

***In vivo* antioxidant activities of *Curcuma longa* and *Curcuma xanthorrhiza*: a review**

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Abstract

Free radicals, reactive nitrogen species (RNS) and reactive oxygen species (ROS) have been known to contribute several degenerative diseases such as cardiovascular diseases, cancers, rheumatoid arthritis, neurodegenerative, and diabetes mellitus. In order to overcome the negative effects of these radicals, some scientist explores natural antioxidants from plants. *Curcuma longa* (Turmeric) and *Curcuma xanthorrhiza* (Javanese Turmeric) have been known as herbs and spices with antioxidant activities due to curcuminoid contained. Antioxidant can be defined as any substances or samples capable of inhibiting free radical reactions in the oxidation reaction. Several chemical and biological methods either *in vitro* or *in vivo* have been proposed, evaluated, and used for antioxidant evaluation of studied samples. Antioxidant activities *in vivo* can be measured by determining antioxidant enzymes which include catalase, glutathione reductase, superoxide dismutase, glutathione peroxidase, and glutathione S-transferase. The antioxidant enzymes increased while the lipid peroxidation decreased for both *Curcuma* species when research using animal models. This present review highlights the potential use of *C. longa* and *C. xanthorrhiza* as natural antioxidants *in vivo*. Based on *in vivo* studies, *Curcuma* species are potential sources of natural antioxidants, which can be used as food supplements.

1. Introduction

Herbs and spices are traditionally defined as any part of plants used in the diet for their aromatic properties with no or low nutritional values (Serafini and Peluso, 2016). Currently, herbs and spices are largely used for health-promoting purposes due to the antioxidant properties of the phytochemicals contained in these plants (Dragland *et al.*, 2003). Therefore, herbs and spices such as *Curcuma longa* and *Curcuma xanthorrhiza* may have a role in antioxidant defense and redox signaling, and there are large numbers of literature reporting the potential benefits of these herbs and spices from the health perspectives (Tapsell *et al.*, 2006; Hosseini and Hosseinzadeh, 2018). As a consequence, natural antioxidants coming from herbs and spices are extensively explored as an alternative to synthetic

antioxidants for safety reasons (Opara and Chohan, 2014).

Natural antioxidants can be defined as the phytochemicals capable of counteracting the damaging effects of the oxidative physiological processes which occur in the tissues (Podsdek, 2007). The antioxidants are believed to play important role in preventing the oxidative stress-related diseases such as cancer, heart disease, diabetes, stroke, rheumatoid arthritis, Alzheimer's disease, aging and cataracts (Kumar *et al.*, 2017). Natural antioxidants are typically derived from plant sources, and its efficacy is depended on several factors, namely plant species and its variety, extraction methods, and the growing conditions. Polyphenols such as phenolic acids, the flavonoids including flavonols, flavones, isoflavones, flavanones, flavanols and the

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anthocyanidins, the stilbenes and the lignans are believed as phytochemicals contributing to act as natural antioxidants in plants (Pandey and Risvi, 2009; Jiang, 2019). The action mode for these phytochemicals as natural antioxidants will vary depending upon the source of plant materials, the presence of synergists and antagonists, and of course the matrix in which the natural antioxidants were applied (Harwort, 2003).

Curcuma, the group of family Zingiberaceae, is an economically important genus due to its use in medicinal and food sectors (Xia et al., 2005). *Curcuma* genus has been reported to comprise 70 perennial rhizomatous species, widely distributed along tropical and subtropical regions around the world. The rhizomes of *Curcuma* species is a source of the yellow dye and historically has been exploited as spices and food preservatives, flavoring agent, and remedy for the treatment of many diseases due to chemical compounds contained (Rajkumari et al., 2017). The main components which are believed to be responsible for several biological activities of *Curcuma* species including antioxidants are curcuminoid (Nugroho et al., 2015), a group of phenolics compounds of curcumin, demethoxycurcumin and bisdemethoxycurcumin (Figure 1). In this review, the antioxidant activities *in vivo* of *C. longa* and *C. xanthorrhiza* were highlighted.

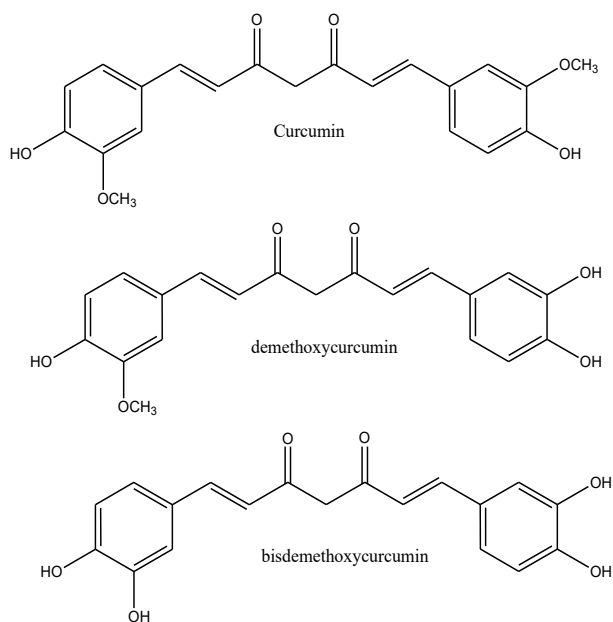


Figure 1. The chemical structures of curcumin, demethoxycurcumin and bisdemethoxycurcumin (Nugroho et al., 2015) in *Curcuma* species and xanthorrhizol in *Curcuma xanthorrhiza* (Ramdani et al., 2016).

2. Methods

Several databases including Scopus, PubMed, and Google Scholar were used for preparing this review. These databases were identified, analyzed and selected to

obtain the most appropriate ones. The keywords used during searching of information was (antioxidant + *Curcuma longa* + *in vivo*) or (antioxidant + *Curcuma xanthorrhiza* + *in vivo*) in the month of May-June 2018.

3. Evaluation of *in vivo* antioxidant activities

Evaluation of antioxidant *in vivo* typically was performed using animal models in which the animals were administrated with tested samples at certain doses along with positive controls such as tocopherols and phenolic compounds (Moniruzzaman et al., 2012). After a specified time of treatment period, the animals were sacrificed and the tissue or blood was exploited for *in vivo* antioxidant assay, and antioxidant enzymes were measured. Several enzymes namely catalase, glutathione reductase, superoxide dismutase, glutathione peroxidase, gamma-glutamyl transpeptidase activity, and glutathione S-transferase were measured. Besides, glutathione levels and lipid peroxidation were also used as indicative of antioxidant activity *in vivo* (Nur Alam et al., 2013).

Catalase (CAT) enzyme converts hydrogen peroxide (H₂O₂) to water and oxygen. This enzyme activity is largely located in peroxisomes, subcellular organelles (Weydert et al., 2010). The activity of CAT is usually determined by involving the incubation of a test tube containing 0.5 mL of H₂O₂ and 0.1 mL of pancreatic homogenate in a water bath at temperature of 37°C for 1 minute. After incubation, the reaction is terminated using 0.5 mL of ammonium molybdate solution. A yellow color due to complex of ammonium molybdate and H₂O₂ is formed. The absorbance is measured at wavelength 405 nm using a spectrophotometer. One unit of catalase is usually defined as the amount of CAT enzyme capable of catalyzing the decomposition of 1 μmol of H₂O₂ per min (Lubrano and Balzan, 2015).

Superoxide dismutase (SOD) is an enzyme which catalyzes the partitioning or dismutation the superoxide radicals (O₂⁻) into either hydrogen peroxide (H₂O₂) or molecular oxygen (O₂). H₂O₂ is also damaging cells because it is easily converted into hydroxyl radical, therefore, H₂O₂ is degraded by catalase. Therefore, SOD and catalase are considered as an important antioxidant defense in nearly all living cells exposed to oxygen (Nur Alam et al., 2013). The procedure of SOD activity measurement is typically carried out using commercial kit (Cayman, MI, USA) which use a tetrazolium salt for the detection of superoxide radicals generated by xanthine oxidase and hypoxanthine. One unit of SOD is defined as the amount of SOD enzyme needed to produce 50% dismutation of superoxide radicals (Erujawa et al., 2010). This assay

measures all SOD types, namely Manganese (Mn), Copper/Zinc (Cu/Zn), and Iron (Fe) SODs.

The levels of glutathione (GSH), glutathione reductase (GR), glutathione peroxidases (GPO), and glutathione S-transferase (GST) are frequently used as *in vivo* antioxidant measures. GSH, an intra-cellular reductant involved in oxidation-reduction processes resulting in the formation of glutathione disulfide (GSSG), has been known to play an important role in metabolism, catalysis, and transport. GSH can protect cells against toxic compounds including free radicals and peroxides (Sapakal *et al.*, 2008). The GSH deficiencies can lead to the formation of certain diseases, therefore, any compounds capable of increasing GSH levels are considered as antioxidant (Nur Alam *et al.*, 2013). In addition, GR is an enzyme catalyzing the reduction of GSSG into GSH, a molecule playing a critical role in oxidative stress resistance and the reducing environment maintenance of the cell. GR activity is assayed using the substrate of oxidized glutathione (GSSG). Briefly, 1 mL of GSSG solution (2.728 mM and 40 μ L of pancreatic homogenate) was incubated in the water bath at 37°C for 5 mins. The reaction is initiated by the addition of 200 μ L of 1.054 mM NADPH solution and the decrease of absorbance is measured at 340 nm using the spectrophotometer and recorded every 30 s during the period of 5 mins. The GR activity is expressed as unit per mg protein based on the molar extinction coefficient of $6.22 \times 10^3 \text{ Lmol}^{-1}\text{cm}^{-1}$. One unit of GR is defined as the amount of enzyme that catalyzes the oxidation of 1 nmol of NADPH per min (Moniruzzaman *et al.*, 2012).

Glutathione peroxidase (GPO), a seleno-enzyme, in which one-third of the enzyme is present in the mitochondria and the remaining in the cytosol. GPO catalyzes the reaction of hydroperoxides with GSH to GSSG and the reduction product of hydroperoxide. The measurement of GPO is particularly important to patients who are under oxidative stress because the low activity of GPO is one of the early consequences of the imbalance between prooxidant and antioxidant (Nur Alam *et al.*, 2013). The activity of GPO is based on the principle of a coupled reaction with glutathione reductase (GR). The oxidized glutathione, GSSG, is formed after the reduction of hydroperoxide by GPO. In the presence of NADPH, GSSH is recycled to its reduced state by GR. The decrease of absorbance value of oxidized NADPH was measured spectroscopically at 340 nm. GPO (one unit) is defined as the amount of enzyme capable of catalyzing the oxidation of 1 nmol of NADPH per min at 25°C (Erejuwa *et al.*, 2010).

Glutathione S-transferases (GSTs) are enzymes involved in detoxifying harmful electrophilic

endogenous and exogenous compounds. These enzymes are involved during the conjugation of glutathione in phase two metabolism. There are some GST classes (with class members of) namely, alpha (GSTA1, GSTA2, GSTA3, GSTA4, GSTA5), kappa (GSTK1), mu (GSTM1, GSTM1L, GSTM2, GSTM3, GSTM4, GSTM5), omega (GSTO1, GSTO2), pi (GSTP1), theta (GSTT1, GSTT2, GSTT4), zeta (GSTZ1), and microsomal (MGST1, MGST2, MGST3) (Commandeur *et al.*, 1995; Sudiby, 2000; Nur Alam, 2013). Each class of GSTs has specific substrates (Sudiby, 2000). The activities of GST are typically determined based on the conjugation of GSH to 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate as reported by Habig *et al.* (1974, based on. Briefly, 2 mL of potassium phosphate buffer 0.3 M (pH 6.35), 75 μ L of CDNB solution 30 mM, 725 μ L of distilled water and 0.1 mL of pancreatic homogenate are pipetted into a test tube, vortexed and incubated at 37°C for 10 mins. After that, the reaction is initiated by the addition of 100 μ L of reduced 30 mM GSH solution and the decrease of absorbance was measured using spectrophotometer at 340 nm every 30 s for the period of 4 min. GST activity is calculated as unit per mg protein based on a molar extinction coefficient of $9.6 \times 10^3 \text{ Lmol}^{-1}\text{cm}^{-1}$. GST (one unit) was defined as the amount of enzyme catalyzing the conjugation of 1 nmol of GSH-CDNB per min.

Lipid peroxidation (LPE) is an autocatalytic process as a consequence of cell death. This process may cause peroxidative tissue damage in inflammation, cancer and toxicity of xenobiotics and aging. One of lipid peroxidation products during the peroxidation process is malondialdehyde (MDA), a product of free oxygen radicals. MDA is a common indicator of lipid peroxidation (Nur Alam *et al.*, 2013). LPE can be determined based on the procedure by Ohkawa (1979). Initially, the tissues are homogenized in 0.1 M buffer (pH 7.4) to obtain MDA: 0.2 mL tissue homogenate is added with 0.2 mL of sodium dodecyl sulfate (SDS) 8.1%, 1.5 mL of acetic acid 20% and 1.5 mL of thiobarbituric acid 8%. The mixture is then heated at 95°C for 60 mins in the water bath. After incubation, the mixture is cooled at ambient temperature and added with butanol: pyridine (15:1 v/v). The mixture is vortexed for 2 mins thoroughly, centrifuged at 3000 rpm for 10 mins, and the upper organic layer is measured spectrophotometrically at 532 nm against a blank. The levels of LPE are expressed as *n* moles of thiobarbituric acid reactive substances (TBARS)/mg protein using molar extinction coefficient of $1.56 \times 10^5 \text{ MLcm}^{-1}$.

4. *In vivo* antioxidant activities of *Curcuma longa*

The rhizome of *C. longa* is probably the most commonly used of *Curcuma* species in the herbal formulation. The antioxidant effects of *C. longa* are ascribed to the presence of curcuminoids, diarylheptanoid compounds of curcumin, demethoxycurcumin, and bisdemethoxycurcumin, which are considered the main active components in *C. longa*, although their bioavailability can be classified as poor due to low absorption, rapid metabolism and systemic elimination (Ireson *et al.*, 2001; Anand *et al.*, 2007).

The antioxidant activity of *C. longa in vivo* has been investigated by Dall'Acqua *et al.* (2016) using a metabolomic approach based on NMR and LC-MS/MS data. The results showed that some urinary levels namely *m*-tyrosine, allantoin, nitrotyrosine and 8-hydroxy-2'-deoxyguanosine decreased in the rats treated the group with orally administered *C. longa* for 33 days thus supporting the antioxidant effect *in vivo* of *Curcuma* extract. Zafir and Banu (2007) also investigated the efficacy of *C. longa* as antioxidant *in vivo* using Swiss Albino rats subjected to restraint stress. Rats were exposed to stress for 4 hrs during the light phase of the cycle and given with aqueous turmeric extract (50 mg/kg) orally. The results showed that administration of *C. longa* extract on stressed animals for 21 days prevented restraint stress-induced oxidative damage, as indicated by significant enhancement of superoxide oxidoreductase, glutathione S-transferase, glutathione-NADP+oxidoreductase and GSH, which were severely depleted by restraint stress in control rats.

The study by Baxla *et al.* (2014) revealed that Wistar albino rats had a significant increase in enzymes of aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT), and lipid peroxidation (LPO), decreased in superoxide dismutase (SOD), reduced glutathione (GSH) and increase in the accumulation of lead in liver. In addition, the treatment of *C. longa* at dose 500 mg/kg BW decreased the levels of ALP significantly ($p < 0.01$), ALT, AST, and LPO levels ($p < 0.05$) as well as increased the levels of GSH compared to group only treated with lead acetate. However, there were no significant differences ($p > 0.05$) in SOD and lead concentration levels in the liver when compared with the group only treated with lead acetate. From this result, it can be concluded that *C. longa* can decrease liver enzymes and increase antioxidant content. Curcumin, the main active component present in *C. longa*, in the form of nano also revealed to increase reduced glutathione levels and decreased reactive oxygen species and thiobarbituric acid reactive

substances levels in rats treated with lead acetate (Flora *et al.*, 2013).

Salama *et al.* (2013) also investigated the protective effects of ethanolic extract of *C. longa* (EECL) on rats treated with thioacetamide as a hepatotoxin. The oxidative stress was evaluated by measuring the levels of malondialdehyde (MDA), urinary 8-hydroxyguanosine (8-OH-dG) and nitrotyrosine levels, while the protective activities of EECL were evaluated through antioxidant enzymes. The results showed that EECL decreased MDA, nitrotyrosine, and urinary 8-OH-dG as well as increased antioxidant enzymes of catalase and superoxide dismutase statistically significant ($P < 0.05$). EECL also revealed to have protective effects in Male Sprague-Dawley rats treated with carbon tetrachloride (CCl₄) as hepatotoxin. The levels of AST and ALT in the serum and hepatic malondialdehyde had decreased significantly in rats group treated with EECL compared to CCl₄-treated group. While, the levels of antioxidant enzymes of SOD, catalase, and glutathione peroxidase activities, as well as glutathione content, increased significantly in EECL group compared to CCl₄-treated group. The levels of detoxifying enzymes of glutathione S-transferase (GST) were also found to have increased significantly in EECL group compared to CCl₄-treated group. These findings indicated that EECL has protective effects against CCl₄-induced hepatotoxicity in rats via antioxidant activities and phase II detoxifying enzymes (Lee *et al.*, 2010). EECL has also been reported to exhibit protective effects in liver injury due to the treatment of CCl₄ through increasing levels of antioxidants enzymes of SOD, glutathione peroxidase activity, and glutathione content in the CCl₄-treated group ($p < 0.05$), leading to a reduced lipid peroxidase level (Lee *et al.*, 2017). *C. longa* extract at the concentration of 0.5% given to Sprague Dawley rats also revealed the antioxidant activity *in vivo*. The oral treatment of *C. longa* extract could decrease the levels of MDA, serum urea and creatinine and increased the levels of GSH, SOD and total protein (Morsy *et al.*, 2008).

The antioxidant properties of fermented *C. longa* (FCL) have been investigated in rats treated with hepatotoxin of CCl₄ which induced oxidative stress. FCL at 300 mg/kg BW orally administered in rats could prevent the elevated levels of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) caused by CCl₄- induced hepatotoxicity. In addition, FCL enhanced the antioxidant capacities with higher activities of catalase, glutathione-S-transferase, glutathione reductase, and glutathione peroxidase, and level of reduced GSH. These findings suggested that FCL could be a developed as an

alternative protective agent against various diseases induced by oxidative stress through elevating antioxidative and decreasing lipid peroxidation (Kim *et al.*, 2014).

5. *In vivo* antioxidant activities of *Curcuma xanthorrhiza*

The main components of *C. xanthorrhiza* are starch (48.18-59.64%), volatile oils (3-12%) such as phellandrene, camphor, tumoral, cineol, borneol, and xanthorrhizol (1.48-1.63%), sesquiterpenes (β -curcumene, α -curcumene, bisabolane, lactone germacone), flavonoids (catechin, epicatechin, quercetin, myricetin, kaempferol, apigenin, luteolin, naringenin), and also curcuminoids (Ramdani *et al.*, 2016). Devaraj *et al.* (2014) have investigated the antioxidant activities of *C. xanthorrhiza* in vivo on Male Sprague Dawley rats (150–200 g) treated with carbon tetrachloride (CCl₄) as hepatotoxin. The hexane fraction of *C. xanthorrhiza* could serve as hepato-protector via antioxidant activities. Rats treated with CCl₄ indicated the decreased levels of glutathione peroxidase (GPx), superoxide dismutase (SOD), glutathione reductase (GR), catalase (CAT), and total protein (TP) and the increased level of malondialdehyde (MDA) levels in the liver compared to rats in control group. But, the treatment of hexane fractions of *C. xanthorrhiza* at doses of 250 mg/kg and 500 mg/kg returned the antioxidant enzymes as in control rats. This indicated that *C. xanthorrhiza* could act as antioxidant and may prevent the peroxidation of lipids induced by CCl₄. *C. xanthorrhiza* also revealed antioxidant activities on rats treated with single dose of β -D-galactosamine (288 mg/kg) intra-peritoneal (Lin *et al.*, 1996) and those treated for CCl₄ (Lin *et al.*, 1995). Devaraj *et al.* (2010) also proved that *C. xanthorrhiza* ethanolic extract (500 mg/kg) could increase antioxidant enzymes in acute hepatic diseases.

Alkiyumi (2013) has investigated the hepatoprotective activities of *C. xanthorrhiza* through evaluation of levels of antioxidant enzymes (SOD and CAT) and level of MDA. *C. xanthorrhiza* extract was subjected to fractionation. Fraction 5 of this extract revealed the highest levels of SOD and CAT on rats treated with thioacetamide. The active compounds identified using LC-MS/MS for these activities is xanthorrhizol. The ethanolic extract of *C. xanthorrhiza* rhizome (EECR) on the levels of GPx and MDA in the brains of trimethyltin (TMT) induced dementia model rats was evaluated by Yuliani *et al.* (2017). There is a significant difference between normal group rats and treated group rats ($p < 0.05$).

6. Conclusion

Curcuma species, especially *C. longa* and *C. xanthorrhiza* contained some phenolics compounds (curcuminoids) which played important roles in antioxidant activities. Using animal models, both *Curcuma* revealed high antioxidant activities as indicated by increasing levels of antioxidant enzymes of catalase, glutathione reductase, superoxide dismutase, glutathione peroxidase, and glutathione S-transferase and decreasing lipid peroxidation levels. *C. longa* and *C. xanthorrhiza* could be used as a preventive agent to any diseases caused by excessive radicals.

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