SYNERGISTIC ANTI-INFLAMMATORY AND ANTIOXIDANT ACTIVITIES OF INDIAN FLAX AND SESAME SEED LIGNANS

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Abstract

Linum usitatissimum (L.), the flaxseed, and Sesamum indicum (L.), the sesame seeds are rich sources of thelignans viz Secoisolariciresinol diglucoside(SDG) and sesamin(Sm), respectively. In the present study, SDG+Sm from Indian flaxseeds(FS) and sesame seeds(SS), respectively, were purified and their synergistic effects anti-oxidant, and anti-inflammatory properties were investigated.The HPLC chromatogramconfirmed the presence of SDG and Sm in seeds of FS and SS, respectively. SDG and Sm were estimated to be 23.9 and 4.7 g.kg-1 from FS and SS, respectively. SDG+Sm quenched free radicals studied by DPPH and reducing power assay indicating its strong antioxidant property. The free radical scavenging effects of SDG+Sm was24.04 while they were 42.56 and 63.14 for SDG and Sm alone, respectively. During15-LOX inhibition, they exhibited lower IC_{50} values (60%) of 35 ± 0.53 when compared to IC_{50} values of SDG (56 ± 0.02) and Sm (58 ± 0.23). Similarly, SDG+Sm showed that they could inhibit COX-2 enzyme at relatively lower concentrations.Taken together,the results encourage the combineduse of a combination of SDG+Sm from FS and SS, respectively, in a wide range of applications as nutraceuticals, functional foods,therapeutic agents with pharmacological effects such as anti-oxidant and anti-inflammatory agents to prevent diseases and promote health.

Keywords:

Polyphenols, Flax seeds, Sesame seeds, Lignans, Synergism

1. Introduction

Plants based nutrients present impressive medical advantages, incompletely owing to their plentiful micronutrients (E.g., polyphenols, including lignans) contents. Currently, there is a developing interest within the sight of lignans in groceries, given the possibly advantageous bioactive properties of the previous (against estrogenic, cancer prevention agent and hostile to cancer-causing exercises) [1]. The central wellsprings of dietary lignans are different vegetables and organic products, vegetables, entire grain oats, and oilseeds [1,2]. Among consumable plant parts, the most thought lignan sources are sesame and flax seeds. In particular, flax seeds contain roughly 294.21 mg/100 g lignan, at present the maximal known substance of any staple. Sesame seeds display the second-most elevated lignan fixation, with sesaminol as the significant constituent, at 538.08 mg/100 g. Flaxseed and cashew nuts are additionally moderately rich in lignans (containing 257.6 and 56.33 mg/100 g, individually) [3].

Flaxseed (*Linum usitatissimum* L.), also known as linseed, is a highly prized seed for its impressive health benefits and disease preventive nutrients. It is composed of secoisolariciresinol diglucoside (SDG), a major lignan in addition to other nutritionally important and health beneficial components like dietary fiber, proteins, minerals, etc. [3]. It also contains 36-40% of oil with a high

content of polyunsaturated fatty acids (PUFAs, 73% of total FA), a moderate amount of monounsaturated fatty acids (MUFAs; 18%); 12-16% linoleic acid (LA, ω-6 FA) and 52-57% α-linolenic acid (ALA, ω-3 FA) and 7.97-12.30% saturated fatty acids (SFAs) of total FAs. In the olden days, flaxseed was mainly used to manufacture linen fabric, underclothes, table linen, and also for weaving bedsheets in Western countries. In India, it is cultivated in Madhya Pradesh, Uttar Pradesh, Bihar, Himachal Pradesh, Andhra Pradesh, and Karnataka. It is mainly cultivated for its seeds, which are ground into a meal oil or pressed into flaxseed oil to make it a nutritional supplement or as timber in wood products. Added to these, more importantly, it has been an integral part of Ayurvedic medicine for many years, and nowadays, it is being commercially available in the form of powder, tablets, capsules and also consumed as dietary supplements[4].

Sesame (*Sesamum indicum* L.) is accepted to be one of the most antiquated crops cultivated by people of Asia and Africa. It contains appreciable amounts of lignans viz sesamin, sesamolin, and sesaminol, and 18-25% protein and carbohydrates, 50-60% oil, which is composed of 36.4-42.1% of LA, an ω-6 FA, 16.3% of SFAs and [6]. About 50% of the sesame seed contains oil than the significant oilseeds, for example, soybean or rapeseed. Sesame oil (SO) is by and large viewed as extravagant and great oil. The sesame seeds (SS) are utilized both as the bakery, confectionery products, sauce, and oil sources. Indians utilized sesame oil for cooking, clinical, massaging, and medicinal purposes from antiquity. The lignans present in the sesame seeds act as natural antioxidants accounting for the stability of the oil [7]. The major active lignan Sesamin present in SS is responsible for many medicinal aspects like antioxidant; anti-inflammatory; lipid-lowering; anti-carcinogenic activities [8].

Secoisolariciresinol diglucoside (SDG) is the principal lignan of flaxseed, and it has been intensively evaluated for its various potential chemopreventive [9, 10] and biological properties [11] such as an antioxidant [12], anti-inflammatory [13], anti-diabetic [14] and nephroprotective roles[15]. Similarly, sesamin(Sm) is a fat-soluble lignan of a sesame seed. Several studies have reported that Sm exerts many health beneficial effects like anti-cancerous, anti-hypertensive, anti-inflammatory, free radical scavenging, and anti-diabetic and anti-lipidemic [16-19].Furthermore, SDG and Sm have exhibited antiviral and antibacterial activity, e.g., in opposition to Gram-positive microorganisms via alteration of biofilm formation, microorganism metabolites, membrane receptors, and ion channels. Concerning anti-inflammatory activity, both the lignans have the potential to inhibit NF-kB hobby (transcription component includes at the expression of inflammatory cytokines) on human mast cells (HMC-1). Thus, decreased pro-inflammatory cytokines production. Furthermore, lignans are capable of suppressing nitric oxide (NO) generation and reduce inflammatory cellular infiltration. Neolignans and flax lignans are reportedly relevant in diabetes, hypercholesterolemia, and cardiovascular disorders. In addition to these, the anti-aging role of lignans has recently been described [6].

In recent years, as synthetic drugs have multiple side effects, there is an upsurge in the consumers' interest towards a novel bio-inspired strategy of cocktailing two or more bioactives at a lower concentration to reduce both side and cost effects, apart from enhancing the positive effects for the development of novel compounds by the food, pharmaceutical, and chemical industries. *Schisandra Chinensis and Ribes fasciculatum* have been demonstrated to offer potential synergistic neuroprotective effects on neuronal cell death and scopolamine-induced cognitive impairment [20]. Quercetin and catechin were combinatorially used as potential anti-inflammatory agents, wherein, they synergistically acted together to drastically reduce some pro-inflammatory molecules like COX-2, TNF-α, IL-1β, NO synthase, and inhibited the activation of TLR4–MyD88-mediated NF-κB and MAPK signaling pathways[21]. A combination of flax oil and astaxanthin reduced the risk of oxidative stress, lipid abnormalities, cholesterol, triglycerol, hepatic steatosis, and inflammation in cardiovascular complicated cases[22].Therefore, in the current investigation, the lignans SDG and Sm from Indian flaxseed and sesame seed respectively were purified, and HPLC and TLC analyses were carried out to check their purity. Additionally, total phenolic contents of SDG and Sm; and their synergistic in-vitro antioxidant and anti-inflammatory properties were evaluated.

2. Methodology

2.1. Collection of Flax and sesame seeds

Five different varieties of each flaxseed (FS) and sesame seeds (SS) were procured from Haveri and Gulbarga of North Karnataka, India, and identified. Out of five identified LCK-5021, N.N, RLC-81, S-36, and T-397 flaxseed, and DS-1(C), DS-5, DS-21, DSS-1, and DSS-9 sesame seed varieties by the Oil Seed Department, University of Agricultural Sciences, Dharwad, Karnataka, India, the varieties N, N and DS-5 of FS and SS, respectively were used in the present study.

2.2. Proximate analysis, and determination of Total Phenolic Content

Proximate analyses of the seed varieties were carried out according to the AOAC method (1995)[23] in triplicates. Flax and sesame seeds were ground and analyzed for the contents of moisture, crude fiber, total protein, crude fat, carbohydrates, and ash.

The total phenol substance of the seed extricates was estimated by the Folin-ciocalteau's (FC) technique [24]. Briefly, 2% Na_2CO_3 was mixed with 100 µl of the sample and it was incubated at room temperature for 2 minutes. 100 µl of 50% FC reagent was added, vortexed thoroughly, and again incubated in the dark at room temperature for 30 minutes. By using a spectrophotometer, the absorbance was measured at 765 nm. The unit of phenol contents was expressed as caffeic acid equivalent per gram (CE/g).

2.3 Isolation of the lignans-SDG andSm

Flax and sesame seeds were pre-cleaned and conditioned. Sesame seeds were washed and desiccated at 70^0C. The flaxseeds were ground into powder using a coffee blender, and further defatted using ether. A 50g of the processed and defatted flaxseed powder was precisely gauged and extricated with 300 ml of 70% aqueous methanol with ceaseless blending for 4 h followed by sonication for 10 min and centrifugation at 10,000×g for 5 min. The extraction procedure was repeated and the total concentrate was subjected to HPLC analysis to identify and distinguish SDG in the flaxseed extract.

2.4. Purification of SDG

The SDG was purified from flaxseed extract as we reported elsewhere [25]. In brief, 10 g of the flaxseed extract (40%) extract was disintegrated in 100 ml of ethanol at room temperature. The extract was filtered and concentrated using a rotary evaporator (Laborota 4001-efficient, Heidolph Instruments, Schwabach, Germany). A 5.6 g of obtained dried concentrate was disintegrated in 80 ml of ethanol and blended with 21.0 g of silica gel (GF 254). After drying, the concentrate (~4g each) was exposed to a flash column chromatography (a glass tube, 20 mm× 300 mm) and washed with 100 ml of ethyl acetic acid derivation/ethanol (8.5:1.5, v/v). Further, the SDG adsorbed by silica gel was eluted with 180 ml of ethyl acetic acid derivation/ethanol (8:2, v/v). The Six parts (30 ml each) obtained were pooled and identified by HPLC. SDG-containing portions were collected and concentrated. SDG was further purified by the preparative TLC, which was prewashed by ethyl acetic acid derivation, with ethyl acetic acid derivation/ethanol (8:2, v/v) as the developing solvent followed by HPLC investigations to confirm its immaculateness. The part with the Rf value of 0.28, comparing SDG was cut and removed by using methanol. After filteration and drying, 1.0 g of SDG was obtained.

2.5. Isolation of Sesamin

As we reported elsewhere[25], the sesamin was isolated from sesame seeds. Briefly, the sesame seeds were flake crushed using a coffee blender and oil was extracted using food grade hexane in a Soxhlet apparatus for 8 h. The dried meal was desolventized under a vacuum in a water bath at 35^0C before being used for the experiments. The oil recovered was mixed with acetone in a ratio of

1:10, allowed for lipid precipitation for 8-12 h at -40^{0}C. The yellow oil obtained with the supernatant was saponified by using 5% alcoholic KOH. Ether extracted unsaponifiable matter was dissolved in methanol, which contained Sm. Furthermore, the methanol fraction was subjected to preparative HPLC analysis and purified sesamin was obtained.

2.6. Thin layer chromatography (TLC)

TLC analysis was additionally performed to assess the purity of the obtained Sm. The lignan was dissolved in chloroform at 1.0mg/ml and spotted on TLC plates alongsideSm standards. To obtain a superior yield of Sm, three to four TLC's were envisioned by stacking 1.0 ml of 10 mg/ml stock as a transgression gel straight streak/band on a 20 cm × 20 cm plate, and TLC was run along with commercially obtained reference Sm. After advancement, the TLC was imaged under UV and the fractions of Sm were collected into vials by scraping. The lignan was then peeled off the silica by dissolving in 20 ml chloroform as a fraction. The lignan Sm from this fraction was re-solidified by the dissipation of the dissolvable at room temperature and labeled as purified Sm.

2.7. Determination of in vitro antioxidant activity of SDG and Sm

2.7.1. DPPH free radical scavenging activity

The free radical scavenging capacity of the SDG and Sm were determined by the DPPH method with minor modifications [26]. The solution of DPPH in methanol (500 µM) was prepared and 1.0 ml of this solution was added to 200 µl of SDG+Sm solution in methanol and water in the ratio of 1:1 at different concentrations (20 to100 µg/ml), and 100 mM Tris-HCl buffer (800 µl, pH 7.4). The mixture was vigorously shaken and allowed to stand at room temperature. After twenty minutes, absorbance was measured at 517 nm. A blank was prepared without adding SDG + Sm. Ascorbic acid and Quercetin were used as standards at various concentrations (20 to100 µg/ml). The lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation.

DPPH Scavenged (%) = ((A control – A test) / A control) × 100

Where "A" control is the absorbance of the control reaction and A test is the absorbance in the presence of the sample of the SDG+Sm or standards. The antioxidant activity of the SDG+Sm is expressed as IC_{50} and compared with the standard. The IC_{50} value is defined as the concentration (in µg/ml) of SDG+Sm that scavenges the DPPH radicals by 50%.

2.7.2. Reducing power assay

The method described by Yen and Hui[27]was used to evaluate the reducing power of SDG and Sm with minor modifications. According to this method, various concentrations of the SDG+Sm (20 to100 µg/ml) in 1.0 ml of deionized water were mixed with phosphate buffer (2.5 ml) and potassium ferricyanide (2.5 ml). The mixture was incubated at 50^{0}C for 20 min. Aliquots of trichloroacetic acid (2.5 ml) were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (0.5 ml). The absorbance was measured at 700 nm. A blank was prepared without adding SDG+Sm. Ascorbic acid and Quercetin at various concentrations (20 to100 µg/ml) were used as standards and compared with SDG+Sm. The higher the absorbance of the reaction mixture the greater is the reducing power.

2.8. Determination of in vitro Anti-inflammatory Activity

2.8.1 Lipoxygenase (15-LOX) inhibition

A spectrophotometric assay for determination of 15-LOX inhibition (5 µg; Sigma- Aldrich, St. Louis, MO, USA) activity with 0.2 µM linoleic acid (substrate; Sigma-Aldrich, St. Louis, MO, USA) in buffer [(0.2 M borate buffer (pH 9.0)] was carried out. The purified SDG, Sm, and SDG+Sm

at 10, 25, 50, 75, and 100 µg.mL−1were used for inhibition studies. The values of hydroperoxide content were estimated. The LOX activity was calculated as computed earlier [26].

2.8.2. Human Cyclooxygenase (COX)-2 inhibition

Human cyclooxygenase (COX)-2 inhibition kit was obtained from Cayman Chemicals, Ann Arbor, MI, USA. COX-2 inhibition by SDG, Sm, and SDG+Sm were measured using a COX-2 inhibitor (human) screening kit (Cayman, Ann Arbor, MI, USA) by colorimetric estimation. The samples (100 µg) of SDG, Sm, and SDG+Sm were used for inhibition studies as per the manufacturer's protocol. The absorbance was read at 415 nm in a microtitre plate reader (Varioskan Flash with SkanIt) with Software RE 2.4.3.

3. Statistical analysis

All experiments and measurements were carried out in triplicate. The values are expressed as the mean ± S.E.M. The results were subjected to variance analysis followed by Tukey's test to analyze differences between SDG+Sm and control conditions. Statistically significant differences (P-value < 0.05 / 0.01) were shown.

4. RESULTS

4.1. Proximate analysis and Total Phenolic Content

Table 1 shows the proximate composition of FS and SS. Phytochemical investigation of the selected FS (N, N) and SS (DS-5) cultivars uncovered the nearness of intriguing mixes. The significant low moisture esteems would permit putting away seeds for quite a while since higher dampness substance could cause deterioration of unsaturated fats by microbial activity.
The total phenolics content in the extracts of FS (N, N) and SS (DS- 5) was 1.84 ± 0.02 mg and 0.86 ± 0.03 mg (Gallic acid equivalent per gram dry weight), respectively.

Table 1. Chemical composition of the flax and sesame seeds

Sl. No.	Parameters	Percent composition	
		Flax seed (N,N)	Sesame seed (DS-5)
1	Moisture	4.8	4.4
2	Total ash	4.0	4.0
3	Total fiber	22.0	6.0
4	Oil	40.4	59.3
5	Protein	21.3	19.0
6	Carbohydrate	7.5	7.3

[a]All the values were means of three mean ± *SD* (*n* = 3) measurements.

4.2. HPLC analysis of SDG and Sm

The contents of the lignans SDG and Sm in their respective seed extracts were identified and evaluated by HPLC analysis (Figure 1). The peak of the standard SDG was eluted at 34.83 min (Figure 1a), while, the peak of sesamin was at 7.77 min (Figure 1c). The presence of these two lignans in the extracts of flax (SDG; Figure 1b) and sesame seed, respectively, (Sm; Figure 1d) were confirmed by comparing with their respective reference standards.

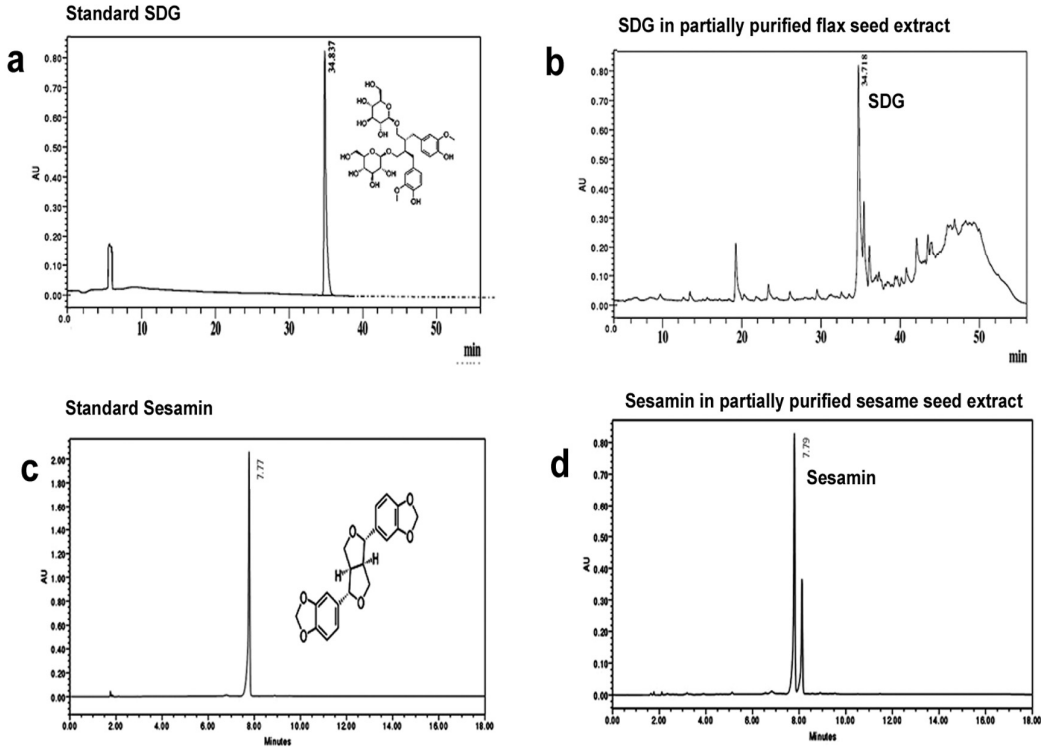

Figure 1. HPLC chromatogram of a. Standard SDG (t_R - 34.83 min), b. SDG in flaxseed extract(t_R-34.83 min), c. Standard sesamin (t_R - 7.77 min) and d. Sm in the Sesame seed extract (t_R –7.79 min). *t_R – Retention time

Semi preparative HPLC performed for the purified SDG and Sm showed their low yield. To get their higher yield, an endeavor was made to run an enormous scope TLC on 20 cm × 20 cm TLC plates on a preparative scale. Three such runs, on recuperation, yielded a higher amount of purified SDG and Sm. The lignan portions confined in such a way additionally demonstrated single peaks in HPLC confirmed their 99% purity (Figure 2 and 3).

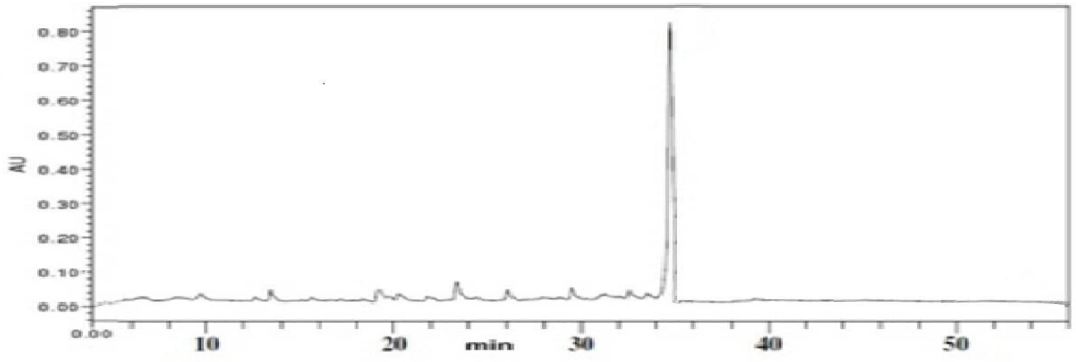

Figure 2. HPLC chromatogram of purified SDG

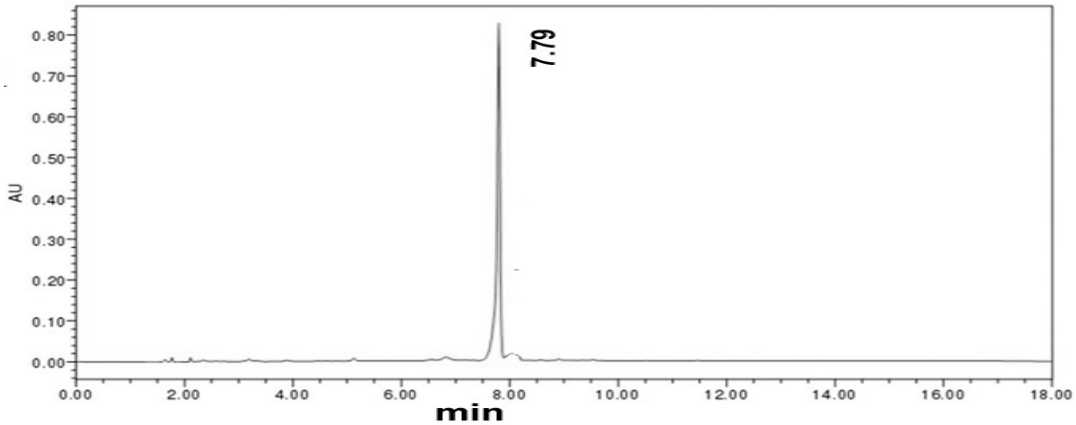

Figure 3. HPLC chromatogram of purified sesamin

4.3. Determination of *in vitro* antioxidant activity of SDG and Sm

4.3.1. DPPH free radical scavenging activity and reducing power assay

Free radical scavenging activity of SDG, Sm, and SDG+Sm, ascorbic acid, and quercetin at varying concentrations (25 to 100 µg) were tested by DPPH radical scavenging assay. The results are shown in figure 4. The IC_{50} (µg.ml-1) values for SDG and Sm alone were 42.56 and 63.14, respectively. For the collegial SDG+Sm, it was 24.04 µg.ml-1 and for the standards, quercetin and ascorbic acid, they were 27.86 and 21.34 µg.ml-1, respectively.

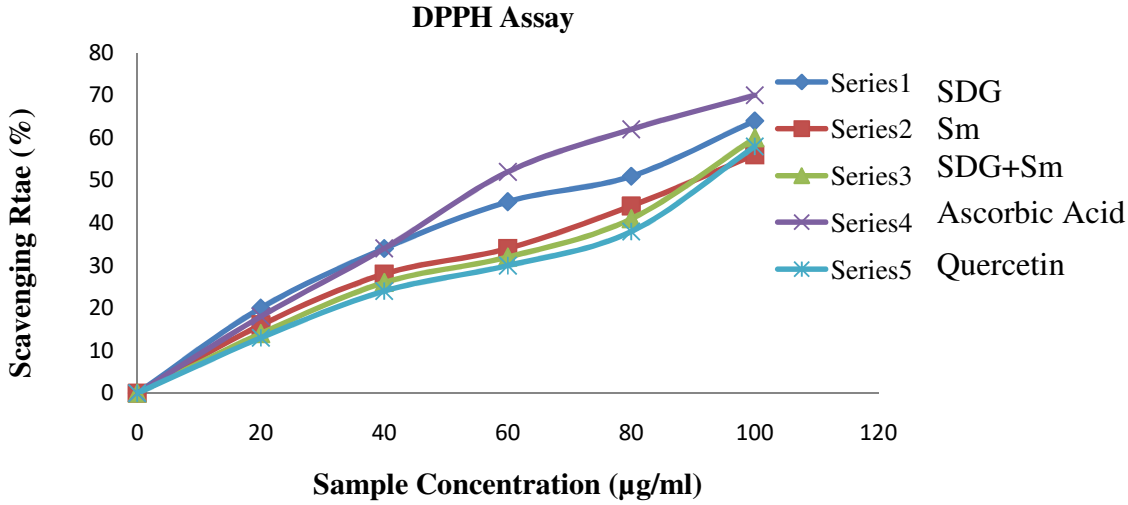

Figure 4. The DPPH radical scavenging activity of SDG, Sm, SDG+Sm, Ascorbic acid, and Quercetin at a concentration of 100 µg.ml-1.
Values are expressed as mean ± SD (N=3)

The reducing power of SDG+Sm was compared with that of ascorbic acid, quercetin, SDG, and Sm at 100 µg concentration. It was observed that the synergism of SDG+Sm showed lesser reducing capacity than ascorbic acid but exhibited a higher reducing capacity than that of quercetin, SDG, and Sm individually (Figure 4).

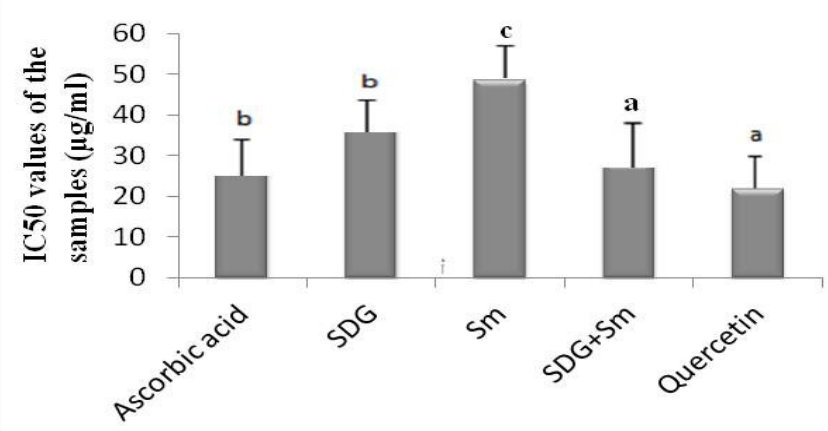

Figure 4. The Reducing ability of standard ascorbic acid, quercetin, SDG, Sm, and SDG+Sm lignans at a concentration of 100 µg.ml-1. Values are expressed as mean ± SD (N=3).

4.4. Anti-inflammatory Activity

4.4.1. Lipoxygenase (15-LOX) Inhibition

The purified SDG, Sm, and their combination-SDG+Sm could inhibit 15-LOX to various capacities (Table 2). The combined formexhibited potential capacity in inhibiting 94.37% LOX activity with IC_{50} of 35 ± 0.53. This was followed by SDG and Smwith 68.26% and 67.07% inhibition, respectively (Table 2).

Table 2.Inhibition of 15-lipoxygenase by purified SDG+Sm

Samples (purified extracts)	Lipoxygenase inhibition in % (100 µg.mL−1 of samples)	IC_{50} (µg)
SDG	68.26	56 ± 0.02
Sm	67.07	58 ± 0.23
SDG+Sm	94.37	35 ± 053

Data are mean ± *SD* (*n* = 3).

a. Human cyclooxygenase (COX)-2 inhibition

The inhibitory effects of the purified SDG, Sm, and their combination, SDG+Sm at 100 µg by *in vitro* enzymatic activities were measured against human COX-2. The results indicated that SDG+sm inhibited human COX-2 by 73.56 ± 2.02%, whereas, SDG and Sm alone were by 62.18 ± 3.14% and 56 ± 2.11 %, respectively (Table 3).

Table 3. Inhibition of cyclooxygenase-2 assay by human COX-2 inhibitor screening kit (Cayman, Ann Arbor, MI, USA) by colorimetric estimation of the purified SDG+Sm

Samples (purified extracts)	COX-2 inhibition in % (100 µg of samples)
SDG	62.18 ± 3.14
Sm	56 ± 2.11
SDG+Sm	73.56 ± 2.02

Data are mean ± *SD* (*n* = 3)

5. Discussion

Nowadays, there is an increasing global interest in traditional herbal medicine. Ayurveda, the Indian traditional medicine, remains the most ancient, yet living customs. Presently, the growing interest of the consumers in polyphenols such as flavonoids, phenolic acids, lignans, and others is generally centered on the commitment of their cell reinforcement action to the anticipation of different disorders, including cardiovascular infection and malignancy.Natural bioactives combinations such as lignans and flavonoids have been widely used as a promising choice for treating inflammation and various oxidative stress-mediated conditions. Lignans, which have a steroid-like structure are characterized as phytoestrogens and proved to act both as agonists and antagonists of phytoestrogens.In the current examination, the HPLC analyses carried out on FS and SS affirmed the presence of SDG and Sm. SDG and Sm have been anticipated to be 23.9 and 4.7g.kg−1 of FS and SS, individually. These are in close concurrence with the previous reports [28, 29]. The vast information acquired by the survey on 1H and 13C NMR and MS of SDG and Sm demonstrated that they are 97 and 98% unadulterated. These are in close agreement with reports of Stefano et al. [30] and Ali et al [31].

Free radicals instigated modifications in DPPH might be principle tailed inside a lymphocyte populace and changes brought about at one fiery site may show an impact on the trustworthiness of ensuing safe reactions. Superoxide anion, H_2O_2, and hydroxyl extremists add to the pathogenesis of an assortment of sicknesses. They are generated in vivo by such instruments, as respiratory redox chain in mitochondria, the respiratory explosion of phagocytes, and the exercises of different oxidases. Despite the fact that the ROS created by actuated macrophages and neutrophils standard anticipate in safe and provocative reactions, oxidative pressure happens when the intracellular centralizations of ROS are higher than physiologic qualities [32]. Human fringe blood lymphocytes presented to both endogenous and exogenous oxidative pressure are more touchy to oxidative pressure and display more harm (DNA strand breaks) contrasted with other cell types [33]. Free revolutionary incited genomic adjustment in lymphocytes may likewise activate cell pathways that lead to fast cell lysis or programmed cell demise [33]. As of late, more consideration is centered around the defensive role of lignans, which are broadly conveyed in plants, assume a significant part as a dietary revolutionary scrounger, and prevent oxidative harm in living frameworks.

The present study demonstrated the IC_{50} (µg.ml-1) values for SDG and Sm alone were 42.56 and 63.14, respectively. Similarly, SDG+Sm exhibited 24.04 µg.ml-1, and the standards viz quercetin and ascorbic acid showed 27.86 and 21.34 µg.ml-1, respectively. Besides, DPPH radical scavenging activity, the reducing power assay of SDG and Sm proved that the synergistic activity of SDG+Sm showed lesser reducing capacity than the reference compounds ascorbic acid but higher reducing than that of quercetin, and SDG and Sm alone. Thus, the results of our present study are in close agreement with previous reports [32, 17].

Polyphenolic compounds, including, lignans are reported to play an anti-inflammatory role notably through regulation of cellular activities in inflammatory cells, radical scavenging activities, and modulation of the activities of enzymes involved in arachidonic acid metabolism (such as COX and PLA2)[33]. Additionally, they can also exert their anti-inflammatory effects by modulating the production of pro-inflammatory molecules and also arginine metabolism (NOS). The possible mechanism for anti-inflammatory activities of polyphenols includes inhibition of enzymes involved in pro-inflammatory activities such as LOX, COX-2, and iNOS, NF-κB and the activation of phase-II antioxidant detoxifying enzymes, mitogen-activated protein kinase (MAPK), nuclear factor erythroid 2-related factor protein-1 (AP-1), and protein kinase-C[34].

Earlier studies have reported that the SDG has cell reinforcement properties and sesamin is to exert potential cancer prevention management [26, 19, and 27]. The current investigation showed that the combined SDG+Sm exhibited lower IC_{50} values (60%) of 35 ± 0.53 when compared to IC_{50} values for SDG (56 ± 0.02) and Sm (58 ± 0.23) when tested against 15-LOX inhibition. Similarly, the results of our present study also proved that the SDG+sm inhibited human COX-2 by 73.56 ± 2.02%,whereas, SDG and Sm alone by 62.18 ± 3.14% and 56 ± 2.11 %, respectively. As a result, the

combined activity or synergism of SDG and Sm i.e.,SDG+Sm could inhibit COX-2 enzyme at relatively lower concentrations than their alone forms. Thus, the outcome of our present investigation is coherent with previous studies [13,17]. Taken together, the results obtained in our study for the first time showed that SDG and Sm possessed synergistic anti-oxidant effects, which may be attributed to their roles in quenching the free radicals; as well as anti-inflammatory effects by inhibiting 15-LOX and COX-2 mediated signaling pathways. The anti-oxidant and anti-inflammatory effects of SDG and Sm were more pronounced in their combined form (SDG+Sm) i.e., synergism than their individual effects (SDG and Sm).

Conclusion

The results obtained in our present investigation for the first time showed that the major lignans Secoisolariciresinol diglucoside and sesamin can be isolated and purified from Indian flaxseed and sesame seeds, respectively. Clinical investigations exhibited the protective part of collegial SDG+Sm against oxidation and oxidation-intervened diseases. In outline, our investigation features the defensive function of synergism SDG+Sm in diminishing oxidative stress. SDG and Sm were able to synergistically quench the free radicals and inhibit the pro-inflammatory mediators such as 15-LOX and COX-2 *invitro*. This new strategy could also pave a way for their use to treat various diseases that have been treated so far using either synthetic drugs or bioactives alone.These results may provide some basis for the combined use of SDG and Sm as effective anti-oxidative and anti-inflammatory agents in the formulation of food, feed, nutraceutical, and functional foods in the future.However, animal experiments are needed to elucidate the molecular mechanism behind the synergistic antioxidant and anti-inflammatoryactions of SDG and Sm in the future.

Conflict of interest

The authors declare no competing financial interest.

Authors' contributions

AB and RJ conceived and designed the study. AB performed the experiments and collected the data. AB and RJ analyzed and/or interpreted the data. AB drafted the manuscript. AB and RJ revised the manuscript critically for important intellectual content. AB wrote the manuscript, and the authors have read and approved the final manuscript before its submission.

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