

Review on *in vitro* antioxidant activities of Curcuma species commonly used as herbal components in Indonesia

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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, aging, certain types of cancers, rheumatoid arthritis, neurodegenerative, and diabetes mellitus. In order to overcome the negative effects of these radicals, some scientists have explored some natural antioxidants from plants and its by-products. The antioxidant can be defined as any substances or samples capable of inhibiting free radical reactions in the oxidation reaction. Due to curcuminoids contained, Curcuma species such as *Curcuma longa*, *Curcuma heyneana*, *Curcuma mangga*, and *Curcuma xanthorrhiza* were commonly used for herbal components in some traditional medicine. Several *in vitro* tests been introduced and used to measure antioxidant activities, namely radical scavenging assay using 2,2'-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS⁺), ferric reducing antioxidant power (FRAP), ferric-thiocyanate, phosphomolybdenum method, cupric ion reducing antioxidant capacity, metal chelating power, beta-carotene bleaching linoleic-ferric-thiocyanate, and thiobarbituric acid methods. This review highlighted the antioxidant activities *in vitro* of *C. longa*, *C. heyneana*, *C. mangga*, and *C. xanthorrhiza* through several tests. To perform this review, several reputed databases were analyzed and used. From this review, it can be stated that Curcuma species have powerful antioxidant activities, therefore they could be potential sources of natural antioxidants and can be used as food supplements.

1. Introduction

Oxidative stress has been defined as the imbalance between the occurrence of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and the endogenous antioxidants present in human body (Persson *et al.*, 2014). When ROS/RNS interacts with various components in the human body, it forms free radicals or compounds capable of producing free radicals. Because free radicals have an unpaired electron, they are highly reactive and unstable. Free radical can react with some biological components (lipids, proteins, and DNA) which resulted in the formation of a new free radical (Lichtenberg and Pinchuk, 2015). The disadvantage

effects of oxidative stress in the human body have become a serious issue. Under oxidative stress condition, the human bodies produce more ROS/RNS such as hydroxyl radicals, nitric oxide radicals, hydrogen peroxide and superoxide anion radicals than endogenous antioxidants either enzymatic antioxidants (e.g., catalase, superoxide dismutase, glutathione peroxidase (GPx), and non-enzymatic ones (e.g., vitamin C, vitamin E, carotenoids, glutathione, and flavonoids) (Pisoschi and Pop, 2015). The imbalance between ROS/RNS and antioxidants leads to the cell damage (Lefer and Granger, 2000; Bhatia *et al.*, 2003; Uttara *et al.*, 2009) and health associated problems (Uchida, 2000; Steer *et al.*, 2002).

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Some degenerative disease is also associated with this imbalance (Rajendran *et al.*, 2014), including cardiovascular diseases (Gerber *et al.*, 2002), several types of cancers (Pool-Zobel *et al.*, 2005; Kaefer and Milner, 2008), degenerative rheumatoid arthritis (Hadjigogos, 2003), neurodegenerative and Alzheimer's diseases (Di Matteo and Esposito, 2003), aging and diabetes mellitus (Avignon *et al.*, 2012). One solution to solve these problems is the use of diet rich in antioxidant compounds contained in plant sources (Krishnaiah *et al.*, 1996). There are some definitions regarding antioxidant. One of the most commonly used and accepted by scientists is that antioxidants are any substances or any compounds capable of delaying or preventing the oxidation of substrate significantly when present at low levels compared with those of an oxidizable substrate. The oxidizable substrate refers to everything found in the living cells which include lipids, DNA, proteins, and carbohydrates (Al-Jaber *et al.*, 2011). In addition, biological antioxidants are defined as any compounds capable of protecting biological systems against the harmful effects of processes or reaction that can cause excessive oxidation. In our body, some endogenous antioxidants namely any substances having the ability to terminate the formation free radicals or to limit the damage effects caused by the oxidative reaction, are found (Halliwell, 1999).

The substances or compounds can be considered as antioxidants due to its activities as: (1) species scavenger which initiate peroxidation or free radical reactions, (ii) chelators of heavy metal ions so that the metals are unable to generate the reactive species, (iii) quencher of singlet oxygen, (iv) the breaker of free radicals chain reaction, and/or (v) reducing agents. Antioxidants include different classes of compounds, namely carotenoids such as carotenes and xanthophylls, vitamins like ascorbic acid (vitamin C) and tocopherols (vitamin E), and polyphenols such as phenolic acids, flavonoids, lignans and stilbenes (Oroian and Escriche, 2015). Endogenous antioxidants, a product from the body's metabolism, can be either enzymatic or non-enzymatic. Some enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), and peroxiredoxins (Prxs). The nonenzymatic antioxidants are represented by Ferritin, Transferrin, Myoglobin, Lactoferrin Metallothioneins, Coenzyme Q10 Ceruloplasmin, Glutathione and Polyamines (Babula *et al.*, 2012). Antioxidants can also be classified as synthetic antioxidants like Butylated hydroxyanisole (BHA), Butylated hydroxytoluene (BHT), *tert*-Butylhydroquinone (TBHQ), propyl gallate and natural antioxidants derived from plants (Shahidi and Zhong, 2010). Due to the safety concerns for synthetic

antioxidants, the natural antioxidants derived from plants are chosen to be used as a food supplement which functions as functional foods (Embuscado, 2018). Curcuma species which include turmeric (*Curcuma longa*), java turmeric (*Curcuma xanthorrhiza*), Temu mangga (*Curcuma mangga*) and *Curcuma henevnea*. The aim of this review was to highlight the antioxidant activities *in vitro* of *C. longa*, *C. henevnea*, *C. mangga* and *C. xanthorrhiza* through several tests of antiradical assay, ferric reducing antioxidant power, ferric-thiocyanate, phosphomolybdenum method, cupric ion reducing antioxidant capacity, metal chelating power, beta-carotene bleaching linoleic-ferric-thiocyanate, and thiobarbituric acid methods.

2. Materials and methods

During performing this review, the abstracts, reports, and research papers related to antioxidant activities of Curcuma species are identified, downloaded from several databases including Scopus, PubMed, and Google Scholar. The keywords used during searching for information was (antioxidant + Curcuma + *in vitro*) in the month of January-March 2019.

2.1 Evaluation of antioxidant activities *in vitro*

Several tests have been used for the evaluation of antioxidant activities *in vitro* (Moniruzzaman *et al.*, 2012), namely (1) radical scavenging methods using several radicals namely 2,2'-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS^{•+}), hydrogen, nitric oxide, and peroxy nitrite radicals, (2) reducing power including ferric reducing antioxidant power (FRAP), ferric-thiocyanate, phosphomolybdenum method, cupric ion reducing antioxidant capacity (3) metal chelating power, (4) lipid peroxide inhibition using beta-carotene bleaching linoleic-ferric-thiocyanate, and thiobarbituric acid (TBA) methods (Nur Alam *et al.*, 2013; Schaich and Xie, 2015). Among antioxidant assays, radical scavenging methods are the most commonly used for antioxidant screening of plant extracts (Permatasari and Rohman, 2016). The parameter of IC₅₀ (concentration of sample solution capable of scavenging 50% DPPH radicals) is used to assess the power of antiradical. The lower the IC₅₀ value, the higher the antiradical (Rohman *et al.*, 2006; Sharma and Bhat, 2016).

The reducing power was based on the capability of plant samples to reduce the oxidation systems. For example, ferric reducing activity power (FRAP) is relied on the capability of antioxidant to reduce the complex of Fe (III)-TPTZ or 2,4,6-tripyridyl-s-triazine to produce the intensely colored Fe (II)-TPTZ, having maximum absorption at 593 nm. The antioxidant capacity using

FRAP method can be evaluated by comparing the absorption change in the test mixture with that obtained from increasing concentrations of Fe^{3+} and expressed as mM of Fe^{2+} equivalents per kg or per L sample (Nur Alam *et al.*, 2013). In the phosphomolybdenum method, the antioxidant activity was measured based on the reduction of Mo (VI) to Mo (V) by the samples and subsequently the complex formation of a green phosphate-Mo (V) at acidic pH (Prieto *et al.*, 1999).

Metal chelating power was also considered as an antioxidant, because the excess of ree metals has implicated in the induction and formation of free radicals in the biological systems (Wong *et al.*, 2014). This assay was based on the measurement of iron-ferrozine complex. Ferro ion (Fe^{2+}), as representative of metal, can form a complex with chelates of ferrozine to obtain red colour having maximum absorption at 562 nm. This reaction is limited in the presence of other chelating agents and results in a decrease of the red color of ferrozine- Fe^{2+} complexes. Measurement of the color reduction due to the addition of evaluated samples for inhibition of complexation reaction Fe^{2+} -ferrozine determines the chelating activity. Ethylene diamine tetra acetate (EDTA) or citric acid can be used as a positive control (Soler-Rivas *et al.*, 2000).

Lipid peroxidation inhibition was based on the inhibition of peroxide formed during initiation step of free radical reaction. This assay can be represented by β -carotene bleaching and linoleic-ferric-thiocyanate (FTC) methods. β -carotene bleaching (BCB) method is rapid method used for antioxidants screening, mainly relied on the principle that an unsaturated fatty acid as represented by linoleic acid (C18:2) was oxidized by reactive oxygen Species, produced by oxygenated water. The oxidized products formed radicals which then oxidized β -carotene to yield discoloration of beta-carotene. The antioxidant components decrease the extent of discoloration, as measured at 434 nm (Kabouche *et al.*, 2007). In FTC method, the level of peroxide at the initiation stage of lipid peroxidation was determined. The peroxides are formed during linoleic acid oxidation. The peroxides oxidize Fe^{2+} to Fe^{3+} . The Fe^{3+} ions formed are complexed with thiocyanate to form red color of $\text{Fe}(\text{CNS}_6)^{3-}$, with maximum absorbance at 500 nm. The lower absorbance value indicated the higher the antioxidant activity (Saraswathy *et al.*, 2008). While, TBA method was based on the reaction between malonaldehyde, representative of secondary oxidation products, with barbituric acid to form red-colored complex with maximum absorbance at 532 nm (Ghani *et al.*, 2017).

2.2 Antioxidant activity of *Curcuma longa*

Curcuma longa, belonging of family Zingiberaceae, is probably a native plant from Southeast Asia. It is cultivated mostly in India followed by Bangladesh, China, Thailand, Cambodia, Indonesia, Malaysia, and Philippines (Ravindran *et al.*, 2007). In Indonesia, *C. longa* is recognized as *kunyit*. The dried rhizomes of *C. longa* are also known as turmeric. *C. longa* has been used as a medicinal plant for centuries. It is a famous medicinal plant and is believed to have many biological activities (Awin *et al.*, 2016).

The rhizomes of *C. longa* have orange-brown, pale yellow, or red yellow in color and it is usually harvested 7-9 months after planting. The powder of *C. longa* rhizomes or ground turmeric is prepared from dried finger rhizomes and it has yellow or red-yellow powder in color (Li *et al.*, 2011). Figure 1 shows the rhizomes and powdered rhizomes of *C. longa*, *C. heyneana*, *C. mangga* and *C. xanthorrhiza*. Turmeric has been widely used as a spice, food, preservative, coloring agent, and cosmetics in most of the South Asian countries. Recently, *C. longa* is extensively used in the treatment of various diseases because of its pharmacological activities. Several publications have reported that *C. longa* has numerous biological activities related to antioxidant, anti-inflammatory, and cancer preventive properties (Dall'Acqua *et al.*, 2016). Nowadays, research on *C. longa* is focused on antioxidant, anti-inflammatory, hepatoprotective, antimicrobial, and anticarcinogenic properties (Ravindran *et al.*, 2007).



Figure 1. The rhizomes of *Curcuma* species.

Members of *Curcuma* especially *C. longa* has the rich contents in curcuminoids that consist of curcumin, demethoxycurcumin, and bisdemethoxycurcumin (Jung

et al., 2012). Figure 2 shows the structure of curcuminoids. The rhizomes of *C. longa* contain the greatest amount of pigments and has been prized for its coloring, flavoring, and digestive properties. Curcumin is a yellow pigment, possesses many pharmacological activities. The active constituents of turmeric are the curcumin (diferuloylmethane) and several volatile oils such as turmerone, atlantone, and zingiberone.

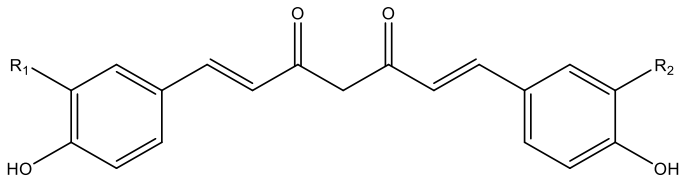


Figure 2. Chemical structure of curcuminoids (Curcumin, R1=R2=OCH3; demethoxycurcumin, R1=OCH3, R2=H; bisdemethoxycurcumin, R1=R2=H) (Rohman, 2012).

C. longa rhizome also contains sugars, protein, and resin (Li et al., 2011). In a standard form, turmeric contains moisture (>9%), curcumin (5-6.6%), mould (<3%), and volatile oils (<3.5%). The other compounds of turmeric contents are a variety of sesquiterpenes, like germacrone, termerone, alfa curcumenone, zingiberene, and curcumenone. Tanvir et al. (2017) have investigated antioxidant activity *in vitro* of *C. longa* from varieties of Kopil moni mura, Kopil moni chora, Chittagong mura, and Chittagong chora in Bangladesh via analysis of DPPH radical scavenging assay and ferric reducing antioxidant power. Among these varieties, the ethanolic extract of *C. longa* variety of Khulna's mura exhibited the highest DPPH radical-scavenging activity with the lowest IC₅₀ value of 1.08 µg/mL, while a variety of Khulna's chora showed the highest FRAP value of 4204.46±74.48 µM Fe(II)/100 g sample. The different fractions of methanolic extract of *C. longa* have been investigated as antioxidant *in vitro*. The fraction of ethylacetate (EtOAc) showed the highest DPPH IC₅₀ of 9.86 mg/mL. EtOAc fraction also revealed the highest ABTS radical scavenging and FRAP values. These activities were concentration dependent and highly correlated with phenolic contents present in that fraction (Choi, 2009). Nahak and Sahu (2011) have compared DPPH antiradical assay of ethanolic extract of *C. longa* and *C. aromatica*. The research showed that among extracts and fractions evaluated, the ethanolic fraction of *C. longa* revealed highest free radical scavenging activity accounting of 74.61±0.02% due to the presence of high amount of curcumin, while the ethanolic extracts and fractions of *C. aromatica* exhibited poor to moderate antioxidants. Unlike vitamin E and vitamin C, the antioxidant properties were not degraded by food processing such as cooking provides benefits (Krup et al., 2013). It is also reported that water-soluble and fat-soluble extracts of *C. longa* and its curcumin component

revealed strong antioxidant activity, comparable to vitamin C (Labban, 2014).

2.3 Antioxidant activity of *Curcuma heyneana* *in vitro*

Curcuma heyneana is widespread in tropical country. In Indonesia, especially in Java, *C. heyneana* (Figure 3) is also known as *Temu giring*. The main important part of this plant which has pharmacological properties is the rhizome. *C. heyneana* rhizome is yellow in color (Widyaningsih, 2013). Rhizomes of *C. heyneana* have some pharmacological properties. The saponin and flavonoid in these rhizomes provide a potent antioxidant and anti-inflammatory. The volatile oil has the ability to kill *Fasciola hepatica* worms (Woelansari et al., 2013). Widyaningsih (2013) reported that ethanolic extract of *C. heyneana* rhizomes could decrease triglyceride level. *C. heyneana* rhizomes have chemical components such as volatile oil 0.8-3%, amylum, lipids, saponin, yellow pigment, and flavonoid. The rhizomes contain curcumin that gives a yellow color, essential oils, starch, resins, fats, tannins, saponins, and flavonoids.

Zulaikha (2017) has investigated the antioxidant activities of chloroform extract of *C. heyneana* and the result showed that *C. heyneana* extract had strong antioxidant activities using DPPH radical scavenging assay with IC₅₀ of 50.93 µg/mL, ABTS DPPH radical scavenging assay with IC₅₀ of 90.90 µg/mL and FRAP value of 85.70 mM.

2.4 Antioxidant activity of *Curcuma mangga*

Curcuma mangga is distributed widely from Asia to Africa and Australia (Sasikumar, 2005). It is closely related to turmeric. *C. mangga* has the characteristics of raw mango aroma. Therefore in some countries, it is more recognized as mango ginger. *C. mangga* is also often called as *Curcuma amada*. The rhizomes are fleshy, buff coloured, 5 - 10 cm long, 2 - 5 cm in diameter and demarcated into nodes and internodes (Sheeja and Nair, 2012). The major chemical components include starch, phenolic acids, volatile oils, curcuminoids and terpenoids. These rhizomes are rich in phenolic contents including caffeic acid and ferulic acid. The terpenoids content are difurocumenonol, amadannulen and amadaldehyde.

Jalip et al. (2013) have compared DPPH radical scavenging activities of methanolic extracts of *C. heyneana*, *C. mangga*, *C. aeruginosa*, *C. phaeocaulis* and *C. purpurascens*. Among these, *C. purpurascens* has the highest antiradical activities with IC₅₀ of 36.30 µg/mL followed by *C. mangga*, *C. phaeocaulis*, *C. heyneana*, and *C. aeruginosa* with IC₅₀ values of 90.42, 110.92, 155.68 and 199.71 µg/mL, respectively. The H₂O₂-scavenging activity of fractions of *C. mangga* has

been evaluated by Pujimulyani *et al.* (2018). Ethyl acetate fraction revealed the highest in H₂O₂-scavenging activity with IC₅₀ of 162.78 µg/mL, compared to positive control of butylated hydroxytoluene (BHT) with IC₅₀ of 179.86 µg/mL. Indis and Kurniawan (2016) have reported that water extract of *C. mangga* has IC₅₀ value of 212.70 µg/mL. The bleaching treatment of *C. mangga* increased the antioxidant activity and quercetin content via hydrolyzing quercetin-3-rutinoside into quercetin (Pujimulyani *et al.*, 2012).

2.5 Antioxidant activities of *Curcuma xanthorrhiza*

Curcuma xanthorrhiza rhizome (Family of Zingiberaceae, Figure 1), also known as Javanese turmeric or *temulawak*, is a widely used traditional plant medicine from Indonesia (Ramdani *et al.*, 2016). The main substances of *C. xanthorrhiza* are starch (48.18-59.64%), volatile oils (3-12%) such as phelandren, camphor, tumerol, sineol, borneol, and xanthorrhizol (1.48-1.63%), sesquiterpenes (β-curcumene, ar-curcumene, bisabolane, lactone germacone), flavonoids (catechin, epicatechin, quercetin, myricetin, kaemferol, apigenin, luteolin, naringenin), and also curcuminoids.

Qader *et al.* (2011) have compared antioxidant activities *in vitro* of ethanolic and aqueous extracts of *C. xanthorrhiza* using DPPH radical scavenging and FRAP methods along with total phenolics compounds (TPC). At a concentration of 1 mg/mL, the ethanolic extract had the higher DPPH radical scavenging percentage (64.0%) than aqueous extract (62.3%) and revealed higher FRAP value of 2955 mmol/g compared to aqueous extract accounting of 358 mmol/g. These FRAP values correlated with TPC present in both extracts, in which ethanolic extract had higher TPC (88.0 mg/g) over aqueous extract (24.3 mg/g). The similar results were also obtained in which ethanolic extract of *C. longa* revealed higher antioxidant activities over aqueous extract using DPPH and ABTS radical scavenging assays, FRAP, and lipid peroxidation inhibition (Tilak *et al.*, 2004).

3. Conclusion

Curcuma species namely *Curcuma longa*, *C. xanthorrhiza*, *C. mangga* and *C. heyneana* which contained some phenolics compounds, mainly curcuminoids, exhibited antioxidant activities *in vitro* through several mechanisms namely radical scavenging, reducing power and lipid peroxidation. *Curcuma* species is also herbal components in several traditional medicine preparations. In addition, *Curcuma* species is widely used as a preventive agent to any diseases caused by excessive radicals.

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