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Review Article

Effect of *Curcuma mangga* and *Curcuma longa* on Oxidative Stress-related Diseases and ROS Level: A Recent Study

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ABSTRACT

Oxidative stress causes various disorders, and an imbalance between the generation of free radicals and the availability of antioxidants in the body increases oxidative stress. External antioxidants are needed to help prevent free radical reactions and cellular damage. *Curcuma mangga* (*C. mangga*) and *Curcuma longa* (*C. longa*) are plants often used as kitchen spices with several benefits such as anti-inflammatory and antidiabetic activities. This review aims to discuss the effect of *C. mangga* and *C. longa* in several oxidative stress-related diseases, their antioxidant activity, and the measurement of their ROS level. The research on *C. mangga* and *C. longa* was gathered using Scopus, PubMed, and Google Scholar in the last five years (2016-2021). Based on the reviews of the research results, the compounds that play a role in the pharmacological activity of *C. mangga* and *C. longa* are phenolic and flavonoid compounds, wherein curcumin is the most common compound found in *C. longa*. In the past five years, most researchers have used maceration extraction methods and ethanol solvents for *C. mangga* and *C. longa* extraction. Some pharmacological activities mentioned in this review include antioxidant, antidiabetic, anti-inflammatory, and anticancer activities. Several *in vitro* studies reported that *C. longa* and curcumin could decrease ROS levels in normal cells even if induced by hydrogen peroxide (H_2O_2) or virus and in cancer cells.

Keywords: Antioxidant, ROS level, *Curcuma mangga*, *Curcuma longa*, Oxidative stress.

Introduction

Free radicals can be produced in cells by losing or obtaining one electron, working as oxidants or reducing agent.[1] Free radicals are unpaired, highly reactive, unpredictable, and can produce a new free radical when reacting with various body components such as lipids, proteins, and DNA.[2] The presence of free radicals can be related to oxidative stress. An imbalance between the free radicals/ROS (in cells and tissues) and the biological antioxidant systems induce oxidative stress.[3] In addition, an imbalance between free radicals and the antioxidant response system's scavenging capacity cause cellular degeneration and functional decline.[2] Besides, chronic metabolic disorders, antioxidant enzyme deficiency, ultraviolet irradiation and radioactivity, and drug and xenobiotic metabolism can increase ROS production.[3] Oxidative stress is involved in several diseases such as diabetes mellitus, inflammation, and cancer.[4-6] The radical scavenging ability of antioxidants can inhibit cell damage caused by oxidation.[6,7]
Antioxidants can be categorized as natural and synthetic antioxidants based on their nature. A natural antioxidant is a substance or molecule that can act as antioxidants and naturally exist in nature or in our body while humans make synthetic antioxidants. Natural antioxidants can be classified as endogenous and exogenous antioxidants based on their origin. Several instances of exogenous antioxidants are vitamins and derivatives, minerals, carotenoids, organosulfur compounds, flavonoids, and phenols.[8-10]

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The antioxidant activity tests are performed by reacting several reagents based on the chemical reaction mechanism. According to Sadeer *et al*.,[11] these tests can be divided into three mechanisms: single electron transfer (SET), hydrogen atom transfer (HAT), and chelation of transition metals.[9-11] However, according to Furger,[12] antioxidant assays based on the mechanism of chemical reactions do not accurately predict the effect of antioxidants in living systems because most of the sample components only react based on an oxidation-reduction mechanism.[12,13]
Furger stated that there are four different cell-based approaches to quantifying the antioxidant effects of plant extracts: the catalase-like assay, the cell antioxidant assay (CAA), the AOP1 assay, and the Nrf2/ARE gene reporter system.[12] In the CAA assay, the antioxidant activity test is carried out using a living organism (cell lines) to consider several factors such as cellular uptake and cell metabolism.[13] Furthermore, the CAA assay is an assay that measures intracellular ROS levels using a 2′,7′-dichlorofluorescein diacetate (DCFH-DA) probe.[12] Several cell lines were used for ROS intracellular measurements, such as Vero cell, MCF-7, HUVECs cell, and RAW 264.7 cell.[14-17] Many studies on in vivo and in vitro antioxidant activity tests of plant extract to explore natural antioxidant sources have been reported.[14,18-21]
Southeast Asia is well-known for its tropical forests. It has a broad distribution and abundant medicinal plants widely used by the community.[22] The Zingiberaceae family, also known as the ginger family, is a large monocotyledon family that produces a distinct odor in the rhizome and is one of the most commonly used plants.[23]
The plant part of this family mainly used for medicinal purposes is the rhizome, rich in curcuminoids (curcumin, demethoxycurcumin, and bisdemethoxycurcumin). They are nontoxic polyphenolic curcumin derivatives with various pharmacological activities.[24] Among the numerous members of the Zingiberaceae family, this review focused

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on discussing *Curcuma mangga* (mango turmeric, temu mangga (Indonesia)), and *Curcuma longa* (turmeric).
Curcuma mangga (*C. mangga*) Val. and Zijp is a 50–200 cm high plant, grows wild, and spreads in Indonesia (especially on the Java island), Malaysia, and Thailand. The part used is a rhizome with a characteristic yellowish-brown exterior, white top, and yellow interior with a mango-like odor.[25] *C. mangga* is used for the traditional treatment such as stomach ache, chest pain, and fever and has antioxidant and anticancer activities.[26,27]
Curcuma longa (*C. longa*) Linn. is a perennial herb that can reach 1 m in height and has pointed leaves and funnel-shaped yellow flowers on a short stem.[28] It can be found in tropical and subtropical forests, specifically in the Asian region. It has been used as herbal medicine for several diseases, including antiseptic, anti-inflammatory, and wound healing.[29] *C. mangga* and *C. longa* contain curcuminoids, whereas curcuminoids have anti-inflammatory and antioxidant activities.[27] Based on the preceding discussion,[11-13,26,27,29] this review aims to summarize several recent studies on the pharmacological activity of *C. mangga* and *C. longa* in a variety of oxidative stress-related diseases and the measurement of their ROS levels.

Methods

This review uses a variety of databases such as Scopus, PubMed, and Google Scholar, based on the research of *C. mangga* and *C. longa* in the last five years (2016-2021). This review used keywords *Curcuma mangga*, *Curcuma longa*, antioxidant, and oxidative stress-related disease. These databases were identified, analyzed, and chosen based on their relevance to the topic. The inclusion criteria uses journals containing the pharmacological activity of *C. mangga* and *C. longa* and their extraction method and the measurement of intracellular ROS levels.

Results and Discussion

Recent study on *C. mangga*, *C. longa*, and their Bioactive Compound
Several studies on the activity of the bioactive components of Curcuma species have been listed in Table 1, along with the solvent and the extraction method used. Table 1 concludes that most researchers use maceration for extraction because maceration is a classic and straightforward method of extracting polyphenols from plant material.[30]
However, Yang *et al*.[14] reported that the total phenolic content obtained from the conventional extraction method was lower than the Ultrasound-assisted extraction (UAE). UAE utilizes cavitation and thermal effect as the driving force. Therefore, the cell wall of the sample ruptures, and the solvent can extract the phenolic compounds efficiently. Thus, extraction with UAE is relatively more effective and efficient.[14]
In addition, Table 1 shows that ethanol is a widely used solvent for Curcuma sp. extraction. The main active constituent, curcumin, was readily soluble in ethanol, explaining this phenomenon.[31] Curcumin is a phenolic compound that dissolves in polar organic solvents. In line with several studies mentioned in Table 1, curcumin was very soluble in ethanol.[31,32] Ethanol is an organic solvent, nontoxic, and has a suitable polarity, so it is widely used for plant extraction. It can dissolve compounds of various polarities, such as phenolic compounds and flavonoids.[18]
Some of the pharmacological activities of *C. mangga* and *C. longa* were reported in several studies listed in Table 1, such as antioxidant, antidiabetic, anti-inflammatory, and antiproliferative activities. The primary contributors to the differences in pharmacological activities may be the antioxidant bioactive contents of the extracts.
Based on Yang *et al*.[14] studies, the UAE *C. longa* extract has a higher total phenolic content and antioxidant activity.[14] This is in line with the results of Sabir *et al*.[20] studies which revealed that *C. longa* ethanolic extract contains much curcumin and various substantial phenolics, which means it has a tremendous amount of antioxidant (radical scavenging) activity.[20]
Research by Muchtaromah *et al*.[18] states that ethanol extract of *C. mangga* had the highest antioxidant activity compared to chloroform and n-hexane extracts. Maryam and Martiningsih[19] state that *C. mangga* has antioxidant activity because of its curcuminoid compounds. Curcumin can scavenge free radicals of lipid peroxidation's initiator (like superoxide anions and hydroxyl radicals).[19]
Differences in antioxidant activity were related to the type of antioxidants present in each extract. Phenols and flavonoids are antioxidant components, and they can affect the antioxidant constituents found in plants.[18,19]
Sabir *et al*.[20] study also shows that *C. longa* extract can inhibit alpha-glucosidase, proving that it can treat diabetes.[20] Curcuminoids, terpenes, and sesquiterpenoids are potent hypoglycemic compounds in turmeric. When an active compound or extract inhibits the alpha-amylase enzyme, carbohydrate digestion, and glucose absorption are reduced because the carbohydrates are still in complex form.[33]
Besides antioxidant and antidiabetic effects, *C. mangga* and *C. longa* possess anti-inflammatory activities, as shown in Table 1. Research from Lee *et al*.[21] evaluated the protective effect of inflammation of 30% ethanol extract of *C. longa* Rhizoma (CLR) in acute reflux esophagitis (ARE) models using Sprague-Dawley rats. The Nrf2 pathway is a critical regulator of oxidative stress and antioxidant defense systems. When SOD, catalase, and GPx are available, they act as antioxidant enzymes, protecting the cell from oxidative stress. The expression of inflammation-related proteins such as NF-κBp65 and p-IκBα and pro-inflammatory enzymes like iNOS and COX-2 were checked. According to research by Lee *et al*.[21] *C. longa* extract can be called an anti-inflammatory agent since it can increase Nrf2 expression and antioxidant enzyme level. Furthermore, *C. longa* extract has been shown to reduce the expression of inflammation-related proteins and pro-inflammatory enzymes.[21]
C. longa and *C. mangga* have anticancer activity. Moreover, *C. longa* has a cytotoxic effect[14] and *C. mangga* serves as a potential anticancer agent, proven by its ability to inhibit MCF-7 cancer cell growth.[22]

Intracellular ROS scavenging activity
Several studies regarding intracellular ROS levels measurement at *C. longa* and curcumin are present in Table 2. The antioxidant effect of *C. longa* L. leaf water extract (TLE) was studied using induction with Hydrogen Peroxide (H_2O_2) *in vitro* (Vero cell) and *in vivo* (zebrafish) with DCFH-DA.[15] In an *in vitro* model, measurements were made by measuring the scavenging capacity of Intracellular ROS. Meanwhile, the antioxidant effect via *in vivo* model was assessed using DCFH-DA fluorescence intensity. This study showed that *C. longa* declined ROS generation significantly in a dose-dependent manner (concentration treatment listed in Table 2). The highest extract concentration showed the most robust ROS scavenging activity. Treatment with the highest dose and two times the highest dose given to Vero cells significantly reduced ROS generation in H_2O_2-induced cell death in zebrafish. As a result, *C. longa* extract can reduce ROS intracellular levels both *in vitro* and *in vivo*.[15] Research by Lin *et al*. measured the effect of curcumin (the main active component in *C. longa*) on ROS intracellular levels in RAW 264.7 that H_2O_2 induced as listed in Table 2. Low-dose curcumin can decrease H_2O_2-induced ROS levels in RAW 264.7 cells.[16]
The study by Lv *et al*.[17] investigated the effect of curcumin on inhibiting ROS in HUVECs cells (infected human cytomegalovirus (HCMV)) with ROS's fluorescence intensity. The result showed that ROS generation was suppressed dose-dependently after curcumin treatment.[17] A study by Lee *et al*.[34] evaluated the effect of curcumin and *C. longa* extract in CCl_4-induced mice to create acute and chronic stress conditions. The result is an increase in ROS levels in conditions of acute stress. Still, endogenous antioxidants (SOD and GPX) will maintain and restore the state until homeostasis occurs. However, in acute stress conditions, the administration of curcumin and extract of *C. longa* can inhibit ROS accumulation in hepatic cell.[34]
Yang *et al*.[14] investigated the effect of Ultrasound-assisted extraction and conventional extraction of *C. longa* on ROS inhibition using an MCF-7 cell line. The promotion of cancer growth can be associated with the elevated intracellular ROS level.[14] The result was that *C. longa* extract decreased MCF-7 ROS levels, demonstrating that antioxidant compounds in *C. longa* can assist in preventing the harmful effects of oxidative stress and oxidative damage caused by ROS.[14]

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Table 1: Several pharmacological studies of Curcuma species

Sample	Extraction method	Solvent	Concentration treatment	Method	Properties	Result / finding	Ref.
Dried rhizome *C. longa* from China	Ultrasound-assisted and conventional solvent extraction	Both of them using ethanol 80%	N/A	TPC, FRAP, DPPH, and ABTS assay	TPC, Antioxidant, Antiproliferative	UAE is higher than conventional and reduce the generation of ROS	14
C. mangga rhizome from Bali, Indonesia	Maceration	90% ethanol	150 - 350 ppm	TPC assay, DPPH assay	Antioxidant	IC_{50} mean : 60.61 ppm (strong), TPC : 87,73 mg/g (good)	19
C. longa rhizomes from Pakistan	Maceration	Ethanol	10-250 μg/mL	DPPH assay, Alpha-glucosidase inhibitory assay	Antioxidant, Antidiabetic	Inhibition of alpha-glucosidase (IC_{50} : 37.1 μg/ml), DPPH assay (IC_{50} : 27.2 μg/mL)	20
C. longa rhizome from India	N/A	Ethanol : distilled water (3:7)	N/A	In vitro (DPPH and ABTS assay, *in vivo* (Acute Reflux Esophagitis Model)	Antioxidant, anti-inflammation	DPPH (IC_{50} : 36,44 μg/mL), ABTS (44,08 μg/mL), ↑Nrf2 expression, SOD, catalase, and GPx-1/2, ↓NF-κBp65 and p-IκBα, suppressed iNOS, COX-2 expressions.	21
C. mangga	Soxhlet extraction	ethanol	DPPH (3.13 – 200 μg/mL), MTT (11.25 - 360 μg/ml)	DPPH assay, MTT assay	Antioxidant, Anticancer	low IC_{50} and has inhibitory effect	22
C. mangga rhizomes from Surabaya Indonesia	Maceration	Demineralized water	5 - 500 mg/L	DPPH assay	Antioxidant	IC_{50}: 212.70 mg/L	25
C. mangga form Yogyakarta, Indonesia	Maceration	• Extraction: ethanol Fractionation (water, hexane, ethyl acetate, and butanol)	• NO scavenging (133.33-2.08 μg/mL) • H_2O_2-Scavenging (6.25-400 μg/mL) alpha-glucosidase inhibitory (3.91 -250 μg/mL)	Alpha-glucosidase activity, H_2O_2, and NO - scavenging activity assays	Antidiabetic, Antioxidant	• Acetate fraction had most increased H_2O_2-scavenging activity hexane fraction has highest alpha-glucosidase inhibitory activity	26
C. mangga	Maceration	Ethanol, Chloroform,	25 - 400 ppm	DPPH assay,	Antioxidant,	ethanol extract has	33

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from Batu, Indonesia		and n-hexane				antioxidant activity higher than other solvent	
Curcuma species from Thailand	Maceration	ethanol	100 µg/mL	TPC assay, DPPH assay	Antioxidant	*C. longa* (Highest TPC)	35
C. longa, C. mangga, from Malaysia	Alkaline and Chemical-based extraction	● Alkaline extraction (NaOH, ethyl acetate solution) ● Chemical extraction (mixture acetone and methanol (7:3))	Curcumin	DPPH assay,	Antioxidant	*C. longa* has the highest curcumin content.	36
C. mangga from Yogyakarta, Indonesia	Maceration	Methanol, then n-hexane, and ethyl acetate was used for fractionation	1 –100 µg/mL	SRB, cytotoxicity assay	cytotoxic activity	Higher in Ethyl acetate extract	37

*Note : TPC (total phenol content), DPPH (1,1-Diphenyl-2-picryl-hydrazyl), NO (nitrite oxide), BHT (butylated hydroxytoluene),
N/A : Not clearly stated in the article

Table 2: The recent measurement of intracellular ROS levels at *Curcuma longa* and curcumin

Sample	**Concentration treatment**	**Cell model**	**Induction/ infected**	**Result/finding**	**Ref.**
C. longa	1.25, 2.5, and 5 µg/mL	MCF-7 cells	-	↓ ROS intracellular levels at dose-dependent	14
C. longa leaf water extract	10, 25, 50 and 100 µg/mL	Vero cells	H_2O_2	↓ ROS intracellular levels at dose-dependent	15
Curcumin	5 µM, 10 µM and 20 µM	RAW 264.7 cells	H_2O_2	↓ ROS intracellular levels at low-dose	16
Curcumin	0.5, 1, 2 µM	HUVECs cells	HCMV	↓ ROS intracellular levels at dose-dependent	17

Conclusion

The antioxidant activity of *C. mangga* and *C. longa* is related to their antidiabetic, anti-inflammatory, and anticancer activities evaluated through *in vitro* and *in vivo* models. Furthermore, *C. longa* and its curcumin content reduced ROS levels in cell lines. In addition, most researchers use the maceration method and ethanol solvent in the extraction process to obtain phenolic (like curcumin) and flavonoid compounds.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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