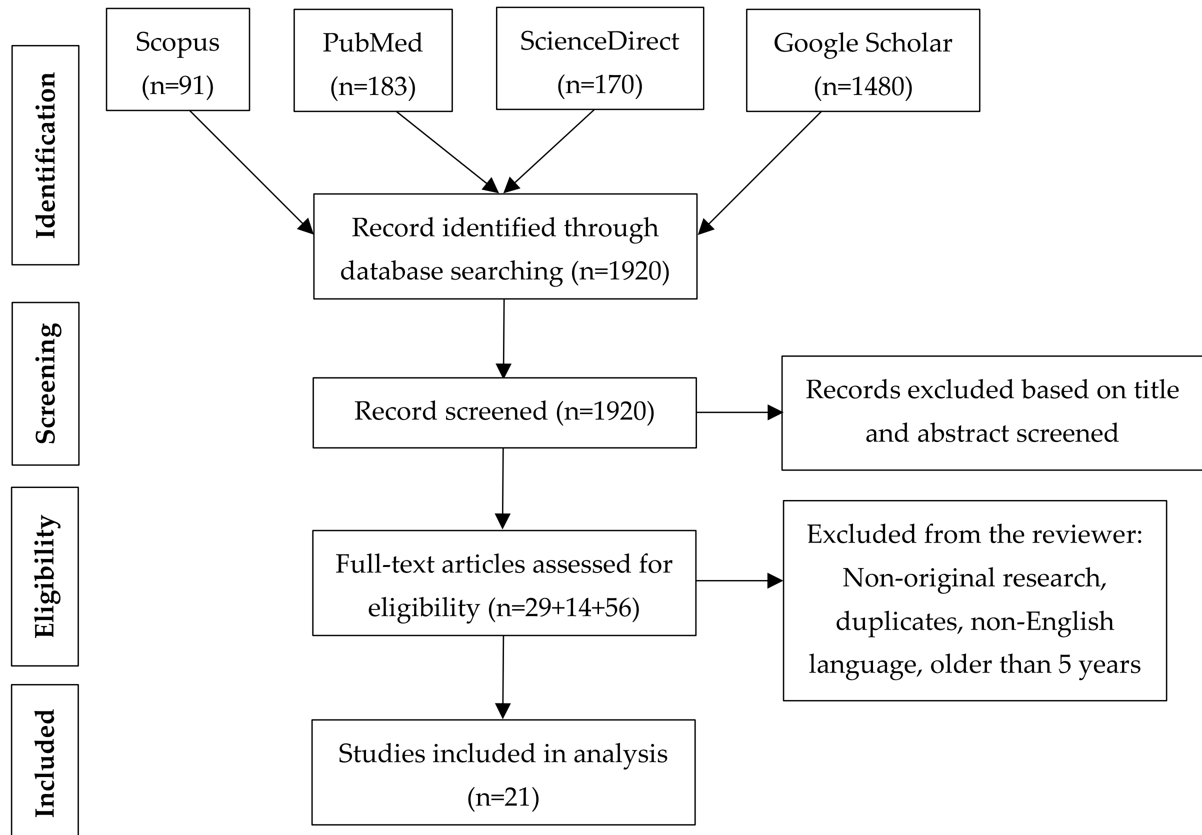

Figure 1. Flowchart of literature review

3. RESULTS AND DISCUSSION

Kaempferia galanga L. (*K. galanga*) is commonly called aromatic ginger, grouped under the Zingiberaceae family. *K. galanga* is probably a native plant from Asian countries including Indonesia, China, Malaysia, Thailand, and Nigeria [19]. Indonesian local people call as "*kencur*" in Bahasa and mix it with white rice as a beverage or "*Jamu Beras Kencur*"[20]. In recent decades, studies have reported that *K. galanga* extracts exhibit various pharmacological effects. The classification of *K. galanga* is explained as follows [21]:

Kingdom	: Plantae
Division	: Magnoliophyta
Class	: Liliopsida
Family	: Zingiberaceae
Order	: Zingiberales
Genus	: Kaempferia
Species	: *Kaempferia galanga* L.

According to a previous study, the methanol extract of *K. galanga* increased total antioxidant and ferrous reducing capacities in a concentration-dependent manner. The methanol extract of *K. galanga* reported an IC_{50} value of 16.58, 8.24, and 38.16 μg/mL using 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and nitric oxide radical scavenging assays, respectively. In addition, the total phenolic and flavonoid content of *K. galanga* was 15.40 ± 0.35 mg/g of gallic acid equivalent and 37.72 ± 0.50 mg/g of catechin equivalent, respectively [22]. The DPPH radical scavenging activity of *K. galanga* crude extracts showed IC_{50} values of 831.82, 492.75, and 424.44 μg/mL in hexane, ethyl acetate, and methanol, respectively. Here, the study concluded that the methanol crude extracts had higher antioxidant activity [23]. The methanol and acetone extracts of *K. galanga* leaves showed IC_{50} values of 611.82 and 702.79 μg/mL, whereas the total phenolic contents were 1.012 and -0.066 mg of gallic acid equivalents/gm [24]. *K. galanga* showed antioxidant activity as determined by various assays of 53.39 ± 0.54, 26.16 ± 2.81, and 42.30 ± 2.96 μM Trolox equivalent/g DW in ABTS, DPPH, and ferric reduction of antioxidant power (FRAP) assays, respectively [25].

Flavonoid-rich *K. galanga* ethanolic extract using ultrasound-assisted extraction shows a 50% scavenging concentration of 0.1084 and 0.1273 mg/mL in DPPH and ABTS assays, respectively [26]. In addition, the antioxidant capacities of *K. galanga* essential oils with an IC_{50} of 7.93 μg/mL for both the DPPH assay and the Trolox C assay [27]. *K. galanga* extraction in 80% methanol solvent exhibited 1.10 ± 0.015 mg AAE/g, 22.15 ± 0.83 %, 1.53 ± 0.02 μg GAE/mg extract, and 0.82 ± 0.01 mg/QE/g of dried sample in ferric reducing antioxidant power activity, free radical scavenging activity, total phenolic, and total flavonoid content, respectively [18].

Kiptiyah *et al*., 2021 [28], evaluated that the ultrasonic extraction method with blanching treatment showed a higher effect on the total phenolic, total flavonoid, and radical scavenging activity of *K. galanga* (67.32 ± 3.05 mg GAE/L, 452.76 ± 9.68 mg EK/L, 56.20 ± 5.04%, respectively). Based on a correlation study, the total phenolic and flavonoid content shows a significant correlation with antioxidant activity. Jamil and Hasyim, 2022 [29], reported the comparison of the rhizome and leaves of *the K. galanga* extraction method. The IC_{50} values of 128.91 and 175.64 μg/mL in *K. galanga* rhizomes with ultrasonic-assisted extraction and hydrodistillation methods, respectively. Whereas, the IC_{50} values of 209.12 and 239.41 μg/mL in *K. galanga* leaves ultrasonic-assisted extraction and hydrodistillation methods, respectively. Muderawan *et* al., 2022 [30], evaluated some varieties of *K. galanga* from Bali, Indonesia. The first one is bigger rhizomes with dark brown epidermis and smaller rhizomes with light brown epidermis, which are thus varieties extracted using steam distillation and maceration methods. The DPPH antioxidant activity found IC_{50} values of 86.10 ± 1.51, 85.24 ± 1.48, 89.19 ± 1.72, and 86.49 ± 2.03 μg/mL in bigger rhizomes using steam distillation, bigger rhizomes using maceration, smaller rhizomes using steam distillation, and smaller rhizomes using maceration, respectively.

Recently, the level of total phenolic content using the Folin-Ciocalteu method and the total flavonoid total of *K. galanga* ethanol extract was 50.35 ± 0.05 mg GAE/100g, and 20.98 ± 0.09 mg QE/100 g, respectively. *K. galanga* ethanol extracts showed IC_{50} values of 626.308 ± 5.06 μg/mL and 139.92 ± 0.51 μg/mL using DPPH antioxidant and xanthine oxidase inhibitory activity, respectively [31]. Nonglang *et* al., 2022 [32], reported that freeze-dried ethanolic extract of *K. galanga* showed IC_{50} values of 1.824 mg/mL and 0.307 mg/mL for the DPPH and the ABTS assays, respectively. Whereas, the

results obtained showed a total polyphenol content of 23.55 ± 0.5 mg GAE/g dry weight of the extract and a flavonoid content of 100 ± 1.414 mg rutin equivalent/g dry weight of the extract.

The current findings indicate that the antioxidant activity of *K. galanga* at 100-500 μg/mL inhibits radical scavenging in the DPPH assay [33]. The total amount of phenolics and flavonoids in the ethanolic extract of *K. galanga* was 55.58 ± 0.54 mg GAE/g extract and 56.16 ± 3.15 mg quercetin equivalent/g, respectively. *In vitro* antioxidant effects of *K. galanga* ethanolic extract with IC_{50} values of 240.80 ± 5.50, 151.64 ± 2.51, 116.49 ± 4.76, and 214.80 ± 6.42 μg/mL for DPPH, ABTS, reducing power, and total antioxidant capacity assays, respectively [34].

Begum *et al*., 2023 [35] showed that the antioxidant activity of *K. galanga* with an IC_{50} value of 15.64 ± 0.263 μg/mL and 21.24 ± 0.413 μg/mL for the DPPH assay, 16.93 ± 0.228 μg/mL and 21.156 ± 0.345 μg/mL for the ABTS assay of ethyl *p*-methoxycinnamate rich *K. galanga* and ascorbic acid, respectively. The reducing power assay showed that ethyl *p*-methoxycinnamate-rich *K. galanga* was superior to ascorbic acid. In addition, the IC_{50} of the metal chelating assay was 19.29 ± 0.805 μg/mL for samples and 30.72 ± 0.834 μg/mL for EDTA. *In vitro*, the antioxidant activity of *K. galanga* extracts showed strong free radical scavenging capacity in DPPH, ABTS, and hydroxyl radical scavenging assays (IC_{50} values of 19.77 ± 1.28, 1.41 ± 0.01, and 3.09 ± 0.34 mg/mL, respectively) and weak reducing capacity in the reducing power assay with an EC_{50} value of 389.38 ± 4.07 mg/mL. In addition, *K. galanga* essential oil at 10 mg/mL showed DPPH radical scavenging activity of 52.70% [36]. Therefore, *K. galanga* has the potential to donate electrons to reactive free radicals.

Srivastava *et al*., 2019 [15], evaluated the ethyl *p*-methoxycinnamate of *K. galanga* inhibited ROS production in H_2O_2-stimulated J774.1 macrophage cells in a dose-dependent manner using an oxidation-sensitive dye (DCFH-DA) assay. In addition, the result of this study showed that the ethyl *p*-methoxycinnamate at 10 μg/mL decreased by more than 50% of the ROS level. Thus, ethyl *p*-methoxycinnamate of *K. galanga* potentially protects the cells from oxidative cellular damage. *K. galanga* rhizome oil and microemulsion formulation indicated moderate UV protective activity and reduced nitric oxide production in lipopolysaccharides (LPS)-induced RAW 264.7 macrophage cells [37]. Moreover, *Kaempferia galanga* L. rhizomes gel formulation has been examined for its wound healing activity by increasing cell viability, cell migration, collagen content, and decreasing nitric oxide production in H_2O_2-induced human dermal fibroblasts [38].

Oxidative stress leads to lipid peroxidation, which produces malondialdehyde (MDA) [39]. Dwita *et al*., 2021 [40], examined the ethanol extract, water fraction, ethyl acetate fraction, n-hexane fraction, and ethyl *p*-methoxycinnamate isolate of *K. galanga* decreases MDA concentration in serum, exudate, and tissue in carrageenan-induced granuloma air pouch inflammation rats.

Based on Men et al., 2022 [34], an *in vivo* antioxidant study showed *K. galanga* ethanolic extract in both 20 mM paraquat and H_2O_2 assays using the *Drosophila melanogaster* model. Under 20 mM paraquat, *K. galanga* increased the longevity of fruit flies through life expectancy (at 18.4 ± 0.69 h), maximum lifespan (at 29.0 ± 1.0 h), and 50% survival (at 15.67 ± 0.58 h). Meanwhile, under the 10% H_2O_2 condition, *K. galanga* shows 50% survival at 30.0 ± 2.0 h, mean lifespan at 36.87 ± 1.35 h, and maximum lifespan at 59.67 ± 0.58 h, as well as under paraquat conditions.

Recently, *K. galanga* essential oil extracts were shown to play a role in antioxidant activity by attenuating ROS generation, cell death, lipid peroxidation, and malondialdehyde [36]. According to Munda et al. (2018), the main essential oils of K. galanga are ethyl p-methoxycinnamate, ethyl cinnamate, 1,8-cineole, borneol, camphene, linoleoyl, methyl-cinnamate, and pentadecane [41].

Treatment of the zebrafish model with H_2O_2-induced oxidative stress reduced intracellular ROS generation, cell death, and lipid peroxidation by 103.31%, 105.04%, and 108.00%, respectively. In addition, the extracts increased superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) levels, as well as the survival rate and heart rate in H_2O_2-treated zebrafish embryos. In contrast, the MDA level decreased by 63.00% compared with that in the H_2O_2-treated group [36]. Shao *et al*., 2023 [42] demonstrated that kaempferide in *K. galanga* inhibited oxidative stress in renal tubule cells to protect against cisplatin-induced acute kidney injury by regulating intracellular ROS, MDA, and SOD.

Here, we summarize the popular design used to evaluate the antioxidant activity of *Kaempferia galanga*. Among *in vitro* antioxidant assays, radical scavenging methods are the most popular. This review found that DPPH, ABTS, FRAP, ROS, SOD, GSH, MDA, and CAT are commonly used for the antioxidant assays of *K. galanga*. Table 2. Commonly, the pharmacology effect of *K. galanga* is on the rhizome. However, this review found that leaves could be used for antioxidant activity.

Table 2. *In vitro*, the antioxidant activity of *K. galanga* using a chemical reaction

No.	Assays	Extracts/Fractions/Compounds	Results [IC_{50}]	Results [EC_{50}]	Ref.
1.	DPPH	Methanol extract	16.58 μg/mL		[22]
		Methanol extract	424.44 μg/mL		[23]
		Hexane extract	831.82 μg/mL		[23]
		Ethyl acetate extract	492.75 μg/mL		[23]
		Methanol extract	611.82 μg/mL		[24]
		Acetone extract	702.79 μg/mL		[24]
		Ethanolic extract	26.16 ± 2.81 μM Trolox equivalent/g		[25]
		Flavonoid-rich ethanolic extract	0.1084 mg/mL		[26]
		Essential oils	7.93 μg/mL		[27]
		80% methanol extract	22.15 ± 0.83 %		[18]
		Water extract (ultrasonic extraction method with blanching treatment)	56.20 ± 5.04 %		[28]
		Essential oil rhizome extract (ultrasonic-assisted extraction pretreatment)	128.91 μg/mL		[29]
		Essential oil rhizome extract (hydrodistillation method)	175.64 μg/mL		[29]
		Essential oil leaves extract (ultrasonic-assisted extraction pretreatment)	209.12 μg/mL		[29]
		Essential oil leaves extract (hydrodistillation method)	239.41 μg/mL		[29]
		Rhizome oil (bigger rhizomes using steam distillation method)	86.10 ± 1.51 μg/mL		[30]
		Rhizome oil (bigger rhizomes using maceration method)	85.24 ± 1.48 μg/mL		[30]
		Rhizome oil (smaller using steam distillation method)	89.19 ± 1.72 μg/mL		[30]
		Rhizome oil (smaller rhizomes using the maceration method)	86.49 ± 2.03 μg/mL		[30]
		Ethanolic extract	626.308 ± 5.06 μg/mL		[31]
		Freeze-dried ethanolic extract	1.824 mg/mL		[32]
		Ethanolic extract	100-500 μg/mL		[33]
		Ethanolic extract	240.80 ± 5.50 μg/mL		[34]

…continued Table 2

		Ethyl *p*-methoxycinnamate-rich essential oil	15.64 ± 0.263 μg/mL		[35]
		Essential oil		19.77 ± 1.28 mg/mL	[36]
2.	FRAP	Ethanolic extract	42.30 ± 2.96 μM Trolox equivalent/g		[25]
		80% methanol extract	1.10 ± 0.015 mg AAE/g		[18]
		Ethanolic extract	116.49 ± 4.76 μg/mL		[34]
		Essential oil		389.38 ± 4.07 mg/mL	[36]
3.	ABTS	Methanol extract	8.24 μg/mL		[22]
		Ethanolic extract	53.39 ± 0.54 μM Trolox equivalent/g		[25]
		Flavonoid-rich ethanolic extract	0.1273 mg/mL		[26]
		Freeze-dried ethanolic extract	0.307 mg/mL		[32]
		Ethanolic extract	151.64 ± 2.51 μg/mL		[34]
		Ethyl *p*-methoxycinnamate-rich essential oil	16.93 ± 0.228 μg/mL		[35]
		Essential oil	1.41 ± 0.01 mg/mL		[36]
4.	Hydroxyl radical scavenging assays	Essential oil	3.09 ± 0.34 mg/mL		[36]
5.	Metal chelating assay	Ethyl *p*-methoxycinnamate-rich essential oil	19.29 ± 0.805 μg/mL		[35]
6.	Phosphomolybdate	Ethanolic extract	214.80 ± 6.42 μg/mL		[34]
7.	Xanthine oxidase assay	Ethanolic extract	139.92 ± 0.51 μg/mL		[31]
8.	NO radical scavenging assays	Methanol extract	38.16 μg/mL		[22]

Note: DPPH (1,1-diphenyl-2-picrylhydrazyl); FRAP (ferric reduction of antioxidant power), ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)); NO (nitric oxide); IC_{50} (inhibition concentration 50%); EC_{50} (effective concentration at which the absorbance was 0.5).

Table 3. Antioxidant activity of *K. galanga* in cell lines and animal models

No.	Compounds	Models	Induction	Results	Ref.
1.	Ethyl *p*-methoxycinnamate	J774.1 macrophage cells	H_2O_2	↓ROS production	[15]
2.	Rhizome oil and microemulsion formulation	RAW 264.7 macrophage cells	LPS	↓NO production	[37]
3.	Rhizomes gel formulation	Human dermal fibroblast	H_2O_2	↑cell viability, cell migration, collagen content ↓NO production	[38]
4.	Ethanol extract, water fraction, ethyl acetate fraction, n-hexane fraction, ethyl *p*-methoxycinnamate isolated	*Wistar* rats	Carrageenan	↓MDA	[40]

…continued Table 2

5.	Ethanolic extract	*Drosophila melanogaster*	20 mM paraquat, 10% H_2O_2	↑life expectancy, lifespan, survival	[34]
6.	Essential oil	Zebrafish	H_2O_2	↓MDA, ROS generation, cell death, lipid peroxidation ↑SOD, CAT, GSH	[36]
7.	Kaempferide	C57BL/6 mice, HK-2 cells	Cisplatin	↓ROS, MDA ↑SOD	[42]

Note: ↑(increase); ↓(decrease); LPS (lipopolysaccharides); ROS (reactive oxygen species); SOD (superoxide dismutase), CAT (catalase); GSH (glutathione peroxidase); MDA (malondialdehyde); NO (nitric oxide); H_2O_2 (hydrogen peroxide)

Generally, the main mechanism of antioxidants is to prevent or detect chain oxidative propagation, stabilize the radicals, and thus reduce oxidative damage in the body [43]. Several assays have been used to evaluate antioxidant activities. Mainly, based on chemical reactions, the mechanisms of antioxidant assay are hydrogen atom transfer (HAT) and single electron transfer (SET). HAT assesses the ability of antioxidants to transfer hydrogen atoms, including the total peroxyl radical trapping antioxidant parameter (TRAP), oxygen radical absorption capacity (ORAC), and total oxy-radical scavenging capacity (TOSC) assays. On the other hand, SET measures the ability of antioxidants to transfer an electron in a pH-dependent manner. SET assays include ferric reduction of antioxidant power (FRAP), cupric ion-reducing antioxidant capacity (CUPRAC), and the Folin-Ciocalteu test (FC). Mixed assays (HAT/SE) were used to eliminate stable chromophores, including 1,1-diphenyl-2-picrylhydrazyl (DPPH) free-radical scavenging activity, Trolox equivalent antioxidant capacity (TEAC)/ABTS radical cation decolorization, and *N, N*-dimethyl-p-phenylenediamine dihydrochloride (DMPD) [9, 39].

DPPH is a stable, long-lived radical that reacts with other radicals or reducing agents, leading to a decrease in absorption at 515 nm. Unfortunately, contrary to peroxyl radicals, antioxidants may react slowly or inertly with DPPH. *K. galanga* may inhibit the color development of the ABTS radical. The formation of ABTS cation radicals from 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate) intensively decreases absorption at 734 nm. However, neither DPPH nor ABTS radicals are exact models of radical reactions in biological systems; instead, they are stable radicals. Fe(TPTZ)2]Cl3 (TPTZ ligand 2,4,6-tripyridyl-triazine) as an oxidizing agent is reduced by a transfer electron from *K. galanga* to produce [Fe(TPTZ)2]Cl2 that absorbs at a wavelength of 593 nm. In addition, the mechanism of the hydroxyl radical scavenging assays of *K. galanga* is reduced to the transfer of a hydrogen atom from *K. galanga* to the peroxyl radicals (ROO•) [45].

K. galanga plays a crucial role in the protection of cells from oxidative damage by inhibiting lipid oxidation. MDA is an indicator of lipid peroxidation caused by oxidative stress, particularly ROS. Hydroxyl radicals and peroxynitrite cause cell membrane and lipoprotein damage via lipid peroxidation. SOD, CAT, and GSH-Px are enzymes that play a role in defense against oxidative stress. SOD, as the first line of defense against free radicals, transforms the dismutation of $O_2\bullet^-$ into H_2O_2 and is then converted into H_2O and O_2 by CAT or GSH-Px [46-47].

As mentioned previously, *K. galanga* can stimulate several antioxidant enzymes, such as SOD, CAT, and GSH [36, 40]. On the one hand, *K. galanga* is shown to reduce MDA and NO production

levels [37, 40]. In addition, the parameter of intracellular antioxidant enzymes plays a key role in homeostasis and the mechanism of ROS signaling pathways [48]. Antioxidant enzymes counterbalance the effects of reactive oxygen species (ROS) and reactive nitrogen species (RNS). In between, radical nitric oxide is produced by nitric oxide synthases, which react with $O_2{\bullet}^{-}$ to result in peroxynitrite formation. The peroxynitrite then reacts with CO_2 to lead to nitrosoperoxycarboxylate, which then breaks down into carbonates and $NO_2{\bullet}$ radicals [49]. Briefly, *K. galanga* inhibits oxidative stress through the suppression of the NF-κB signaling pathway [50]. NF-κB plays a role in cell death, ROS, RNS, and immune responses. Therefore, the regulation of NF-κB as a target may be proposed for protection from ROS. *K. galanga* acts as an antioxidant by reducing ROS production, hence decreasing the activation of IκBα Kinase β (IKK β), as a regulator of the transcription factor NF-κB [51].

Interestingly, a review found that the zebrafish is the modern *in vivo* model that is used to evaluate the bioactivity of compounds because of its easy handling, short generation time, and transparent embryos [52]. In a previous study, an H_2O_2-induced zebrafish model was used to evaluate the *in vivo* antioxidant effect of *K. galanga* [36]. *Drosophila melanogaster* (*D. melanogaster*) fruit flies were used to evaluate the antioxidant activity of herbal medicine. A previous study reported that approximately 75% of *Drosophila* genes are homologous to human disease genes. *Drosophila* has been successfully applied as an *in vivo* model to assess antioxidant studies [53, 54].

The high antioxidant activity of *K. galanga* may be attributed to its high *ethyl p-methoxycinnamate* concentration [35]. This review is an effort to recapitulate recent and well-established research on effective *K. galanga* antioxidants, which would be helpful for the modern prevention of acute and chronic disease advancement in this field.

4. CONCLUSION

K. galanga contained phenolic and flavonoid compounds that exhibited antioxidant activities. The antioxidant activity of *K. galanga* occurs through several mechanisms, such as hydrogen atom transfer and single electron transfer, with the radical scavenging mechanism being the most popular. In addition, *K. galanga* exhibited antioxidant effects by regulating ROS, MDA, SOD, CAT, and GSH-Px levels. However, the feasibility of the antioxidant activity of *K. galanga* is based on the combination of various mechanisms. In the future, *K. galanga* could be used as a preventive agent against oxidative stress-associated diseases.

Funding: This review did not receive any specific grants from the funding agencies.

Acknowledgments: None.

Conflicts of interest: The authors declare no conflict of interest.

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