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Antioxidant Potential of Secoisolariciresinol Diglucoside Isolated from Different Fractions of Flaxseeds

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ABSTRACT

Flaxseed lignan (secoisolariciresinol diglucoside; SDG) was isolated from dehulled flaxseed fractions such as hull, endosperm and flour of LVF-01 and GVF-03 varieties and were analyzed using HPLC. The flaxseed fractions containing SDG were evaluated for 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and reducing power. An EC_{50} of 12.6, 16.4 and 0.016 mg/mL were observed for hull fractions of LVF-01, GVF-03 and standard SDG, respectively. The flour fractions of both varieties showed moderate activity compared to hull fractions with an EC_{50} between 24 and 27 mg/mL, respectively. Least activity was observed in the endosperm fraction of LVF-01 (EC_{50} = 320 mg/mL) and GVF-03 (EC_{50} = 330 mg/mL) varieties. Further, dose dependent reducing power was observed in all the fractions. The hull fraction of LVF-01 showed 1.79 and 7.87 fold higher activity compared to their flour and endosperm fractions, respectively. Similarly, the hull fraction of GVF-03 showed a 1.41 and 7.31 fold higher activity than flour and endosperm fractions, respectively. In addition, at 3.0 mg dosage both LVF-01 and GVF-03 hull fractions offered protection to DNA against hydroxyl radical induced damage suggesting the antioxidant potential of SDG in flax seed fractions.

Keywords: endosperm, flour, hull, lignan, *Linum usitatissimun*, reducing power
Abbreviations: DPPH, 1, 1-diphenyl-2-picrylhydrazyl; **EC_{50}**, effective concentration for 50% DPPH radical scavenging activity; **NDGA**, nordihydroguaiaretic acid; **SD**, standard deviation; **SDG**, secoisolariciresinol diglucoside; **SPE**, solid-phase extraction

INTRODUCTION

Several diseases such as cancer, hypertension, obesity, and cardiac dysfunctioning involve oxidative processes mediated by free radicals (Fujita *et al*. 2007; Pennathur and Heinecke 2007; Roy *et al.* 2007). Plants are known to produce various phytochemicals of therapeutic importance. Therefore, in recent years, the importance of the search for and exploitation of natural antioxidants, especially of plant origin, has increased (Jayaprakasha and Jaganmohan Rao 2000).

Flaxseed (*Linum usitatissimun*) is an important oilseed crop grown for its oilseed and fiber, which contains important phytochemicals including ω-3-fatty acids (*n*-3) and lignans (Wanasundara and Shahidi 1998). The interest in flaxseed has been increasing at an alarming rate from the food industries, because of the presence of bioactive compounds of therapeutic value. Flaxseeds are incorporated into foods for its proposed health benefits. It is typically processed to obtain oil and meal, but unlike many other oilseeds, flaxseed is not dehulled before oil extraction. Whole flaxseeds are incorporated into poultry rations to obtain ω-3-fatty acid enriched eggs in the form of a value added product or functional food. SDG, a mammalian lignan precursor is metabolized into enterodiol and enterolactone. In addition to high content of SDG, flaxseed is also known for other lignan precursors such as matairesinol relatively in lower level (Mazzur *et al.* 1996).

Lignans are bioactive compounds with beneficial effects in humans (Adlercreutz and Mazzur 1997) as phytoestrogens, anti-carcinogenic, anti-estrogenic and estrogenic effects (Menkes *et al.* 1980; Bingham *et al.* 1998). Lignans like sesamin, sesamolin, and schisandrin from different plant sources are known for their antioxidant activities (Willett *et al.* 1984). There are reports on beneficial effects of antioxidants such as β-carotene and vitamin E in the prevention of certain types of cancers like breast cancer (Menkes *et al.* 1980; Horvath and Ip 1983). SDG has structural similarity in its aglycone form, secoisolariciresinol with the known antioxidant nordihydroguaiaretic acid (NDGA). Previous studies reported the hydroxyl radical scavenging activity of SDG from flaxseed in aqueous, *in vitro* tissue homogenate as well as *in vivo* (Lu and Liu 1992; Prasad 2002). SDG is hydrolyzed to enterodiol and enterolactone by the action of gut microflora, and also exerts antioxidant and anticarcinogenic effects (Kirk *et al.* 1981; Kitts *et al.* 1999). Also, SDG appears to be effective against breast and colon cancers (Borriello *et al.* 1985), hyper cholesterolaemia, atherosclerosis, diabetes (Prasad 2004; Zhang *et al.* 2008), lupus nephritis, immune and inflammatory reactions (Cox and Wood 1987; Clark and Anwar 1998). Bakery products such as cookies and biscuits made using lignans from flaxseed and with genistein (soy) have also been implicated in cancer prevention (Jenkins *et al.* 1999). The *in vivo* antioxidant potency of flaxseed has recently been reported (Rajesha *et al.* 2006). However, none of the studies have determined the antioxidant activity of individual flaxseed fractions of Indian flaxseed cultivars obtained upon dehulling. Hence, the present investigation was undertaken to evaluate the antioxidant properties of SDG extract of hull, endosperm and flour fractions of two Indian flaxseed cultivars that were obtained upon dehulling.

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Original Research Paper

MATERIALS AND METHODS

Chemicals

Folin-Ciocalteau reagent, 1,1-diphenyl-2-picryl hydrazyl (DPPH), ascorbic acid, agarose and calf thymus DNA were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Standard secoisolariciresinol diglucoside (SDG) was provided by Dr. Neil Westcott (Saskatoon Research Center, Agriculture and Agri-Food Canada, Saskatoon, Canada) as a generous gift. Ferric chloride, Potassium ferricyanide, Trichloroacetic acid and general solvents used in the experiments were of analytical grade and purchased from Ranbaxy Fine Chemicals Ltd., India.

Flaxseed material and its fractions

Two flaxseed (*L. usitatissimu*m) cultivars grown in Ranebennur and Gadag, North Karnataka, India, were collected (15 samples each from different plots) and preserved under dry conditions at room temperature for analysis. The University of Agricultural Sciences, Hebbal, Bangalore, Karnataka, India authenticated and identified the seed varieties as LVF-01 and GVF-03. Flaxseeds were processed by the combination of conditioning, dehulling, sieving and aspiration. The seed samples (10 kg) were cleaned and subjected for dehulling in a dehuller (Kisan Krishi Yantra Udyog, Kanpur, India) at the Department of Grain Science and Technology, CFTRI, Mysore, India. The fractions such as hull, endosperm and flour were obtained after dehulling process of both seed cultivars LVF-01 and GVF-03. The fractions were labeled as hull, LVFH and GVFH; endosperm, LVFE and GVFE; flour, LVFF and GVFF.

Extraction of SDG from flaxseed fractions

The SDG extracts were prepared from flaxseed fractions such as hull, endosperm and flour by the Klosterman method described by Rickard *et al*. (1996). About 100 g of hull, endosperm, and flour fractions obtained from flaxseeds were defatted by extracting with petroleum ether and chloroform (1:6, v/v). The defatted flaxseed fractions were extracted with 10 mL of 1, 4-dioxane and 95% ethanol (1:1, v/v) in screw-capped test tubes for 16 h at 60°C in a circulating water bath. The supernatant was separated from the residue by centrifugation at 2000 × *g* for 30 min. After separation, solvent was gently evaporated under vacuum at 40°C using a flash evaporator (Rotavapor-laborata-4000, Heidolph, Heizbad, WB, Germany). The extracts were then treated with 0.3 M aqueous sodium methoxide in anhydrous methanol and concentrated. The concentrates were acidified to pH 3.5 by adding sulphuric acid and further eluted through silica column with chloroform: methanol: water (65: 35: 10, v/v/v). Then, the SDG rich extracts were subjected to Solid-Phase Extraction (SPE) to remove salts and phenolics according to the method of Johnsson *et al*. (2000) using RP-C18 reversed SPE columns (Thermo Electron Co. ODS Hypersyl, 6 mL/g, USA) prior to HPLC analysis.

High Performance Liquid Chromatography (HPLC) analysis of SDG

High Performance Liquid Chromatographic analyses were carried out using Shimadzu model LC-10A (Shimadzu, LC-10A, Japan) fitted with solvent delivery system, guard cartridge column, photodiode array detector and integrator. The column was a Shimpack RP-C_{18} column with 5 µm particle size and 4.6 mm inside diameter and 250 mm length, the detector was a SPD 10 M AVP photodiode array detector and C-R7A integrator with class-10A real time analyzer software. The linear gradient mobile phase was 1% (v/v) acetic acid in water and methanol (Muir and West Cott 2000). The instrument was run for 3-5 min at initial gradient before injecting the next sample. The column thermostat was set to 40°C and the eluent was detected at 280 nm. A series of 5 different standard solutions of SDG ranging from 0.0625-1.0 mg/mL was used to obtain linearity curve. The correlation coefficient (r = 0.9953) linearity curve for all the SDG solution was obtained. The SDG peaks of samples were identified and quantified by comparison with those of standard SDG solutions. Linearity curve for standard SDG was prepared by injecting 10 µL of standard solutions, the amount of SDG was determined from the linear regression equation. The repeatability of the method was evaluated by injecting standard solutions of SDG six times and percent relative standard deviation was calculated. The retention time for SDG was 31.09 min. A scan of the SDG peak was also performed at 240-600 nm. Amount of SDG present in various flaxseed fractions were calculated.

DPPH radical scavenging assay

The model system of DPPH radical scavenging ability is a simple method to evaluate the antioxidant activity of phytochemicals. DPPH was used to determine the proton-radical scavenging action of the lignan fractions as it possesses a proton free radical, which shows a characteristic absorption at 517 nm. DPPH radical is scavenged by antioxidants through the donation of an electron forming reduced DPPH (1,1-diphenyl-2-picrylhydrazine). The color changes from purple to colourless after reduction and the degree of discoloration indicates the scavenging potential of the antioxidant extract, which can be quantified at 517 nm. The effect of flaxseed SDG extracts on DPPH radical was estimated according to the method of Lai *et al.* (2001). An aliquot (200 µl) of the flaxseed SDG extracts (4–20 mg/mL) or standard SDG (4-20 µg/mL) was mixed with 100 mM Tris-HCl buffer (800 µL, pH 7.4) and then added to 1 mL of 500 µM freshly prepared DPPH solution in ethanol (final concentration of 250 µM). The mixture was shaken vigorously and allowed to stand for 20 min at room temperature in dark. The absorbance of the resulting solution was measured in a UV-Visible spectrophotometer (Genesys-5, Milton Roy, NY) at 517 nm. The percent DPPH radical scavenging ability was calculated according to the following equation:

DPPH radical scavenging activity (%) =
$[(A_{control} - A_{sample})/A_{control}] \times 100$

The DPPH radical scavenging activity was plotted against concentration of flaxseed SDG extracts and effective concentration for 50% DPPH radical scavenging activity (EC_{50}) was calculated.

Measurement of reducing power

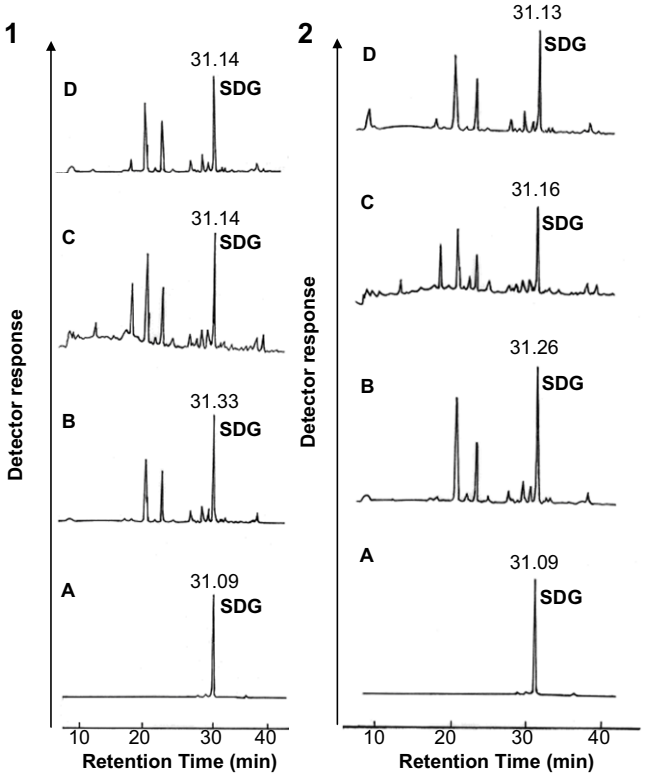
The reducing power of flaxseed SDG extracts and standard SDG were determined according to the method of Yen *et al*. (1995). The flaxseed SDG extracts (4–20 mg/mL) or standard SDG (4-20 µg/mL) was mixed with an equal volume of 0.2 M phosphate buffer, pH 6.6 and 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min. An equal volume of 10% trichloroacetic acid was added to the mixture and then centrifuged at 650 × *g* for 10 min. The upper layer of solution was mixed with distilled water and 0.1% $FeCl_3$ at a ratio of 1:1:2 and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

DNA protection assay

DNA protection activity was performed using calf thymus DNA according to the method of Rodriguez and Akman (1998) with minor modifications. Briefly, calf thymus DNA (1 µg) was treated with Fenton's reagent (0.3 mM H_2O_2, 0.5 µM ascorbic acid and 0.8 µM $FeCl_3$) in presence and absence of flaxseed SDG extracts (3 mg) in addition to standard SDG (3 µg). The final volume of the mixture was brought up to 20 µL, incubated for 30 min at 37°C and the DNA was analyzed on 1% agarose gel followed by ethidium bromide (0.5 µg/mL) staining. Gel was documented (Herolab, Germany) and the intensity of bands was determined (Easywin software).

Statistical analysis

All experimental results of HPLC (n = 4) and spectrophotometric (n = 3) analyses were expressed as mean ± standard deviation (SD). One-way analysis of variance and Duncan Multiple Range Test (DMRT) was conducted to identify differences among means. Statistical significance was determined at $p < 0.05$.



Figs. 1 and 2 HPLC profiles of SDG fractions of LVF-01 (Fig. 1) and GVF-03 (Fig. 2) varieties of flaxseed. Standard SDG (**A**), hull (**B**), endosperm (**C**) and flour (**D**).

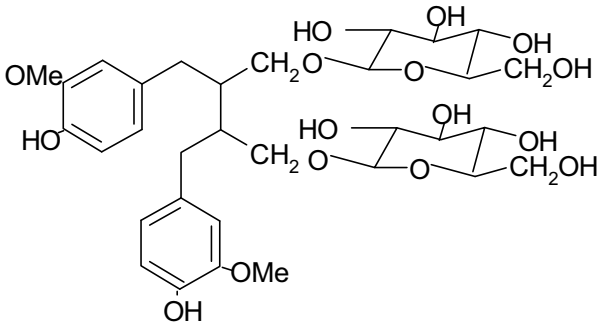


Fig. 3 Structure of secoisolaricinol diglucoside (SDG, 2, 3-bis [(4-hydroxy-3-methoxyphenyl) methyl]-1, 4 butane-diglucoside).

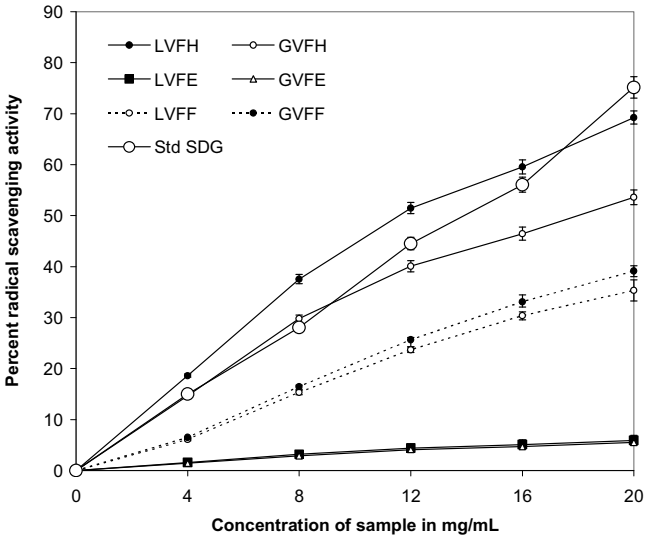


Fig. 4 Effect of SDG extracts from different fractions of LVF-01 and GVF-03 varieties on DPPH radical scavenging activity. Concentration of standard SDG (in μg/mL). Values are mean ± SD (n = 3).

RESULTS

HPLC analysis of SDG

The HPLC pattern of the SDG extracts of different flaxseed fractions are shown in **Figs. 1, 2**. The HPLC patterns of the SDG extracts of different fractions from two different India flaxseed varieties are comparable. HPLC analysis revealed the presence of SDG (**Fig. 3**) in all the flaxseed fractions of both varieties. A retention time of 31.09 min was observed for standard SDG and based on this retention time the SDG contents in different fractions were calculated. Our results showed higher SDG content in the hull fractions of both varieties. The SDG content of the LVF-01 hull fraction (16.9 g/kg) was higher compared to the GVF-03 hull fraction (12.5 g/kg). The SDG content of LVF-01 in all three fractions, namely hull, endosperm and flour, was higher than that of GVF-03 fractions.

DPPH radical scavenging activity

Free radical scavenging potentials of flaxseed SDG extracts of different fractions at various concentrations were tested by DPPH method, and the results are shown in **Table 1**. It indicates the comparative EC_{50} value for DPPH radical scavenging activity of different lignan fractions of GVF-03 and LVF-01 varieties of flaxseed with that of standard SDG. An EC_{50} of 12.6 and 16.4 mg/mL was observed for LVFH and GVFH fractions respectively which showed higher activity than all other fractions except standard SDG (EC_{50} = 16 μg). LVFF and GVFF showed moderate activity compared to the hull fractions with an EC_{50} of 27 mg and 24 mg extract respectively. Least activity was observed in the endosperm fractions of LVF-01(EC_{50} = 320 mg) and GVF-03 (EC_{50} = 330 mg) variety. In addition the radical scavenging activity at different doses (4–20 mg/mL) showed a dose dependent increase in activity (**Fig. 4**). The activities at 20 mg dosage were in the order LVFH > GVFH > GVFF > LVFF > LVFE > GVFE.

Table 1 EC_{50} of hull, flour and endosperm SDG fractions of LVF-01 and GVF-03 flaxseed varieties.

Sample	EC_{50} (in mg)
Standard SDG	0.016 ± 0.001 c
LVF Hull	12.6 ± 0.1 bc
GVF Hull	16.4 ± 0.21 bc
LVF Endosperm	320.0 ± 21.8 a
GVF Endosperm	330.0 ± 13.5 a
LVF Flour	24.0 ± 3.6 b
GVF Flour	27.0 ± 1.7 b

Values are mean ± SD (n = 3).
Different letters indicate significant differences (p < 0.05) according to DMRT.

Reducing power

Further, we investigated the reducing power of different SDG extracts of various fractions (**Figs. 5, 6**) by measuring the formation of Perl's Prussian blue at 700 nm in Fe^{3+}/ferricyanide complex system. The reducing power (indicated by increased absorbance at 700 nm) of flax seed fractions at equal concentration (10 mg/mL) compared with that of known SDG standard (20 μg/mL) showed that all the fractions had lesser reducing power ability than the standard (**Fig. 5**). The hull fraction showed higher reducing power compared to the endosperm and flour fractions in both varieties. At different dosage (4–20 mg/mL) all the fractions of the flaxseed showed an increased activity with increase in concentration of the extract (**Fig. 6**). LVFH showed 1.79- and 7.87-fold increased activity over LVFF and LVFE respectively, while 1.41- and 7.31-fold higher activity in GVFH was observed over GVFF and GVFE fractions, respectively.

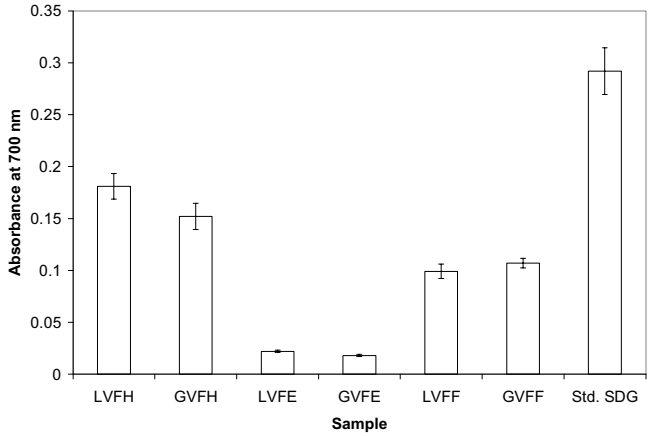


Fig. 5 Comparative reducing power of SDG extracts from different fractions of LVF-01 and GVF-03 varieties (at 10 mg/mL) and standard SDG (0.02 mg/mL). Values are mean ± SD (n = 3).

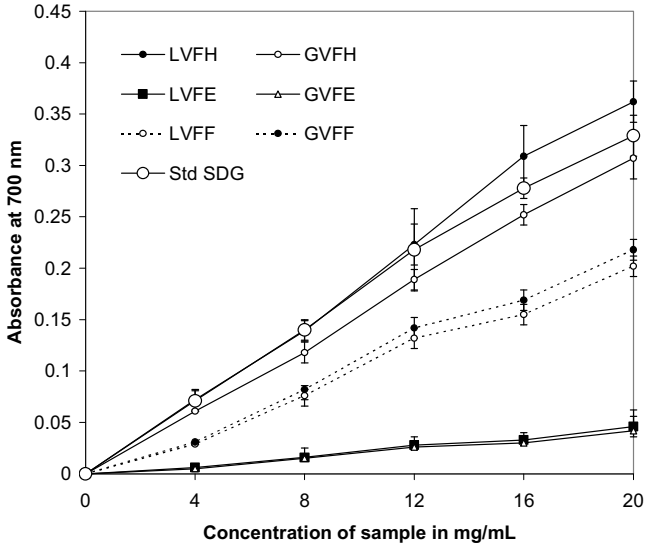


Fig. 6 Reducing power of SDG extracts from different fractions of LVF-01 and GVF-03 varieties. Concentration of standard SDG (in μg/mL). Values are mean ± SD (n = 3).

DNA protection

In addition, as the hull fraction (LVFH and GVFH) of both flaxseed varieties showed good antioxidant activity, DNA protective activity was also carried out. To examine the protective effect of different lignan fractions, calf thymus DNA was subjected to oxidation in the presence and absence of hull and flour fraction of both varieties using Fenton's reagent, the extent of damage and protection offered by the lignan fractions was assessed by the relative electrophoretic mobility of the oxidized and lignans treated DNA on 1% agarose gel compared to that of native DNA. **Fig. 7** shows that the addition of Fenton's reagent caused fragmentation of DNA (lane 2) and increased mobility of DNA. Since the DNA used was from calf thymus, streaks were observed (even in case of native DNA) in all the lanes indicating the presence of a mixture of DNA fragments. However, the fragmentation of DNA was more in oxidant treated samples. The DNA bands in all the lanes corresponding to the band of native DNA with maximum intensity were considered in determining the protection by lignan fractions. At 3.0 mg dosage LVFH showed an increase in DNA protection (lane 4) compared to its flour fraction (lane 3). Also, the GVF-03 variety showed a similar pattern of DNA protection with higher DNA protecting ability in hull fraction compared to its flour fraction. DNA protection of standard SDG (3 μg) was almost equal to that of native DNA. However, DNA protection was not evident in endosperm fraction of both varieties at this concentration (Results not shown).

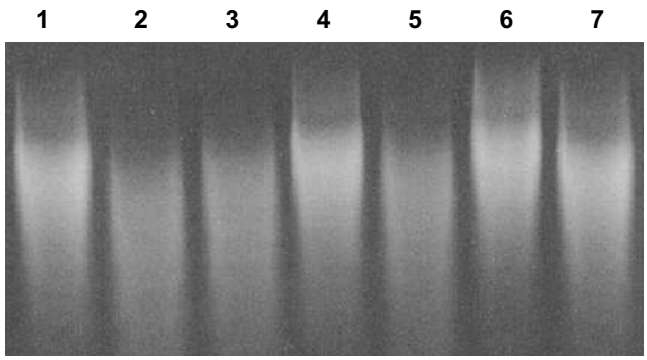


Fig. 7 Effect of SDG from different fractions of LVF-01 and GVF-03 varieties on hydroxyl radical induced DNA damage. Lane 1 – native DNA; Lane 2 – oxidized DNA; Lane 3 – DNA + LVFF + oxidant; Lane 4 – DNA + LVFH + oxidant; Lane 5 – DNA + GVFF + oxidant; Lane 6 – DNA + GVFH + oxidant; Lane 7 – DNA + Std SDG + oxidant.

DISCUSSION

The lignans are phytoestrogens and are similar to estrogen, due to their structural analogy and they bind to the estrogen receptor. Therefore, they are believed to act both as agonists and antagonists for estrogens, there by having similar and opposing actions in comparison with endogenous estrogen (Setchell and Cassidy 1999). Like other phytochemicals, lignans also possess antioxidant properties. They have been shown to suppress tumor promoter induced hydrogen peroxide and superoxide anion formation (Lu and Liu 1991; Kurzer and Xu 1997). In addition to its antioxidant properties, they also enhance the activity of a number of antioxidant enzymes including catalase, glutathione peroxidase, glutathione reductase and superoxide dismutase (Rajesha *et al.* 2006). Lignans play a role in scavenging reactive oxygen species *in vitro* and *in vivo* studies (Hu *et al.* 2007). They are reported to have protective effects on chemical toxicity in hepatic cells in mice (Ip *et al.* 1995). Lignans have been shown to suppress tumor-promotor induced hydrogen peroxide and superoxide anion formation (Thompson *et al*. 1996a). SDG scavenges hydroxyl radical, which causes a decrease in the level of oxygen free radicals and leads to lower risk factors for the development of hyper cholesterolemic atherosclerosis (Prasad *et al*. 1994).

It is well known that free radicals are one of the causes of several diseases, such as Parkinson's disease, coronary heart disease, and cancer (Adams and Odumbe 1991; Hertog *et al*. 1993; Cerutti 1994). The chemoprotective ability of flaxseed and its lignan SDG against colon (Jenab and Thompson 1996) and mammary (Thompson *et al.* 1996b) cancer has been reported. SDG, a plant lignan found in flaxseed, has been shown to be converted to mammalian lignans enterodiol and enterolactone in both *in vitro* fermentation studies with human colonic bacteria (Rickard *et al.* 1996) and possesses antioxidant property (Kitts *et al.* 1999). Although antioxidant activity has been described for SDG (Lu and Liu 1992), there is lack of knowledge on the composition of SDG in hull, flour and endosperm fraction and their antioxidant activities of flaxseed varieties. Therefore, in the present study, we evaluated the antioxidant activity of SDG extracts in different *in vitro* model systems and their SDG content in different flaxseed fractions of LVF-01 and GVF-03 varieties, which are being consumed at lower levels as food substitutes. Added to this, both LVF-01 and GVF-03 varieties are widely being cultivated in Northern Karnataka.

The HPLC chromatogram showed the presence of SDG as one of the major components among the organic molecules of seed extracts, which showed maximum absorbance at 280 nm along with other lignans (**Figs. 1, 2**). The two samples that contained the highest SDG levels were LVFH

and GVFH. In contrast, LVFF and GVFF exhibited lower content of SDG.

The DPPH radical is widely used to investigate the radical scavenging activities of several natural phenolic compounds and also crude extract of plants (Julie *et al.* 2002). In this study, DPPH radical scavenging activity was found in both the varieties of flaxseed fractions. These results agreed with the previous studies using a different method to assess the antioxidant activity of flaxseed lignans (Prasad *et al.* 1994). Compared to standard SDG, all the fractions showed less activity. Among the fractions tested for activity endosperm and flour fractions of both varieties were similar with an EC_{50} of 320–330 and 24–27 mg/mL, respectively. The hull fraction of LVF-01 showed comparatively higher activity than GVF-03. Consequently, hull fractions are the main fractions of flaxseeds that would contribute significantly to their higher antioxidant capacity. The other two dehulled fractions endosperm and flour may be cross contaminated with hull. This could be the reason for the fact that endosperm samples LVF-01 and GVF-03 exhibited a moderate activity and flour fractions showed lesser activity. This indicates that the flaxseed fraction of both the varieties has good potential as a source for natural antioxidants especially the hull fractions of LVF-01 and GVF-03.

To substantiate the results of DPPH radical scavenging activity of flaxseed extracts, the reducing power of the same extracts were also evaluated. The electron donating capability of flaxseed fractions as evaluated by the reduction of ferric chloride and potassium ferricyanide complex showed high activity in standard SDG and comparatively less activity in fractions of both varieties of flax seeds, as evidenced by their lower absorbance at 700 nm. The greatest reducing power was observed in LVF-01 and GVF-03 hull fractions compared to other fractions. Therefore, flaxseed fractions were electron donors especially the hull fractions and can react with free radicals to convert them to more stable products and terminate radical chain reaction.

The damaging action of hydroxyl radicals is the strongest among free radicals. In biochemical systems, superoxide radical is converted by superoxide dismutase to hydrogen peroxide, which can subsequently generate extremely reactive hydroxyl radicals in the presence of certain transition metal ions such as iron and copper (Hu and Kitts 2000). In addition, hydroxyl radicals can attack DNA to cause strand scission and leads to mutations (Park *et al*. 2004). In the present study, we used Fenton's reagent as a source of hydroxyl radical. However, addition of hull fraction LVF-01 and GVF-03 substantially diminished the DNA damage as evidenced by the intensity determination, while flour fractions did not show significant protection. The endosperm fractions of both flaxseed cultivars did not show any protection against hydroxyl radical induced DNA damage. These results indicate that, hull fractions of LVF-01 and GVF-03 protect DNA from hydroxyl radical induced DNA damage and may have a significant role in preventing free radical induced genetic diseases.

In conclusion, SDG was mostly present in hull portion, whereas endosperm and flour fractions contained low SDG content. Hull fractions exhibited highest antioxidant potency than endosperm and flour fractions. The activity in endosperm and flour fraction may be related to low SDG content and other minor lignan contents. From a quantitative aspect, LVF-01 is the type of cultivar, which has a significant level of SDG. However, GVF-03 also has a considerable amount of SDG in its hull portion. The antioxidant activity as assessed by the DPPH radical scavenging, reducing power and DNA protection studies revealed higher DPPH radical scavenging, electron donating and hydroxyl radical scavenging (DNA protective) ability in hull fractions of both varieties. The antioxidant activity was attributed to the SDG content, which was comparable to the increase in activity to the SDG content in all the activities tested and has potential in preventing free radical induced diseases. This work provides evidence that lignans are strongly associated with the antioxidant capacity in various flaxseed fractions and may find use in food and feed industries to produce health beneficial food products with high antioxidant potential. Further, it is interesting and valuable to investigate the potential effectiveness of flaxseeds in different *in vivo* models.

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