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Phytochemical, Antioxidant and Antiproliferative Properties of *Linum usitatissimum* (Flax) Seed Extract

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Abstract: In animal studies, flaxseed (*Linum usitatissimum*) is one of the most widely examined diets for possible ties to breast cancer, although clinical data is sparse. Antioxidant and anti-proliferative effects of flaxseeds were examined in this study. Dried seed extracts included 20.4± 0.48 mg GAE and 3.4±0.55 mg QE, respectively in the phytochemical analysis, as well as flavonoids, tannins, carbohydrate, proteins, and amino acids, as well as anthraquinones, phenols, coumarin, and quinones. Nutritional analysis of seed extract from *L. usitassimum* indicated that it contained 18.4 g protein; 31 g of carbohydrate and 38.5 g fat. *L. usitassimum* seed extract scavenged 83% of DPPH radicals at a concentration of 1000 g/ml. The IC_{50} value was discovered to be 638.70 g/ml. At a concentration of 1000 g/ml, 81 per cent of the ABTS radical was scavenged, and the IC_{50} value was 679.77 g/ml. More than 86% of cell growth was inhibited at the highest dosage of ethanolic extract (20-200 g/ml). MCF7 breast cancer cells were significantly suppressed by ethanolic extract of *L. usitassimum*.

Keywords: *L. usitassimum*, Phytochemicals, Antioxidant, Antiproliferative, Flaxseed, DPPH

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Introduction

Small, flat seeds that vary in colour from golden yellow to reddish brown may be found in the annual plant *Linum usitassimum*, a member of the Lineaceae family. Flaxseed has a crunchy texture and nutty flavour (Rubilar *et al.*, 2010). It is the stem that creates fibres which are very strong and durable. Additionally, it has been used to produce food and medication. Flaxseeds are rich in

phytoestrogenic lignans in addition to ALA, short-chain polyunsaturated fatty acids (PUFA), soluble and insoluble fibres, protein, and other antioxidants (Ivanova *et al.*, 2011). Flaxseed-derived edibles include whole flaxseed, ground flour, and products manufactured from extracted oil or mucilage.

Flaxseed has antioxidant and hepato-protective properties. Flaxseed meal's ability to decrease cholesterol has long been documented. However, lignan precursors from mammals are the most effective in preventing the establishment of new tumours. During the last phases of carcinogenesis, flaxseed and its oil inhibit the development of tumours. Flaxseed lignans, on the other hand, help prevent various types of cancer. Hormone-sensitive tumours, in particular, are extremely well protected by these drugs. Antioxidant characteristics of flax lignans are regarded to be the fundamental rationale for their anticancer effect (Prasad, 1997). Flaxseeds have been reported as nephroprotective, anti-diarrheal, anti-fungal and arthritic pain, anti-bacterial and cancer fighting. They also have antioxidant characteristics that may help prevent diabetes. In this study flaxseeds' antioxidant and antiproliferative activities were evaluated in light of their nutritional and therapeutic significance.

Materials and Methods

Preparation of Extract:

L. usitassimum seeds were collected, cleaned well under running water, and rinsed again with distilled water before being used in this experiment. The seeds were dried in shade. In order to mill the seeds, an electric grinder was used. Overnight, a 75 ml solution of ethanol and 100 g of dry seed powder were stirred in a shaker. After drying, the powder was treated with an ethanol-based Soxhlet solution. The ethanolic extract was stored in an airtight container for future use.

Preliminary Phytochemical Screening:

The ethanolic extract of *L. usitassimum* seeds were subjected to preliminary phytochemical screening (Harborne, 1998; Kokate, 2007).

Determination of total phenolic and total flavonoid content:

The Folin-Ciocalteu colorimetric technique was used to evaluate the total polyphenol content in the ethanol extract of *L. usitassimum* seeds (Kumazawa *et al*., 2002). When extracting *L. usitassimum* seeds, Quettier *et al.* (2000) technique (with minimal changes) was used to find out how much total flavonoid content was contained in the extract.

*Determination of Carbohydrate and Protein Content***:**

The carbohydrate content was measured by the 3, 5-dinitrosalicylic acid method (Miller, 1959) and the estimation of proteins was carried out by the Lowry (1951) method.

Fat content:

The Soxhlet technique was used to figure out how much fat was in each serving. Thimbles were used to transport 4 g of sample to condenser in Soxhlet apparatus. In a round-bottom flask, 100 cc of petroleum ether was heated for 4 h. The conical flask holding the extract had been pre-weighed. Keeping the conical flask underwater helped the petroleum ether to evaporate. A vacuum pump was used to remove petroleum ether leftovers.

Assays for free radical scavenging:

Test for radical scavenging by DPPH:

The ethanolic extract's capacity to neutralise free radicals was tested using the DPPH method (Brand Williams *et al.*, 1995). In order to make the DPPH solution, 95 per cent methanol was used. The stock *L. usitassimum* seeds extract solution was poured in five test tubes containing 200, 400, 600, 800, and 1000 µg/ml, respectively. Incubated for 10 min, the seed extract was added to 0.5 ml of a freshly produced DPPH solution and incubated. A spectrophotometer set at 517 nm was used to measure the absorption. The ascorbic acid standard was used as a point of comparison.

ABTS assay:

The scavenging ability of *L. usitassimum* seed ethanolic extract to neutralise ABTS radicals was tested using the Re *et al.* (1999) procedure. After 1 min, the absorbance at 734 nm was measured after various quantities of seed extract in ethanol (200–1000 g/ml) were added to 3.0 ml of diluted ABTS+ solution.

Analysis via Gas Chromatography Mass Spectrometry:

A GC-MS (Model: QP 2010 Plus, Shimadzu, Tokyo, Japan) equipped with a VF-5 ms fused silica capillary column of 30 m in length, 0.25 mm in diameter, and 0.25 film thickness was utilised to analyse the ethanolic extract of *L. usitassimum* seeds. The GC-MS procedure took 39 min in total. The relative percentage of each extract component was presented as a percentage after peak area normalisation. Retention indexes and mass spectrum patterns were used as a guide to identify bioactive components in an extract of ethanol by comparison with Wiley 8 (Wiley 8). The seed extract from *L. usitassimum* possessed anti-proliferative properties.

Culture of Cells:

The MCF7 (breast cancer) cells were procured from NCCS, Pune, India. The cells were cultured in DMEM with 10% FBS, 1% antibiotic and antimycotic solution, and 5% CO_2 in a 25-cm 2 flask at 37°C. Replacement of the cell culture media was carried out twice a week. Once the cells had reached confluence, they were used for cytotoxicity testing 25 to 29 passages.

Assessment of Cytotoxicity (*MTT Assay*):

L. usitassimum seed extract cytotoxicity was assessed through a modified MTT assay (Mosmann, 1983). In 96 well plates with flat bottoms, about 15,000 cells were cultivated (37°C and 5% CO_2). DMEM with 10% FBS, 1% antibiotic and antifungal solution was then administered to the animals. Use of the Trypan blue exclusion approach allowed us to ascertain the cell count. At concentrations of 25, 50, 75, 100, 125, 150, 200, 300, 400, and 500 µg/ml, the cells were treated with ethanolic extract from *L. usitassimum* seeds. 10 µl of MTT was added to the plate after 24 h of incubation and incubated for 4 h at 37°C and 5% CO_2. Formazan crystals were dissolved in DMSO and their violet colour was quantified using a microplate reader following this procedure (BIORAD). The absorbance of untreated cells was believed to be 100% viable. Following formula was used to calculate the cytotoxicity:

$$\% \text{ Cytotoxicity} = 1-[ATC/ANT] \times 100$$

Where ATC= mean absorbance of treated cells; ANT= mean absorbance of negative control

Statistics:

Each experiment was run in three separate sets, each set including a duplicate. The mean and standard error mean (SEM) were used to represent the data.

Results and Discussion

Analysis of *Linum usitassimum* seeds is shown in Table 1. Flavonoids, tannins, carbohydrates, proteins, amino acids and anthraquinones were identified using a phytochemical study. Total phenolic and flavonoid components per gram of dried seed extract were found to be 20.4± 0.48 mg GAE and 3.4± 0.55 mg QE (Table 2). There are several biological benefits of plant phenolics, such as anti-inflammatory, anti-cancer, antioxidant, and antibacterial activities (John and Grohmann, 2001).

Flaxseeds have a significant phenolic chemical concentration, according to research. Antioxidant and cancer-fighting properties are well-known for the phenolic compounds. Flaxseeds include three major groups of phenolic chemicals phenolic acids, flavonoids, and lignans. Defatted flaxseed contains ferulic acid (10.9 mg/g), chlorogenic acid (7.5 mg/g), and gallic acid (2.8 mg/g) as the main phenolic acids. Phenolic acid glucosides include four-hydroxybenzoic acid, p-coumaric acid and hydroxycinnamic acid glucosides (Beejmohun *et al*., 2007).

Table 1: Phytochemical analysis of *L. usitassimum* seed extract

PHYTOCONSTITUENTS	INFERENCE
Alkaloids	-
Flavonoids	+
Saponins	+
Tannins	+
Phlobatannins	-
Carbohydrate	+
Proteins and amino acids	+
Triterpenoids	-
Glycosides	-
Anthraquinones	+
Phenols	+

+ Presence; - Absence

Table 2: Total phenolic compounds and Total flavonoids of *L.usitassimum* extract

Total Phenol Content	20.4 ± 0.48 mg GAE /g of dry seed extract
Total Flavonoid	3.4 ± 0.55 mg QE/g of dry seed extract

Plant phenolics are the body's primary antioxidants or free radical scavengers. Flavonoids are made up of phenolic compounds. These chemicals may be responsible for flaxseed's powerful antioxidant properties since they were detected in the extract. Therapeutic properties are derived from the medicinal plant's secondary metabolites (e.g. phytochemicals) and chemical ingredients.

Value of flaxseeds as food:

Table 3 shows the nutrient composition of *L. usitassimum* seed extract. Flaxseed has been recognised as a possible functional food due to its high content of lignans, soluble fibre, phenolic compounds, alpha-linolenic acid, and high-quality protein among functional foods (Oomah 2001). Flaxseed contains 18.4 g of protein, 38.6 g of fats, and 31 g of carbs (Morris, 2007).

Ethanolic Extract of Linum usitassimum Seeds: GC-MS Analysis:

We used GC-MS to analyse the chemical composition of *Linum usitassimum* in this study. When compounds were eluted, the GC displays the concentrations of different chemicals in relation to retention time. The peak heights represent the relative concentrations of the seed components. Analysis of ethanolic extracts of *Linum usitatissimum* yielded the results shown in Figure 1.

Table 3: Nutritional composition of *L.usitassimum* extract

Parameter	Amount per 100 g of edible flaxseed
Carbohydrates	31.0
Protein content (g%)	18.4
Fat (g)	38.6

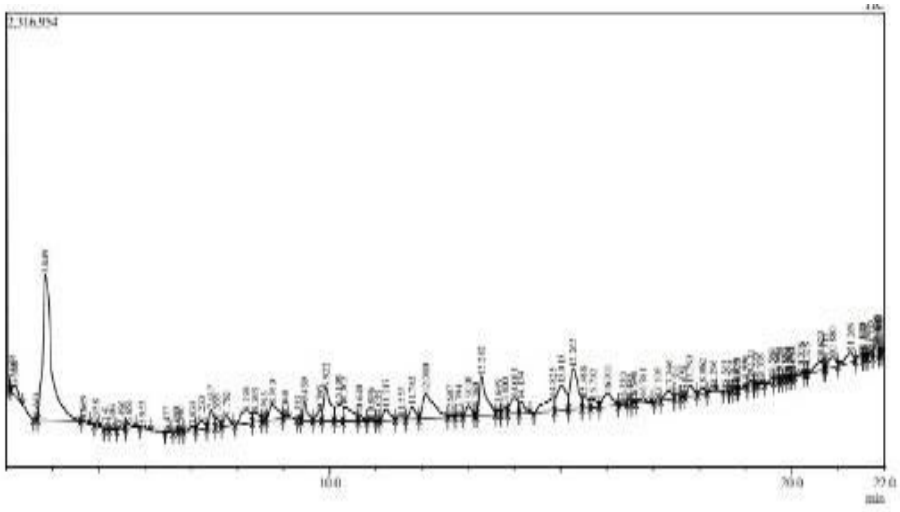


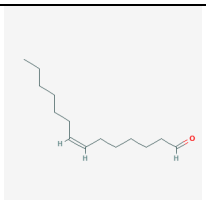
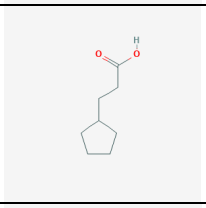
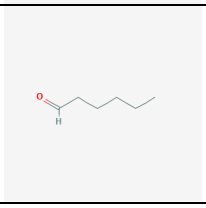
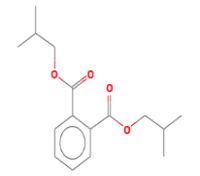
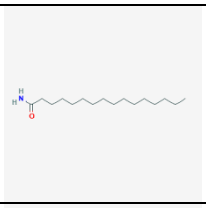
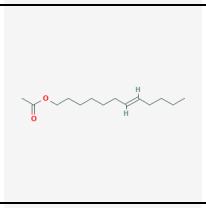
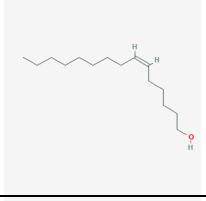
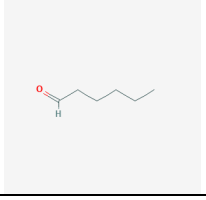
Fig. 1: GC-MS analysis of *Linum usitassimum* extract

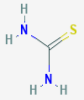
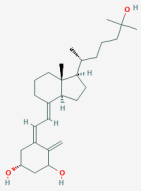
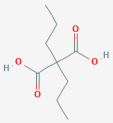
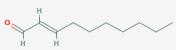
The phytocompounds found in *Linum usitatissimum* are shown in Table 4 for composition and biological function. *Linum usitassimum*'s GC-MS analysis uncovered 189 new chemicals that may be connected to the plant's therapeutic properties. The peak area, retention time, and structure were used to identify the phytochemical constituents. The Dr. Duke's Phytochemical and Ethnobotanical Databases, created by Dr. Jim Duke of the Agriculture Research Service/USDA serve as the foundation for the biological activities mentioned. 3.428 min was the retention time of the first chemical, silanediol. (E)-3,7-Dimethylocta-2,6-diene-1-thiol's prolonged retention duration was 44.368 min. For the most part, this stuff is made up of flavonols and terpenoids and aromatic carboxylic acids and sugar and other hydrocarbons, fatty acids, alcohols and phenols. Anti-urolithiasis, antibacterial, antioxidant, anti-inflammatory, antifungal, and antiparasitic characteristics are only a few of the pharmacological effects linked to these phytochemicals.

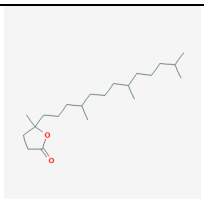
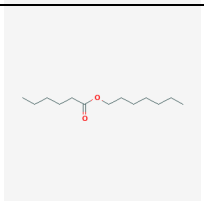
Flax seed antioxidant activity:

Flaxseed extract is efficient in scavenging DPPH and ABTS radicals (Figs. 2, 3). The amount of DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity that antioxidants were able to remove was measured. Similar to this, the antioxidant-suppressed ABTS radical cation content was used to estimate ABTS radical activity. *L. usitassimum* seed extract scavenged 83% of the DPPH radical at a concentration of 1000 g/ml. This is the IC_{50} value, which is 638.70 g/ml. 81% of the ABTS radical was scavenged at a

Table 4: Structure and biological activity of compounds in *Linum usitassimum*

S. No.	R. TIME	NAME	AREA %	NATURE OF THE COMPOUND	STRUCTURE	BIOLOGICAL ACTIVITY
1	27.450	Z-7- tetradecenal	6.06	Aliphatic compound		Anti fungal, Anti bacterial
2	27.142	3-Cyclopentylpropionicacid	1.00	monocarboxylic acid		Antiashmatics
3	3.849	Hexanal	3.37	alkyl aldehyde		Antibacterial, Antiseptic, flavor, pesticide
4	27.760	Hexadecanoicacid,ethyl ester	1.70	Fatty acid ethyl ester		Anti- oxidant, Hypocholesterolemic
5	23.434	1,2- benzenedicarboxylic acid bis(2-methylpropyl) ester	1.19	fatty ester		Antimicrobial, Antifouling
6	27.880	Hexadecanamide	2.41	fatty amide		Antioxidant activty, analgesic, Anti-inflammatory, Anti-bacterial
7	39.310	7-deceo-1-ol-acetate	1.13	monoenic *acetate*		Anti pesticide
8	29.732	(6z)-6-pentadecen-1-ol	2.02	Alkane hydrocarbon		Anti- bacterial
9	27.284	Hexanoic acid	0.70	saturated fatty acid		Anti diabetic, Anti cancerous activity

10	30.570	Oleamide	1.44	Amide		Anti oxidant, Anti infective agent
11	15.265	Undecenal	1.15	fatty aldehyde		Antibacterial, Antifungal, Nematicidal
12	30.284	Nonenal	0.73	unsaturated aldehyde.		Anti- malarial
13	40.067	Thiourea	0.85	organo-sulfur compound		Antimicrobial, Anticancer
14	26.849	Oxalic acid	0.77	dicarboxylic acid		Antiseptic, Antibacterial
15	30.025	9,12-Octadecadienoic acid	0.76	Fatty acid		Anti- oxidant
16	30.440	1,25-Dihydroxyvitamin D3	0.60	fat-soluble secosteroids		Osteoblast
17	29.894	di-n-propyl malonic acid	0.56	dicarboxylic acid		Anti bacterial
18	13.284	2-decenal	1.05	aldehyde		Anti viral, flavouring agent, nematicide

19	30.174	4,8,12,16-Tetramethylheptadecan-4-olide	0.88	C_{21} isoprenoid γ-lactone		Anti-viral, Anti-cancer
20	27.284	Heptyl hexanoate	0.70	Ester		Herbicidal

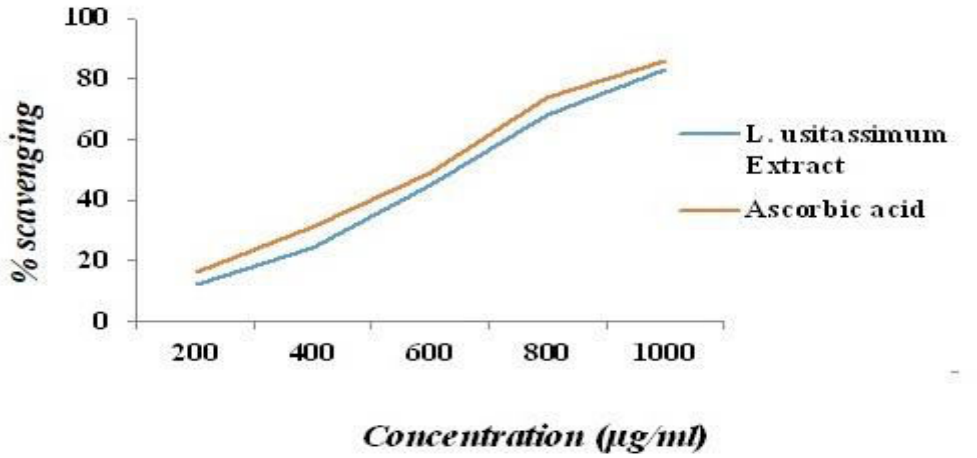


Fig. 2: DPPH scavenging potential of *L.usitassimum* extract.

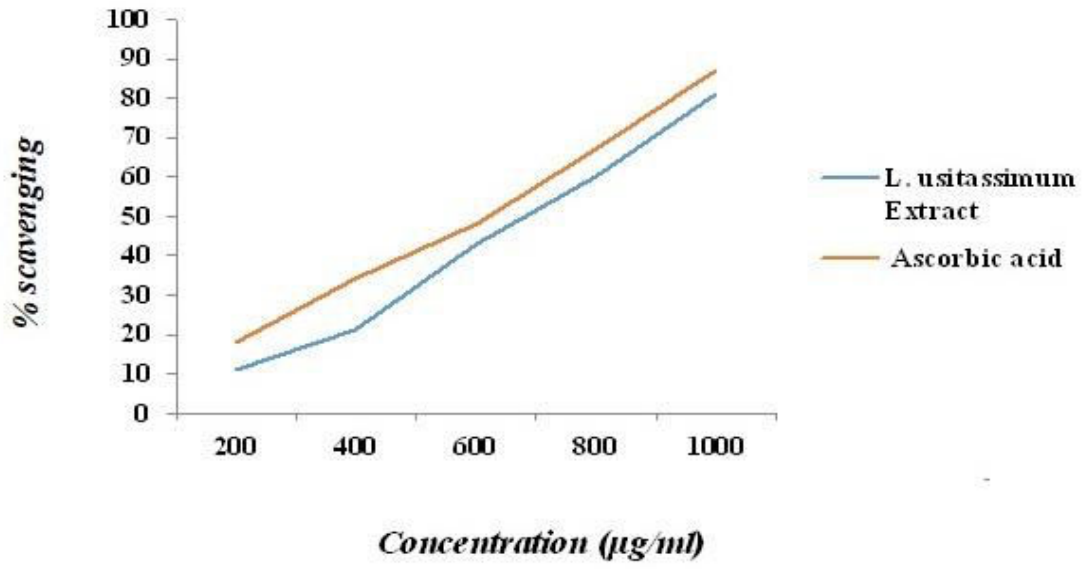


Fig. 3: ABTS scavenging potential of *L.usitassimum* extract

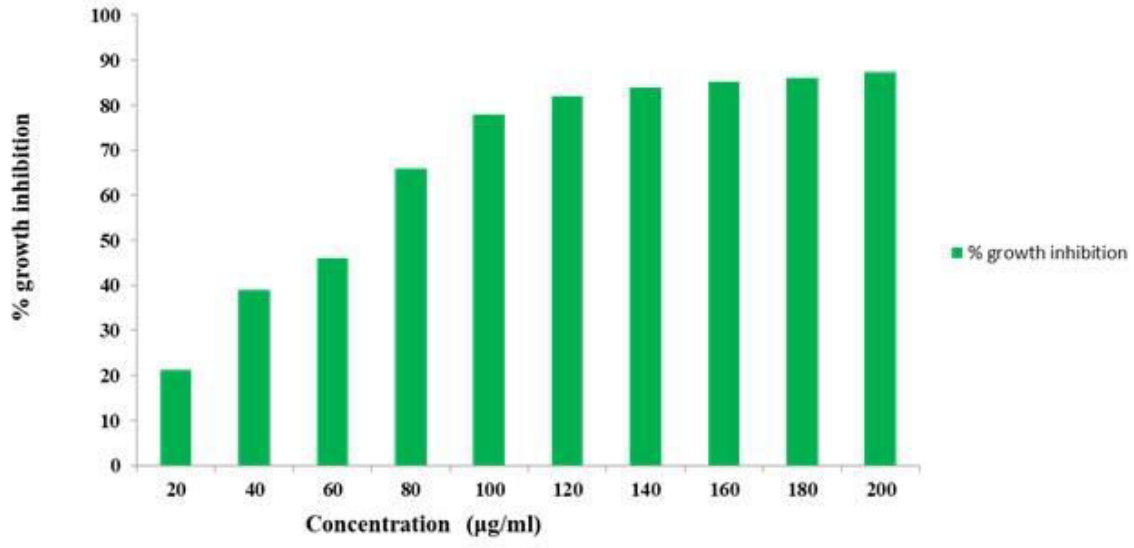


Fig. 4: Toxicity effects of the ethanolic extract of *Linum usitassimum* seed extract against cancer cell line (MCF-7) after 24 h of incubation.

concentration of 1000 µg/ml, and an IC_{50} of 679.77 µg/ml was discovered.

Flax seed anti-proliferative properties:

The ethanolic seed extract of *L. usitassimum* demonstrated substantial cytotoxicity against MCF7 breast cancer cells in 24 h due to its strong antiproliferative effect (Fig. 4). More than 86% of cell growth was inhibited at the highest dosage of ethanolic extract (20-200 g/ml). Ethanolic extract considerably slowed the growth of MCF7 breast cancer cells. Human trials and cell culture experiments have been used to evaluate the beneficial anti-oncotic effects of milled flaxseed. There is speculation that flaxseed's anti-cancer benefits may be explained by its high concentrations of both SDG and ALA. Breast cancer mortality is lowered by 33–70% and overall mortality is reduced by 40–53 per cent when lignans like SDG are used. Flaxseed oil enhanced with ALA, the anti-tumor effects of oncotic therapy medicines (Mason *et al*., 2014). For breast cancer patients with epidermal growth factor receptor 2 positive, combining trastuzumab with flax oil dramatically increased the anti-tumor effects of trastuzumab and lowered the dosages of trastuzumab required to remove tumours in athymic mice (Mason and Thompson, 2010). Combining flaxseed with the anti-tumor medication tamoxifen had similar outcomes (Calado *et al*., 2018). A wide spectrum of pharmacological actions may be identified in flaxseed's phyto-constituents. Flax seeds have also been subjected to GC MS analysis for the detection of a wide range of compounds known to have biologically significant properties.

Conclusion

Omega-3 fatty acids, alpha-linolenic acid, lignin secoisolariciresinol diglucoside, and fibre have all been found in flaxseeds. Additionally, flax seed is recognised to have anti-inflammatory qualities. As a result of their bioactivity, these chemicals may assist both people and animals maintain a healthy balance of lipids and inflammation, which is beneficial to their overall health. Flaxseed extract has antioxidant and antiproliferative properties on the MCF7 breast cancer cell line, according to the results of the current investigation. When it comes to flaxseed, phytoconstituents present in the seed have an array of pharmacological effects, and the seed itself is an excellent source. It is said that flax seeds are rich in omega-3 fatty acids, dietary fibre, alpha linolenic acid, and the lignin

secoisolariciresinol diglucoside, as well as lignans. Anti-inflammatory, antioxidant, and lipid modulator properties provide these compounds bioactivity that is good for people and animals alike. The present study showed thatFlaxseed extract has antioxidant and antiproliferative properties on the MCF7 breast cancer cell line.

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