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Mini Review

Flaxseed phenolics as natural antioxidants

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Abstract

Flaxseed is a rich source of different types of phenolics such as lignans, phenolic acids, flavonoids, phenylpropanoids and tannins. To date, extensive investigations has been undertaken to demonstrate antioxidant potential of flaxseed phenolics. However, antioxidant potential of all the flaxseed phenolics has not been collectively discussed. Hence, the aim of this article is to highlight the various types of phenolic components reported in the flaxseed; and to conduct a review of studies on antioxidant potential of flaxseed and its phenolic components. Future research perspectives pertaining to antioxidant potential of flaxseed phenolics and their possible commercial exploitation are also briefly discussed.

Introduction

In plants, phenols play an important role in protection against photo-oxidation and disease resistance (Antolovich *et al*., 2000). Phenolic compounds in general possess an aromatic ring bearing one or more hydroxyl substituents and may be found in free state, conjugated with sugars or esters or polymerized (Shahidi, 2000). They are not evenly distributed in tissues or cells of plants, and can be associated with components of the cell wall such as polysaccharides and proteins (Nackz and Shahidi, 2004). There are more than 8000 different known phenolic compounds with diverse structures (Robbins, 2003). In general, plant phenols on the basis of their basic structure, can be divided into different types: simple phenols, phenolic acids, coumarins and isocoumarins, naphthoquinones, xanthones, stilbenes, anthraquinones, flavonoids, lignans, lignins and tannins. Among these, phenolic acids and flavonoids are more common (De beer *et al*., 2002; Dykes and Rooney, 2007). In addition to protective effect, phenolics are responsible for color, taste, organoleptic properties of the plant origin foods (Yáñez *et al*., 2004).

Antioxidants are generally classified into two types, synthetic and natural antioxidants. Presently, use of antioxidants for the stability of food and food products is more common. It has been well established that fat and oil containing poly unsaturated fatty acids (PUFAs) are highly susceptible to oxidative deterioration. This oxidative deterioration resulting in rancid odors, flavors and decreased nutritional quality. Further, these oxidation products may be potential toxic compounds. Hence, there is a need to add antioxidants externally to preserve flavor, color loss and to avoid oxidative destruction of food. Most frequently used antioxidants for the preservation of food and food stuff are butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tert-butyl hydroquinone (TBHQ), vitamin C and E. Among the synthetic type antioxidants, BHT and BHA are widely used. However, they have been restricted recently because of serious concerns about their carcinogenic potential and liver damage (Gülçin *et al*., 2007; Wichi, 1988). Therefore, now days there is great interest in finding new and safe antioxidants from natural sources (Reddy *et al*., 2005).

Antioxidants can be classified as primary (chain-breaking) antioxidants or secondary (preventive) antioxidants (Decker *et al*., 2000). Primary antioxidants most often act by donating a hydrogen atom, while secondary antioxidants may act by binding metal ions able to catalyze oxidative processes, by scavenging oxygen, by absorbing UV radiation, by inhibiting enzymes or by decomposing hydroperoxides (Schwarz *et al*., 2000).

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Free radicals, reactive oxygen and nitrogen species (ROS/RNS) are continuously generated in the human body during metabolic processes; and are essential for energy supply, detoxification, chemical signaling and immune function (Dimitrios, 2006). However, over production of these species owing to an exposure of external oxidant substances or a failure in the body's own defense mechanisms such as endogenous enzymes (superoxide dismutase, glutathione peroxidase, catalase), leads to damage of valuable biomolecules such as DNA, lipids and proteins (Aruoma, 1998; Dimitrios, 2006). It is well recognized that these damages are associated with an increased risk of various degenerative diseases like cardiovascular disease, cancer etc. Phenolic compounds are excellent natural antioxidants. They are known to counteract with the excess free radicals, ROS; and nullified their pathological effects. The antioxidant capacity of phenolic compounds is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers or metal chelators (Demiray et al., 2009).

Linum usitatissimum L. (family: Linaceae), commonly known as 'Flaxseed' or 'Linseed'. Since antiquity, it has been cultivated for oil and fiber (Madhusudhan, 2009). Today, flaxseed is being cultivated in more than 50 countries; the majority of them are in the northern hemisphere. Canada is the main flax producer, followed by China, United States and India (Rubilar *et al*., 2010). Day by day, incorporation of flaxseed in food and in food products has been increasing due to its high content of essential omega-3 fatty acid, alpha-linolenic acid (ALA), dietary fiber and natural phenolic antioxidants (Prasad *et al*., 1998). On the basis of cultivar and growing conditions, flaxseed has been reported to contain 40-50% of oil, 23-34% of protein, 4% of ash, 5% of mucilage, and 0.9 -3% lignan precursors (Muir and Westcott, 1996; Muir and Westcott, 2003; Tour´e and Xueming *et al*., 2010). Flaxseed oil comprises about 73% of PUFAs. Further, PUFAs of flaxseed oil constitutes 51–55% essential omega-3 fatty acid, ALA (Hettiarachchy *et al*., 1990; Oomah and Mazza, 1993).

Extensive studies has been undertaken to demonstrate antioxidant potential of flaxseed and their phenolics. However, antioxidant potential of all the flaxseed phenolics has not been collectively discussed. Hence, the aim of this article is to highlight the various types of phenolic components reported in the flaxseed and to conduct a review of the studies on *in vivo* and *in vitro* antioxidant potential of flaxseed extract, fraction, and its individual purified phenolic components in different studied models. Future research purpose, scope and direction in the context of flaxseed phenolic antioxidants and their possible commercial exploitation are also briefly discussed.

Flaxseed phenols

Lignans

Plant lignans are the biologically important class of phenolic compounds. They belong to a group of phenols which are characterized by coupling of two phenylpropanoid units (Willfor *et al*., 2006). The levels of lignans in food vary widely; the richest source is flaxseed. The prevailing lignan in the flaxseed is secoisolariciresinol diglucoside (SDG) (Bambagiotti-alberti *et al*., 1994; Cardoso Carraro *et al*., 2012). Secoisolariciresinol (SECO) is the aglycone of SDG (Mazur *et al*., 1996). SDG-β-D-glucosidase hydrolyses the glucopyranoside bond of SDG and release SECO (Obermeyer *et al*., 1995). SDG was first time isolated from flaxseed by Bakke and Klosterman (1956). It has molecular formula, $C_{32}H_{46}O_{16}$, (Stasevich *et al*., 2009) and molecular weight 686.71 (Prasad, 1997). SDG results from the coupling of the 8 and 8′ C-atoms of the side chains of two coniferyl alcohol moieties (Davin *et al*., 1997) exists in two isomeric forms in the flaxseed (Bambagiotti-alberti *et al*., 1994). In flaxseed, SDG is stored in an ester-linked with 3-hydroxy-3-methylglutaric acid (HMGA) and other phenolic compounds such as p-coumaric acid and ferulic acid glycosides to form SDG oligomers of unknown (Yuan *et al*., 2008). The content of SDG varies between 6-29 g/kg in the defatted flaxseed powder (Johnsson *et al*., 2000; Johnsson *et al*., 2002; Charlet *et al*., 2002; Eliasson *et al*., 2003; Beejmohun *et al*., 2007). The lignan content in the flaxseed differ between varieties dependent on growing location and year (Thompson *et al*., 1997; Westcott and Muir, 1996). In addition to SDG, smaller quantities of other type lignans such as matairesinol, isolariciresinol, lariciresinol and pinoresinol have also been identified in the flaxseed (Meagher *et al*., 1999; Sicilia *et al*., 2003) (Figure 1). SDG is the converted into mammalian lignans, enterodiol (ED) and enterolactone (EL) by colon bacteria (Borriello *et al*., 1985; Wang *et al*., 2000) (Figure 2).

Phenolic acids

It is well known that the phenolic acids are the derivatives of benzoic and cinnamic acid; and are generally classified into two types, hydroxybenzoic and hydroxycinnamic acid. Flaxseed was reported to contain 8-10 g/kg total phenolic acids, about 5 g/kg of esterified phenolic acids and 3-5 g/kg of etherified phenolic acids (Oomah *et al*., 1995). They

Figure 1. Chemical structures of flaxseed lignans (Meagher *et al*., 1999)

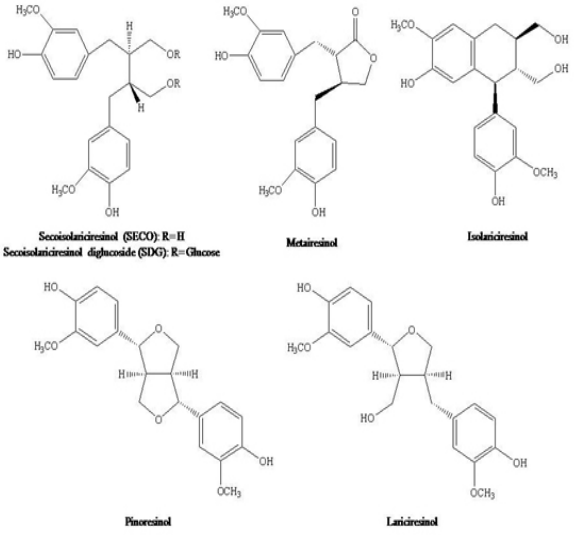


Figure 2. Structures of enterodiol and enterolactone (Meagher *et al*., 1999)

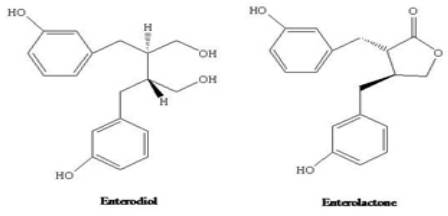
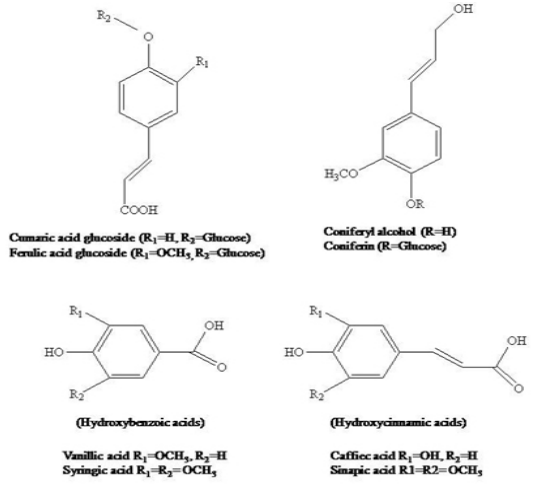


Figure 3. Chemical structures of some phenolic acids reported in the



are either in free and/or bound forms. Free phenolic acids are mainly composed of trans and cis-sinapic, o-coumaric, p-droxybenzoic, trans-p-coumaric and vanillic acids (Kozlowska *et al*., 1983; Babrowski and Soulski, 1984) (Figure 3). However, most of the flaxseed phenolic acids such as p-hydroxybenzoic, trans-ferulic and trans-p-coumaric acids are ester bound. Among these phenolic acids, ferulic and p-coumaric acid glucosides were accumulated at high concentrations in the flaxseed (Beejmohun *et al*., 2007). In addition, phenolic acid like caffiec acid and their glucosides were also reported in the flaxseed (Babrowski and Soulski, 1984). Variations in phenolic acid content in flaxseed were largely attributed to seasonal effects (Oomah *et al*., 1995).

Flavonoids

Flavonoids are the polyphenols, with C_6-C_3-C_6 skeleton that consists of two aromatic rings joined by a three-carbon link. Flavonoids generally include anthocyanins, flavanols, flavones, flavanones and flavonols. Depending upon growing and cultivar conditions, flaxseed possesses about 0.3-0.71 g of total flavonoids per kg of flaxseed (Oomah *et al*., 1996). In the flaxseed, flavonoids are in the form of their glucoside such as herbacetin 3, 8-O-diglucopynanoside, herbacetin 3, 7-O-dimethyl ether, and kaempferol 3, 7-O-diglucopyranoside (Qiu *et al*., 1996) (Figure 4). Herbacetin diglucoside (HDG) are ester linked in the lignan macromolecule via 3-hydroxy-3-methylglutaric acid (HMGA) (Struijus *et al*., 2007).

Phenylpropanoid glucoside

Phenylpropanoids are naturally occurring phenolic compounds having aromatic ring attached to three-carbon side-chain (C_6-C_3). Flaxseed reported to contain phenylpropanoid glucoside linusitamarin, together with other phenylpropanoid glucosides, linocinamarin and daucosterol (Luyengi *et al*., 1993) (Figure 5).

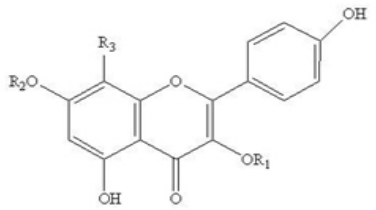
Tannins

In addition to above phenolics, presence of tannins along with phenolic acid and their glycosides were reported in n-butanol fraction of flaxseed (Kasote *et al*., 2011a; Kasote *et al*., 2011b).

Antioxidant potential of flaxseed phenols

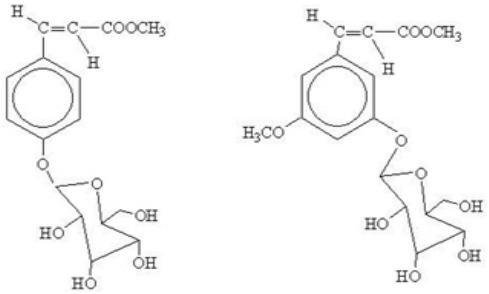
Accumulating evidences suggest that flaxseed is a rich source of natural antioxidants. Thus far, antioxidant potential of flaxseed and their phenolic constituents have been studied in both *in vitro* and *in vivo* models. In most of the studies, antioxidant potential of whole flaxseed or their extracts has been tried to correlate with their phenolic content. Velioglu *et al*. (1998) was studied antioxidant activity of flaxseed methanolic extract in β-carotene bleaching method. In this study authors investigated correction between total phenolics and antioxidant activity; which was found to be statistically significant. In another study, Zanwar *et al*. (2010) assessed *in vitro* antioxidant activity of ethanolic extract of *L. usitatissimum* (EE-LU) by using various methods such as DPPH radical scavenging, reducing power, superoxide scavenging, hydroxyl radical scavenging, hydrogen peroxide scavenging and metal chelating

Figure 4. Flaxseed flavonoids (Qui, 1999)



Herbacetin 3,8-O-diglucopyranoside: R_1=Glc, R_2=H, R_3=OGlc
Herbacetin: R_1=R_2=H, R_3=OH
Herbacetin 3,7-O-dimethyl ether: R_1=R_2=CH_3, R_3=OH
Kaempferol 3,7-O-diglucopyranoside: R_1=R_2=Glc, R_3=H

Figure 5. Chemical structures of phenylpropanoids glucosides reported in the flaxseed (Luyengi, 1993)



Limusitamarin **Linocinamarin**

assay at doses 100, 200,300, 400 and 500 µg/ml. EE-LU showed dose dependant antioxidant activity in different studied models, maximum at 500 µg/ml. Authors concluded that phenolic compounds seem to be the main components responsible for the observed antioxidant activity. Amarowicz *et al*. (2006) fractionated ethanol extract of defatted flaxseed meal into four major fractions, according to their maximum UV absorption and studied their antioxidant potential in a β-carotene–linoleate model system. Out of four major fractions, fraction I of phenolic compounds with maximum UV absorption at 290 nm was found to be most active.

SDG is most studied flaxseed phenolic compound pertaining to its in vitro antioxidant potential so far. Flaxseed lignan, SDG exhibited antioxidant activity by either direct radical scavenging or by inhibition of lipid peroxidation. Prasad (1997) studied hydroxyl radical scavenging potential of SDG. The ability of SDG to scavenge exogenously generated hydroxyl radical (•OH) was investigated by using ultraviolet (UV) light photolysis of H_2O_2, and by studying ability to prevent OH-induced lipid peroxidation in biological system. Results indicated that SDG scavenges concentration-dependent produced •OH radical and also prevented the lipid peroxidation of liver homogenate in a concentration-dependent manner. In addition to this, lipid peroxidation inhibitory potential of SDG, along with ED and EL was reported in linoleic acid emulsion model system (Kitts *et al*., 1999). Results showed that SDG, ED and EL were effective inhibitors against lipid peroxidation of a linoleic acid emulsion model system at 10 and 100 µM concentrations, although SDG and EL were more effective than ED. Moreover, Hosseinian (2006) evaluated *in vitro* antioxidant potential of SECO and SDG in a model of lipid peroxidation using 2, 2'-azobis (2-amidinopropane) dihydrochloride (AAPH). Results indicated that antioxidant mechanisms of both SECO and SDG would be based on their hydrogen donation ability to trap AP (Carbon-centre free radical of AAPH) radicals; and consequently inhibiting radical chain propagation. Moreover, antioxidant potential of SECO, SDG, SDG polymer and BHT was investigated by using two model systems, peroxyl radical-mediated liposomal oxidation system and canola oil oxidative degradation system (Hosseinian *et al*., 2006). Results of this study demonstrated that there was no significant difference between SECO, SDG and BHT; and suggested that flaxseed lignans could be good alternatives for oil stability. Furthermore, Hu *et al*. (2007) assessed plasmid DNA and phosphatidylcholine liposomes damage preventing 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and 2, 2'-azobis (2-amidinopropane) dihydrochloride (AAPH)-initiated peroxyl radical scavenging activities of SDG, SECO, ED and EL. These findings showed that the flaxseed lignans SDG and SECO exhibited strong antioxidant and protective effects in quenching the DPPH. Stable free radical and inhibiting AAPH peroxyl-radical-mediated damage of plasmid DNA and phosphatidylcholine liposomes at potentially feasible physiological concentrations (25–200 µM); whereas, the mammalian lignans ED and EL were ineffective, but observed to be effective latter when present at peroxyl radical: antioxidant ratios ≥1:1. Similarly, Prasad (2000) investigated antioxidant activity of SECO, ED, and EL by using chemiluminescence (CL) of zymosan-activated polymorphonuclear leukocytes (PMNL). Activation of PMNL is known to generate oxygen-free-radicals. Results exhibited that SDG, SECO, END, ENL, and vitamin E at concentration 2.5 mg/mL, produced 23.8%, 91.2%, 94.2%, 81.6%, and 18.7% reduction of zymosan-activated PMNL respectively and confirmed that SECO, END, and ENL are comparatively three times potent than SDG.

Besides lignan SDG and their metabolites, antioxidant activities of other flaxseed phenolics have been also studied. Phenolic acids such as p-coumaric acid and ferulic acid glucosides were accumulated at high concentrations in the flaxseed found to possess antioxidant properties (Schoenrock *et al*., 1997; Yuan *et al*., 2008). In addition to phenolic acids, flavonoids such as herbacetin 3, 8-O-diglucopynanoside, herbacetin 3, 7-O-dimethyl ether, kaempferol 3,7-O-

diglucopyranoside and lignan SDG, (-)-pinoresinol diglucoside were isolated from saturated n-butanol fraction of flaxseed and studied their antioxidant potential by using DPPH free radical scavenging assay (Qui *et al*., 1999). Results showed that among studied flavonoids, non-glycosylated herbacetin and herbacetin 3, 7-O-dimethyl ether possess relatively potent free radical scavenging activity. In our previous study, we selectively isolated ether insoluble phenolic components such as tannins, caffiec acid from n-butanol fraction of defatted flaxseed meal free from lignans and flavonoids (Kasote *et al*., 2011a; Kasote *et al*., 2011b). *In vitro* antioxidant potential of these molecules was evaluated by various methods such as total antioxidant activity, reducing power, DPPH free radical, hydrogen peroxide and nitric oxide scavenging activity. The results of this study outlined that ether insoluble phenolic components of n-butanol fraction (EPC-BF) possesses different levels of antioxidant activities. EPC-BF had significant *in vitro* lipid peroxidation inhibitory property and reducing power close to the standard antioxidants, BHT and BHA. However, observed free radicals such as DPPH, nitric oxide and hydrogen peroxide scavenging activity of EPC-BF was moderate when compared to standard antioxidant BHT and BHA ((Kasote *et al*., 2011a).

At present, there is no any direct *in vivo* evidence regarding the effect of flaxseed phenols on raising the levels of endogenous antioxidant defenses such as superoxide dismutase (SOD), catalase and glutathione peroxidase. However, Rajesha *et al*. (2006) was studied *in vivo* antioxidant activity of whole flaxseed supplementation by feeding weanling albino rats with 5% and 10% of flaxseed (constituting approximately 0.75 and 1.5 g/kg) for 14 days followed by challenging animals with 2.0 g/kg b.w. carbon tetrachloride (CCl_4) as toxin. Then antioxidant potential was assessed by measuring activities of hepatic marker enzymes like catalase, SOD and peroxide dismutase. Results of this study clearly indicated that beneficial flaxseed antioxidant components helps to restore the elevated activity of hepatic enzymes at almost normal level i.e. detoxify CCl_4 induced free radicals. Further, in another study it was demonstrated that dietary flaxseed supplementation increases antioxidant defenses through both reduced ROS generation and increased ROS detoxification (Lee *et al*., 2008).

Bhatia *et al*. (2007) studied radioprotective and antioxidative potential of flaxseed oil was studied against radiation-induced hepatotoxicity in mice. Prophylactic effect of flaxseed oil supplementation after exposure of single dose of gamma radiation was assessed by estimating biochemical parameters such as lipid peroxide, reduced glutathione, total protein, aspartate aminotransferase (AST), alanine aminotransferase (ALT), acid and alkaline phosphatase. Results showed that the radiation-induced deficits in body and organ weight were significantly reduced or prevented in flaxseed oil pretreated mice; and the observed protective effect could be due to omega-3 fatty acids and phytoestrogenic lignans.

Conclusion and future perspectives

Flaxseed represent valuable source of phenolic antioxidant. However, *in vitro* and *in vivo* antioxidant efficacies of all the flaxseed phenolics have not been thoroughly documented. In this article an attempt was made to discuss flaxseed phenolics together with their antioxidant potential. Flaxseed lignans SDG, SECO, ED and EL are found to be equal or somewhat more potent than BHT, vitamin E. Thus, they could have commercial potential as an alternative to these antioxidants. Flaxseed lignans could be the good choice of natural antioxidants for oil stability. Flaxseed antioxidant may have potential application in food and heath industry as food stabilizer, nutraceutical etc. However, there is need to investigate the safety and efficacy these of molecules for making them excellent natural antioxidants. Moreover, further research in the context of *in vitro* and *in vivo* antioxidant potential of flaxseed phenolics such as flavonoids, phenolic acid, phenylpropanoids and tannins is also essential, as limited research attention has been received by them so far.

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