Secoisolariciresinol Diglucoside - A Phytoestrogen Nutraceutical of Flaxseed: Synthesis and Evaluation of Antioxidant Potency

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ABSTRACT

This paper evaluates critically the science base that underpins the argument that oxidative damage is a significant causative factor in the development of human diseases and that antioxidants are capable of preventing or ameliorating these disease processes. Lignans, as polyphenol compounds, have been reported to serve as good and healthy nutrients. By reason of possessing many biological activities, it takes a major part in curing degenerative diseases. Secoisolariciresinol diglucoside (SDG), the chief lignan found in flaxseed, is believed to reduce serum cholesterol levels, delay the onset of type II diabetes and decrease formation of breast, prostate and colon cancers. These health benefits of SDG may be partially attributed to its antioxidant properties. Retrospectively of the above information, in the present study, SDG was synthesized for the first time and screened for its antioxidant properties. The synthetic route of SDG used the coupling of two molecules of 3,4-dimethoxy toluene with dibromobutanediol as the key step to yield the SDG skeleton. The synthesized SDG was found to have potential antioxidant activity. It scavenges the DPPH radical at EC_{50} of 76.67μg/ml which is comparable to that of antioxidant references namely Ascorbic acid, Std-SDG and α-tocopherol (60.64, 66.57, 83.24μg/ml) respectively. Further, its dose dependent reducing power was observed. The synthesized SDG showed 1.25 times higher activity compared to α-tocopherol and lesser activity (0.70, 0.75 times) compared to Ascorbic acid and Std-SDG respectively. Additionally, it was found to protect DNA from hydroxyl radicals generated by an oxidant agent as Fenton's reagent at a concentration of 0.5 mg/ml.

INTRODUCTION

The relationship between antioxidant diets and degenerative diseases has attracted the attention of functional food and nutraceutical industries. Improvement in dietary antioxidant intake in human populations is expected to result in lowering of the risk of a number of degenerative diseases. Oxidation and production of free radicals are an integral part of normal cell metabolism. Free radicals such as superoxide anion, hydroxyl radicals, hydrogen peroxide, singlet oxygen, nitric oxide and peroxy nitrite are formed as a part of the normal metabolic processes. An imbalance of these reactive oxygen species and antioxidant defence systems may lead to chemical modifications of biologically relevant macromolecules and cellular components, such as lipids, proteins and DNA. This imbalance provides a logical pathobiochemical mechanism for the initiation and development of several disease states. It is believed that several diseases, for instance cancer, diabetes, ageing, hypertension, obesity and cardiac dysfunction and other degenerative diseases in humans which involve oxidative processes are mediated by free radicals.[1-4] Various phytochemicals including lignans and their related natural antioxidants from tea, spices and herbs, have intensively been studied and reported for their most important part in suppression of reactive oxygen species.[5] Lignans are widely distributed polyphenol compounds isolated from diverse plants, trees or seeds. Being an essential class of natural products and second class of phytoestrogens, they have attracted considerable interest due to their fascinating biological activities. Physiological activities of compounds are

believed to be affected by their molecular structures. Hence, the various types of bonding of the C6-C3 units have promoted lignans to be major objective compounds for organic synthesis and biological research. Moreover, it is believed to be an important component in foods and medicines from plants. Lignans formally arise biosynthetically from two cinnamic acid residues, as well-defined originally by Howarth in 1936 [6] and originated in roots, stems, leaves, fruits and seeds of the plants. Lignans exhibit a wide range of biological activities, including antioxidant, antitumor, anti-inflammatory, immunosuppressive, cardiovascular, neuroprotective, neurotrophic and antiviral actions.[7] SDG, the major lignan in flaxseed has gained significant attention in nutritional and functional foods due to its substantial biological activities. It has structural similarity in its aglycone form, Secoisolariciresinol (SECO) with the known antioxidant nordihydroguaiaretic acid (NDGA). SDG is metabolized by colon bacteria and converted into mammalian lignans enterodiol and enterolactone, which also exert antioxidant and anticarcinogenic effects. [8,9] SDG, via various in vivo and in vitro studies was found to be a potential antioxidant agent.[10-12] It also appears to be effective against breast and colon cancer,[13] hyper cholesterolemic atherosclerosis, diabetes,[14] lupus nephritis, immune and inflammatory reactions.[15,16] In addition, SGD is found to possess a good antibacterial activity against gram positive and negative bacterial species.[17] Despite their attractive and proven biological properties, only few of the studies approached the chemical synthesis of lignans, for instance alpha-hydroxylated lactone lignans,[18] erythro-nordihydroguaiaretic acid [19] and olive type lignans.[20] Nevertheless, none of the studies has approached the synthesis of SDG from commercial compounds. Altogether, the above information, and to better understand, the present work approached the synthesis of SDG and screening of its antioxidant properties by different in vitro studies.

MATERIALS AND METHODS

Chemicals

The chemicals 1,4-butenediol, 3,4-dimethoxy toluene and 2,3,4,6 tetra-o-acetyl α-D glucopyranosyl bromide, 1,1-diphenyl-2-picryl-hydrazyl (DPPH), Ascorbic acid, and α-tocopherol, Folin-Ciocalteu reagent, Calf thymus DNA, Gallic acid and Agarose were purchased from Sigma Aldrich Chemicals Co.(St. Louis, MO, USA). Other reagents and chemicals used were of analytical grade and obtained from Ranbaxy Fine Chemicals Ltd., India. Melting points were determined on a capillary melting point apparatus (Büchi B-545) and are uncorrected. IR spectra were obtained from a Bruker Vector 22 infrared spectrometer using KBr pellets. ^{1}H NMR and ^{13}C NMR spectra were measured on a Varian Unity 400 spectrometer using tetramethylsilane as internal standard and the chemical shifts are reported in δ units relative to TMS. MS were recorded on a Finnigan Mat LCQ spectrometer (APCI/ESI). Purities of the synthesized compounds were checked on pre-coated silica gel G_{254} TLC plates (Merck) and the spots were visualized under UV light and by exposing them to iodine vapors. Column chromatographic purification was generally performed on Merck silica gel (60–200 mesh).

Chemistry

SDG was chemically synthesized from the commercially available compounds 1,4-butenediol and 3,4-dimethoxy toluene via a novel five-step synthesis sequence as follows:

Synthesis of 2,3-dibromo 1,4-butandiol (2)

The initial step of the synthesis is the bromination of alkenes, a reaction that has been used frequently in the past. The one equivalent of 1,4-butenediol (5g) was brominated using (12g) bromine solution to yield (9.71g, 71.18%) compound-2 (Liq.). ^{1}H-NMR (CD$_3$OD) δ 3.66 (2H, d, J=4.1Hz,H1/H4-OH) , 3.87 (2H, d, J=6.7 Hz,H1/H4a), 4.10 (2H, d, J=6.9 Hz,H1/H4b), 3.92 (2H,J=7.4 Hz,H2/H3-Br). ^{13}C-NMR (CD$_3$OD) 65.2 (C1/C4), 49.9 (C2/C3). MS (APCI): m/z 248 (M+1).

Synthesis of 2,3-bis(3,4-dimethoxybenzyl)butane-1, 4-diol (4)

To a stirred solution of two equivalent (10g) of 3,4-dimethoxy toluene (compound-3), and one and a half equivalent (2.2g) of n-butyl lithium in 10 ml of DMF, one equivalent (8.15g) of 2,3-dibromo 1,4-butanediol (compound-2) was added. The mixture was heated to reflux for 19 hrs and it was monitored every three hours by thin layer chromatography. The reaction mixture was cooled to room temperature, transferred to separating funnel and worked up. The solvent was evaporated from the crude product to yield (2.7g, 34.29%) compound-4 (oil). ^{1}H NMR (CD$_3$OD) δ 6.78 (2H,d,J=1.9Hz,H-2,2'), 6.80 (2H,d,J=8.4 Hz,H-5,5'), 6.70 (2H,dd,J=1.8,8.0 Hz,H-6,6'), 2.60 (2H,dd,J=8.2,13.8 Hz,H-7a,7a'), 2.38 (2H,dd,J=6.7,13.6 Hz,H-7b,7b'), 1.94 (2H,m,H 8,8'), 3.62

(2H,dd,J=5.6,9.7 Hz,H-9a,9a'), 3.37 (2H,dd,J=6.6,9.8 Hz,H-9b,9b'), 3.84 (3H,S,H-OCH3), 3.65 (2H,dd,J=7.6,7.8 Hz, H-9,9'-OH). ^{13}C NMR (CD$_3$OD) 131.9 (C1,1'), 113.2 (C2,2'), 149.2 (C3,3'), 147.1 (C4,4'), 112.4 (C5,5'), 122.4 (C6,6'), 35.3 (C7,7'), 42.8 (C8,8'), 65.8 (C9,9'), 56.2 (C-OCH3). MS (MALDI): m/z 390.5 (M+1).

Synthesis of 2,3-bis(3,4-dimethoxybenzyl)butane-1,4-O-tetra acetyl glucose (5)

In 100 ml round bottomed flask, one equivalent (2.7g) of compound-4 was added to two equivalents (5.55g) of 2,3,4,6 tetra-o-acetyl α-D glucopyranosyl bromide and dissolved using 10 ml of ethanol. To this mixture, 5 ml of conc. HCl was added. The mixture was heated to reflux for 14 hrs. The reaction was monitored every three hours by thin layer chromatography. The reaction mixture was cooled to room temperature, transferred to separating funnel and worked up. The solvent was evaporated, and the crude product was recrystallized to yield compound-5. Appearance of some other spots on the TLC plate after recrystallization, indicate existence of some impurities and un-reacted reactants. Hence, the crude product was taken for further purification using silica gel column chromatography (100-120 mesh) and mixture of chloroform and hexane to yield (3.5g, 47.76%) compound-5. ^{1}H NMR (CD$_3$OD) δ 6.77 (2H,dd,J=1.8 Hz,H-2,2'), 6.83 (2H,d,J=8.2 Hz,H-5,5'), 6.72 (2H,dd,J=1.8,8.0 Hz,H-6,6'), 2.61 (2H,dd,J=8.5,13.8 Hz,H-7a,7a'), 2.41 (2H,dd, J=8.4, 13.6Hz,H-7b,7b'), 2.18 (2H,m,H 8,8'), 3.62 (2H,dd,J=5.4,10 Hz,H-9a,9a'), 3.37 (2H,dd,J=6.6,10 Hz,H-9b,9b'), 3.83 (3H,S,H-OCH3), 3.65 (H,d,7.1,H-9,9'-O-Glu anomeric), 2.21-4.51 (H-9,9'-O-Glu). ^{13}C NMR (CD$_3$OD) 131.9 (C1,1'), 113.2 (C2,2'), 149.2 (C3,3'), 147 (C4,4'), 112.4 (C5,5'), 122.7 (C6,6'), 39.7 (C7,7'), 37.8 (C8,8'), 72.7 (C9,9'), 56.1 (C-OCH3), 59.7 (C9,9'-O-Glu anomeric), 20.7-170.2 (C9,9'-O-Glu). MS(APCI): m/z 1050 (M+1).

Synthesis of 2,3-bis(3,4-dimethoxybenzyl)butane-1,4-O-glucose (6)

3.0g of compound-5 was placed in a 25 ml Erlenmeyer flask and a solution of 0.75g NaOH in water and ethanol was added, and then the mixture was stirred and heated in water bath. After heating, the reaction mixture was cooled to room temperature and the compound-6 was extracted from the mixture by diethyl ether, the solvent was evaporated to yield (0.8g, 44.95%) compound- 6 (solid). ^{1}H NMR (CD$_3$OD) δ 6.77 (2H,dd,J=1.8 Hz,H-2,2'), 6.80 (2H,d,J=8.2 Hz,H-5,5'), 6.74 (2H,dd,J=1.8,8.0

Hz,H-6,6'), 2.63 (2H,dd,J=8.3,13.8 Hz,H-7a,7a'), 2.38 (2H,dd, J=8.4,13.6Hz,H-7b,7b'), 2.18 (2H,m,H8,8'), 3.44 (2H,dd,J=5.6,10 Hz,H-9a,9a'), 3.19 (2H,dd, J=6.4,9.8 Hz,H-9b,9b'), 3.83 (3H,S,H-OCH3), 4.1 (H,d,7.1,H-9,9'-O Glu anomeric), 3.2-3.90 (H-9,9'-O-Glu).^{13}C NMR (CD$_3$OD) 132.6 (C1,1'), 113.3 (C2,2'), 149 (C3,3'), 146.9 (C4,4'), 112.2 (C5,5'), 122.5 (C6,6'), 39.6 (C7,7'), 40.6 (C8,8'), 71.7 (C9,9'), 56.1 (C-OCH3), 104 (C9,9'-O-Glu anomeric), 63.8-79.3 (C9,9'-O-Glu). MS (ESI): m/z 714 (M+1).

Synthesis of secoisolariciresinol diglucoside (7)

Partial demethylation of compound-6 was carried out according to the regioselective method of Arvind et.al 2006 [21] with minor modification in the reagent quantities. 0.75g of compound-6 and 1g of Lewis acid (stannous chloride) were added to 20 ml of dichloromethane. The reaction mixture was stirred approximately for 3 hrs at 40^{0}C. 10 ml of dilute HCl was added into the reaction mixture, further stirring the mixture for 10 min to obtain the demethylated compound-7. The reaction mixture was washed with water, and the organic layer was dried over anhydrous sodium sulphate, and chromatographed through silica gel column to obtain (0.41g, 41.1%) of the demethylated compound-7, literature m.p 118-120 ^{0}C and practical m.p 117-119 ^{0}C.^{1}H-NMR (CD$_3$OD) δ 6.59 (2H, d, J=1.9Hz) , 6.64 (2H, d, J=8.0 Hz), 6.56 (2H, dd, J=1.8, 8.0 Hz), 2.61 (2H, dd, J=8.2, 13.8 Hz), 2.68 (2H, dd, J=6.9, 13.8 Hz), 2.12 (2H, m), 4.06 (2H, dd, J=5.6, 9.9 Hz), 3.47 (2H, dd, J=6.5, 9.9 Hz), 3.73 (2H, s), 4.23 (2H, d, J=7.8 Hz), 3.10-3.90 (2H, m).^{13}C NMR δ (CD$_3$OD) 133.9 (C1/1'), 113.59 (C2/2'), 148.7 (C3/3'), 145.4 (C4/4'), 115.7 (C5/5'), 122.9 (C6/6'), 35.6 (C7/7'), 41.2 (C8/8'), 71.2 (C9/9'), 56.3 (C-OCH3), 104.8 (C-O Glu'.), 62.8, 78.2 (C-O Glu.). MS (APCI): m/z 687.6 (M+1). As represented in figures (1, 2).
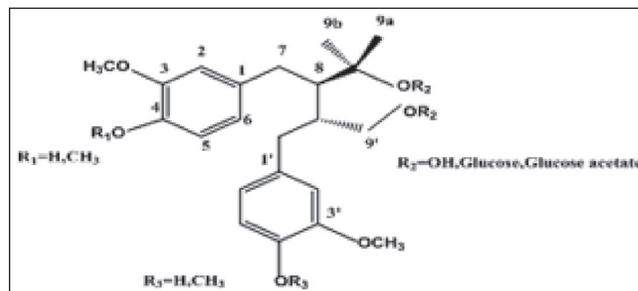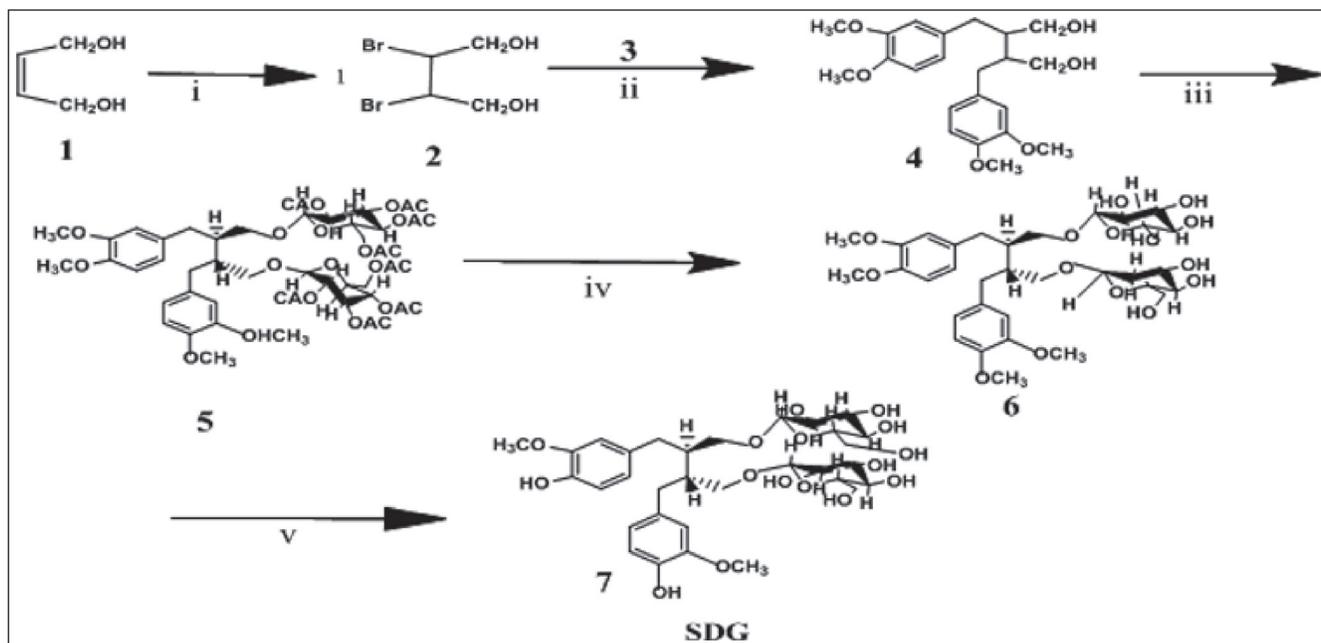

Figure 1. Chemical structure of SDG skeleton representing the position of the carbon and hydrogen atoms.

Figure 2. Scheme. Reagents and conditions: (**i**) Br₂, ACOH, stirring; (**ii**)2(3,4-dimethoxy toluene), n-But Li, DMF, reflux, 19h; (**iii**) 2(2,3,4,6 tetra-o-acetyl α-D glucopyranosyl bromide),HCl, ethanol, reflux, 14h; (**iv**) NaOH, ethanol, diethylether; (**v**) dichloromethane, stannous chloride, stirred,3h,40ºC, dil-HCl.

Determination of Total Antioxidant Activity

DPPH radical scavenging assay

The effect of synthesized SDG on DPPH radical was estimated according to the method of Lai et.al 2001.[22] An aliquot of the SDG 200 µL of various concentrations (20-100 µg/ml) were 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. Ascorbic acid, Std-SDG and α-tocopherol of various concentrations (20-100µg/ml) were used as standards. The absorbance of the resulting solution was measured in a UV-Visible spectrophotometer at 517 nm. The percent DPPH radical scavenging ability was calculated according to the following equation.

$$\% \text{ Inhibition} = \frac{\text{Abs}_{control} - \text{Abs}_{sample}}{\text{Abs}_{control}} \times 100.$$

Further, a dose response curve was plotted to determine the EC₅₀ values. EC₅₀ is defined as the concentration sufficient to obtain 50% of a maximum scavenging capacity. All tests and analyses were run in triplicate and averaged.

Measurement of reducing power

The reducing power of synthesized SDG was determined according to the method of Yen and Chen 1995.[23] The SDG fractions (20-100 µg/ml) were 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 X g for 10 min. The upper layer of solution was mixed with distilled water and 0.1% FeCl₃ at a ratio of 1 : 1 : 2 and the absorbance was measured at 700 nm. Ascorbic acid, Std-SDG and α- tocopherol of various concentrations (20-100 µg/ml) were used as standard and compared with synthesized SDG. Increased absorbance of the reaction mixture indicates the increased reducing power.

DNA protection assay

DNA protection activity was performed using calf thymus DNA according to the method of Henry & Akman 1998 [24] with minor modification. Briefly, calf thymus DNA (1 µg) was treated with Fenton's reagent (0.3 mM H₂O₂, 0.5 µM ascorbic acid and 0.8 µM FeCl₃) in presence and absence of synthesized SDG (0.5mg) in addition to standard DNA and standard antioxidant compound gallic acid (50µg/ml). 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 subsequently ethidium bromide (0.5µg/ml) staining. The intensity of the bands was determined using easy-win software.

Statistical Analysis

All the experimental results of spectrophotometric analyses were expressed as mean ± standard deviation (SD). Data were analysed and EC_{50} of all the samples were calculated using dose response curves, nonlinear regression equation using MasterPlex ReaderFit (Hitachi Solutions America, Ltd., USA) and SPSS version 16.0 (SPSS, Chicago, USA).

RESULTS AND DISCUSSION

Chemistry

Our earlier studies have proved various biological activities such as antioxidant, anti-cancer, anti-diabetic, anti-inflammatory and antibacterial properties of SDG. Thus, these studies prompted us to take up synthesis and evaluation of biological potency of SDG as challenge. Synthesis of this promising compound was carried out for the first time by a novel five-step synthesis sequence using commercially available materials obtained from Sigma Aldrich Chemicals Co. (St. Louis, MO, USA). Firstly, halogenation of the alkene (1,4-butendiol) is an important reaction in the chemical industry through electrophilic addition to yield compound-2. Oxidative coupling of compound-2 with two molecules 3,4-dimethoxy toluene is the second step to yield the skeleton of SDG compound-4. Thirdly, the two hydroxyl group of compound-4 were glycolated using two molecules of 2,3,4,6 tetra-o-acetyl α-D glucopyranosyl bromide to yield the compound-5 as viscous oil. Decactelation of the acetyl groups of the glycoted skeleton was the fourth step using sodium hydroxide to yield the compound-6. Further, the Para methoxy groups of this compound (6) were demethylated following the regioselective method of Arvind et.al 2006 to yield the compound of our interest SDG and this was the fifth and final step in the scheme. All the synthesized compounds were checked for purity and their structural analyses were confirmed using different techniques include IR, MS and [1]H&^{13}C NMR.

Determination of Total Antioxidant Activity

DPPH radical scavenging assay

The DPPH radical is widely used to investigate the radical scavenging activities of several natural as well as synthetic phenolic compounds and also crude extract of plants. [25] The scavenging activity has been studied in the process of hydrogen atom transfer to the stable free radical DPPH to compare the activity of compounds under investigation with that of widely known antioxidant parameter. Free radical scavenging activity of DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become stable diamagnetic molecule. The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm, which is induced by antioxidants.

The present study was carried out to determine the hydrogen donating capabilities of the synthesized SDG. Free radical scavenging activity of synthesized SDG, Ascorbic acid, α-tocopherol and Std-SDG of various concentrations (20-100 µg/ml) was tested by DPPH radical method, the result is summarized in **Fig.3**. It indicates the comparative EC_{50} values for DPPH radical scavenging activity of synthesized SDG with that of different standards. An EC_{50} of 76.67 µg/ml was observed for synthesized SDG which showed higher activity than α-tocopherol (EC_{50}=83.24 µg/ml) and lesser activity than Ascorbic acid (EC_{50}=60.64 µg/ml) and Std-SDG (EC_{50}=66.57 µg/ml). Moreover the results of DPPH test at 20^0C showed that the activity depends strongly upon the presence of phenolic group in the compound. In other words, the compound showed dose dependent increase in activity (**Fig.3**). With respect to previous studies the crude extract SDG showed an EC_{50} =12.6 mg/ml at concentration of 20 mg/ml,[11] which is lesser than that of synthesized SDG in our current study. These findings supported our synthesized compound with the fact that synthesized compounds can be more effective and active with fewer amounts than the extracted one.
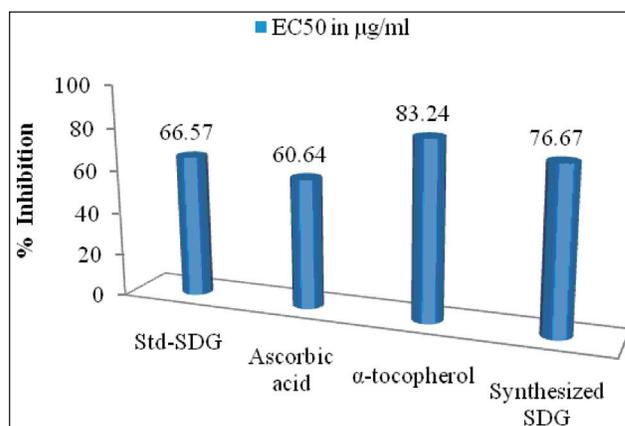
Figure 3. Scavenging activities of synthesized SDG, Ascorbic acid, α-tocopherol and Std-SDG against the 1,1-diphenyl-2-picryl-hydrazil (DPPH·) radical.

Reducing power assay

To substantiate the results of DPPH radical scavenging activity the reducing power of the same compounds was also evaluated. Reducing power assay was determined based on the principle that Substances, which have reduction potential react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form ferric-ferrous complex that has maximum absorption at 700nm. The reductive capabilities of the synthesized SDG were compared with standards for the reduction of the Fe^{3+}, Fe^{2+} transformation in the presence of the synthesized SDG. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity.

The electron donating capability of the tested compounds as evaluated by the reduction of ferric chloride and potassium ferricyanide complex revealed high activity in Ascorbic acid and std-SDG, comparatively less activity in both of synthesized SDG and α-tocopherol, as evidenced by their lower absorbance at 700 nm.

The reducing power (indicated by increased absorbance at 700 nm) of the synthesized SDG compared with that of Ascorbic acid, Std SDG and α-tocopherol of equal concentrations (20-100 μg/ml) was investigated and it showed intensity dose-dependent property of Perl's Prussian blue at 700 nm. At the concentration of 100 μg/ml the synthesized SDG showed 0.75 and 0.70 fold lesser reducing power ability than Ascorbic acid and Std-SDG respectively, while 1.25 fold higher activity was observed over α-tocopherol (**Fig.4**). While in earlier investigation the crude extracted SDG (10 mg/ml) was found to exert 0.60 fold reducing power activity compared with std-SDG (0.02mg/ml).[11] In agreemen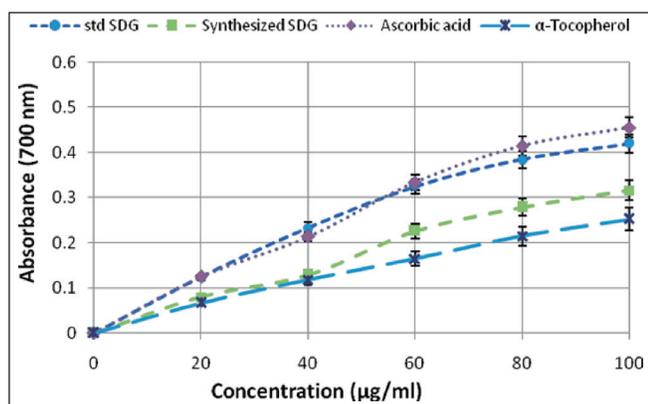t with the DPPH results the synthesized SDG exhibited higher reducing power activity with minimum concentration compared to the extracted one from flaxseed. All the examined compounds showed an increased activity with increase in their concentration, in other words, showed concentration dependent activity, therefore, they were electron donors, especially synthesized SDG, and it can react with free radicals to convert them to more stable products and terminate radical chain reaction.

DNA protection assay

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.[26] In addition, hydroxyl radicals can attack DNA to cause strand scission and leads to mutations.[27] This assay was accomplished in order to estimate the protective effect of synthesized SDG and compared it with authentic antioxidant compounds as well as Std-SDG. The calf thymus DNA was subjected to oxidation in the presence and absence of synthesized SDG using Fenton's reagent, the extent of damage and protection offered by the synthesized SDG was assessed by the relative electrophoretic mobility of the oxidised and SDG treated DNA on 1% agarose gel compared to that of native DNA. **Figure 5** displays
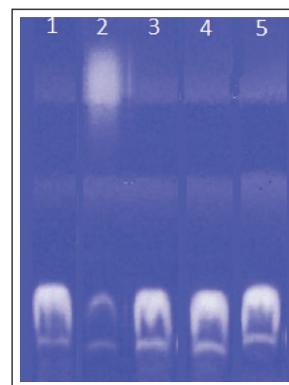

Figure 5. Effect of synthesized SDG on the protection of DNA against hydroxyl radical generated by the H_2O_2 Lane 1- native DNA; Lane 2-native DNA + Fenton's reagent (DNA damage control); Lane 3- DNA + Fenton's reagent + Gallic acid (Standard) (50μg/ml); Lane 4- DNA + Fenton's reagent + SDG (Standard) (50 μg/ml); Lane 5- DNA + Fenton's reagent + synthesized SDG (0.5mg/ml).

Figure 4. Reducing power of synthesized SDG, Ascorbic acid, α-tocopherol and Std-SDG.

that the addition of Fenton's reagent caused fragmentation of DNA and increased mobility of DNA. Lane-2 which is an oxidant treated sample shows a maximum fragmentation of DNA compared to other lanes. The DNA bands in all the lanes corresponding to the band of native DNA with maximum intensity were considered in determining the protection offered by these compounds. At 0.5 mg dosage the synthesized SDG substantially diminished the DNA damage as evidenced by the intensity determination, and displays almost an equal pattern of DNA protection with higher DNA protecting ability as DNA protection influence carried out by Gallic acid and Std-SDG. Compatible with former findings crude extracted SDG at 3.0 mg dosage showed an increase in DNA protection.[11] However, this activity is lesser than that of synthesized SDG. These results indicate that, synthesized SDG protect DNA from hydroxyl radical induced DNA damage and may have a significant role in preventing free radical induced genetic diseases.

CONCLUSION

This work provides evidence that lignans are strongly associated with the antioxidant capacity in various studies due to the resemblance of their structure to that of our body estrogen. Lignan phytoestrogens bind to the estrogen receptor. As a result, they are believed to act both as agonists and antagonists for estrogens. Lignan, especially SDG is also found to possess antioxidant, anti-diabetic, anticancer properties and some other biological activities. Isolation of this lignan is very laborious, sensitive and time consuming process. This could be the reason for the fact that crude extracted SDG exhibited lesser activity than the synthesized one. Apart from this, the bioavailability of isolated SDG is very low. In view of all these, chemical synthesis of SDG using commercially available compounds is taken up as a challenged approach. This approach was found to be an efficient, easy and high yielding method. The synthesized SDG is believed to be a good source of antioxidant properties. It exhibited significant antioxidant potency challengeable to the known antioxidant agents. The *in vitro* antioxidant studies revealed that synthesized SDG possesses higher DPPH radical scavenging activity, reducing power, hydroxyl radical scavenging ability and potential DNA protecting property as well. These conclusions established a potential for SDG to impact on antioxidant status and to possibly provide further mechanistic evidences to serve as a good source of anticancer and other degenerative diseases, and further investigations are needed in this direction.

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