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Chemo-Modulatory Potential of Flaxseed Oil as Natural Anticancer Therapeutic

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Abstract

Background: Cancer is a disease which is characterised by uncontrolled cell proliferation and development. Surgery, chemotherapy, radiotherapy, and photodynamic therapy are the most frequent cancer treatments. On the other hand, there are many negative health impacts of radiation and chemotherapy that limit the efficient use of these therapies.

Methods: This scenario needs natural treatments that are cost-effective and has no adverse effects. Flaxseed oil can be used as a nutraceuticals for the management of cancer. The current research was aimed on the exploration of the flaxseed (*Linum usitatissimum*) oil for in vitro anticancer activity as a natural therapy for the management of cancer.

Result: The flaxseed powder contained 36.6±0.04% oil contents with an average yield of 36.6±0.03% by using hexane as solvent for extraction. Moreover, the oil contained polyunsaturated fatty acid with omega 3 fatty acid (alpha-linolenic acid) as a dominant content of the oil. In-vitro anticancer activity of flaxseed oil was observed by Cytotoxic (3T3 cell line) and Prostate Cancer (PC3 cell line) indicating that the oil possessed anticancer activity which was dose-dependent.

Conclusion: On the basis of results, it was concluded that the flaxseed or its oil can be used for the management of cancer as a natural therapy by using optimized dose levels for a different types of cancer.



Introduction

Cancer is a disease caused by the uncontrolled cell division. The development and progression of cancer cell are basically due to the change in the DNA cell. Under different circumstances, the tumour cell migrates, attacks, and proliferate to other organs and tissue of the body [1]. Overall, worldwide about 19.3 million people diagnose cancer every year and cause 10 million deaths. In developed countries, it is the second disease after cardiovascular with a death rate of 10% globally [1]. Every year, around 3 million new cases of cancer were recorded, in Asia, more than 2 million mortalities due to cancers have been reported. There are different types of cancer and show different symptoms and causes. Some common cancer is breast, kidney, lung cancer, prostate, colorectal, liver, bladder cancer, melanoma and non-Hodgkin lymphoma. Cancer cells start to develop abnormally and increase rapidly to form lumps [2]. Depending on the type of tumour many anticancer treatments are used. The most common treatments used for cancer are surgery, radiotherapy, photodynamic therapy, and chemotherapy. Photodynamic therapy is the combination of photosensitizer agent's e.g., temoporfin or porfimer sodium with a specific kind of light in an oxygen-rich atmosphere. When photosensitizer agents are exposed to light oxygen is produced and they kill the tumour cell [2]. These treatments show good results but on the other hand, there are many side effects of this therapy. Thromboembolism, nausea, stomatitis, neuropathy, alopecia, myelosuppression, myalgias, and fatigue are the short time effects. And the long-term effect is emesis infertility, weight gain, cardiac dysfunction, cognitive dysfunction and Leukaemia/MDS [3]. A major side effect of these treatments is a failure in ovarian and infertility [4]. Natural treatment is cost-effective, easy to access, highly effective, and has no side effects. Natural compounds extract from plants played significant role in the development of several clinically useful anticancer agents [5]. Herbal drugs or extracts contain many active compounds which interact with other given pharmaceutical drugs to increase the therapeutic effect. Different phytochemicals (quercetin, genistein, curcumin, and catechins) are present in herbal drugs, these phytochemicals increase the therapeutic effect and enhance the bioavailability of the other drug [6,7]. Compounds obtained from a natural source show active potential toward cancer treatment in the past many drugs derived from natural sources uses for the treatment of cancer [8].

Cheese, egg, milk, walnut, fish, and flaxseed are rich sources of omega 3 fatty acids. Omega 3 fatty help to decrease the proliferation of cancer. Flaxseed is one of the highest plant sources of soluble mucilage and α -linolenic acid (omega 3 fatty acid) [9]. Flaxseed contains

approximately 36 to 40% of oil content it is become much common nowadays as a functional food due to its many health benefits and help in many diseases coronary heart disease, hormonal conditions, many types of cancer, nerve [10]. Flaxseed oil is a rich source of ALA, which is around 55 to 60% of total fatty acids [11,12].

Keeping in view increasing the incidence of various cancer types all over the world and potential health benefits of flaxseed oil the current study were planned to expect flaxseed oil to cold press and solvent extraction techniques. The extracted oil after analysis was subjected to assess the anticancer potential against prostate cancer.

Methods

Compositional analysis of flaxseed and oil extraction and analysis

The percentage composition of flaxseed was determined for crude protein, moisture content, ash, crude fibre, and crude fat according to the method of AOAC [13]. The oil was extracted by two different techniques. For this purpose, the flaxseed was divided in two equal parts. The first portion was used for the oil extraction by solvent extraction method by using n-Hexane whereas the other portion was used for the extraction of oil by the cold press method. In cold press method, the screw presser (Carver Press, USA) was used to extract the oil for 20 min at 40°C-60°C and pressure range of 29.4-49.0 MPa. The oil yield was measured by the following equation.

$$\text{Flaxseed oil yield (\%)} = \frac{\text{Oil wt (g)}}{\text{Wt. of flaxseed sample (g)}} \times 100$$

Saponification and peroxide value

Saponification and peroxide value of flaxseed oil was estimated according to the method of AOCS [14]. The equations used for quantification are as under.

$$\text{Saponification value} = \frac{(B - S) \times N \times 56.1}{W}$$

$$\text{Peroxide value} = \frac{(S - B) \times N}{W} \times 1000$$

Free Fatty Acids and Refractive index

The free fatty acids and refractive index of flaxseed oil was determined by refractometer as per the recommendations of AOCS [14]. Free fatty acids were calculated by the following formula:

$$\text{FFA\%} = \frac{V \times N \times 282}{W} \times 100$$

Fatty acid profile

The fatty acid profile of flaxseed oil was determined by the method of National Institute of Standards and Technology [15]. According to this method, first fatty acid methyl esters were prepared using methanol. With

the combination of mass spectroscopy detector (GC-MS) system, 1µL sample of FAME were injected into the gas chromatography (Shimadzu GC-17A). The temperature of the program was set around 70°C to 280°C and helium gas used as a carrier gas. Capillary column (DB-23; 30m x 0.32mm x 0.50µm) performed the separation with flow rate of 0.8mL/min. Firstly, the temperature of the oven was set at 190 °C for the time period of only 1 min then afterwards increased up to 230 °C at a rate of 3 °C min⁻¹. After attaining this temperature, maintained it for 10 minutes. The injection and detector temperatures were kept at 240 and 250 degrees Celsius, respectively. The chromatogram peaks were obtained using retention data from analysed standard samples. Finally, detailed fatty acid content was estimated/calculated as a percentage (%).

Total phenolic contents

The Folin-Ciocalteu assay was used to quantify the total phenol content (TPC) of flaxseed oils [16].

Antioxidant assay

Antioxidant activity of flaxseed oil was determined through spectrophotometer (company, Cecil Instruments limited and model, Aquarius CE 7400S), following method described by Anwar *et al.* [17]. Antioxidant activity was computed by the given formula:

$$\text{DPPH radical scavenging (\%)} = ((\text{AB} - \text{AE})/\text{AB}) \times 100$$

FTIR Analysis

Infrared spectrum of absorption of flaxseed oil was measured from Fourier-transform infrared spectroscopy (FTIR) techniques model Cary 630 [18].

In-vitro anticancer property of flax seed oil

Cytotoxic (3T3 cell line) and prostate cancer (PC3 cell line) was brought from (HEJ), Husain Ebrahim Jamal Research Institute of Chemistry Karachi. The in-vitro anti-cancer activity of flaxseed oil was determined through the method of Gavamukulya *et al.*, [19].

Statistical Analysis

The data attained during whole study was analysed through analysis of variance (ANOVA) using STATISTIX (Version 8.1) software as recommended by Steel *et al.* [20], to validate the physio-chemical parameters, anticancer activity of flaxseed oil.

Results

The present research evaluated proximate analysis of flaxseed and then extraction of flaxseed oil was done by two techniques, physio-chemical characterization of flaxseed (*Linum usitatissimum*) oil and in-vitro anticancer property of flaxseed oil. The results indicated

that the flax seed contained 7±0.02%, 18.4±0.04%, 36.6±0.04%, 16±0.06% and 18.2±0.04% of moisture, protein, fat, total minerals and fibre, respectively (Table 1). The oil recovery was 36.6±0.03% and 29.2±0.07% in case of solvent extraction and cold press method, respectively. Similarly, the “L”, “a*” and “b*” values for color of flaxseed oil were relatively higher in the oil extracted through solvent extraction method rather than that of cold press method extracted oil. The “L”, “a*” and “b*” values for solvent extracted oil were 55.2±0.09, 9.10±0.02 and 40.2±0.04 (Table 2). The significantly lowest moisture content was observed in oil extracted by solvent extraction method with mean values of 2.10±0.01% and 0.93±0.01%, correspondingly. The peroxide value was relatively higher in the oil extracted through hexane method rather than that of cold press extracted oil with mean values of 4.13 ± 0.09 mg KOH/g and 3.24± 0.02 mg KOH/g, correspondingly. The highest refractive index value was observed in the oil extracted through cold press method whereas significantly the lowest refractive index value was observed in case of oil extracted through solvent extraction method with mean values of 1.58± 0.01 and 1.17± 0.01. The saponification value of flaxseed oil through hexane was observed to be 181.23± 0.55mg KOH/g whereas saponification value of cold press oil was found to be 177.63± 0.45 mg KOH/g.

Parameter	Flaxseed powder (%)
Moisture	7±0.02
Protein	18.4±0.04
Fat	36.6 ±0.04
Mineral	16±0.06
Fiber	18.2±0.04

Table 1: Proximate analysis of flaxseed Powder.

Parameters	Solvent Extracted Oil (SEO)	Cold Press Extracted Oil (CPO)
Total Yield	36.6±0.03%	29.2±0.07%
Physical Appearance	Yellow brown	Light yellow
Color L*, a*, b*	55.21±0.09, 9.14±0.01, 40.28±0.04	51.06±0.07, 7.99±0.02, 14.69±0.02
Moisture (%)	0.93±0.01%,	2.10±0.01%,
Peroxide value (%)	4.14 ± 0.09	3.24± 0.02
Rancidity		
Saponification value (mg KOH/g)	181.23± 0.55	177.63 ±0.45
Refractive index	1.17 ± 0.01	1.58 ± 0.01
Total Phenolic Contents (TPC) (mg GAE/g)	158.9±1.16	90±0.58
Antioxidant assay (%)	105±0.58	66.97±0.25

Table 2: Physio-Chemical analysis of flaxseed oil extracted by solvent and cold press.

The total phenolic contents of flaxseed oil through hexane was observed to be 158±1.16 (mg GAE/g) whereas the total phenolic contents of cold press oil was found to be 90±0.58 (mg GAE/g). The antioxidant activity of flaxseed oil through hexane was observed to be 105±0.58 whereas saponification value of cold press oil was found to be 177.63 ±0.45 (Table 2). The results further exposed that the fatty acid profile of flaxseed oil

through hexane was observed to be ALA (50±0.04%), linolenic acid (13±0.02%), oleic acid (18±0.02%) and palmitic acid (3±0.01%) whereas fatty acid profile of cold press oil was found to be ALA (48±0.03%), linolenic acid (11±0.01%), oleic acid (17±0.01%) and palmitic acid (2±0.01%) as shown in table 3.

Fatty acids	Solvent extracted oil%	Cold press oil %
C18:3 (n-3) a-Linolenic acid (ALA)	50±0.04	48±0.03
C18:2 (n-6)-Linoleic acid	13±0.02	11±0.01
C18:1 (n-9)-Oleic acid	18±0.02	17±0.01
C16:0-Palmitic acid	3±0.01	2±0.01

Table 3: Fatty acid profile of flaxseed oil extracted by solvent and cold press.

The results further indicated that the a-linolenic contents were higher than others fatty acids in both oils with mean values of 50±0.04% (SEO) and 48±0.03% (CPO) (Table 3). The oils were also subjected to FTIR analysis and the result obtained are shown in Fig. 1 cold press oil and solvent extraction (hexane) oil as chromatogram.

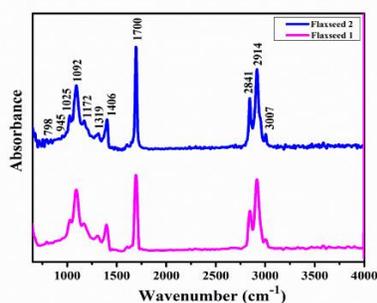


Figure 1: FTIR of flaxseed oil extracted through cold press and solvent extraction. Flaxseed 1(Cold press) and Flaxseed 2 (Solvent extraction).

Oil	Anticancer Activity (Prostate Cancer) (PC3 cell line)	Cytotoxic (3T3 cell line) Activity
Cold Press	Yes	Yes
Solvent Extraction	Yes	Yes

Table 4: Anticancer activity of flaxseed oil extracted through solvent and cold press.

The peaks above the 3000 cm⁻¹ for alkene and below 3000cm⁻¹ for alkane. The spectra shows that below 1172 cm⁻¹ saturated fatty acid were present, from 1319 cm⁻¹ to 1700 cm⁻¹ monounsaturated fatty acid and from 2841 cm⁻¹ to 3007cm⁻¹ polyunsaturated fatty acid present. Flaxseed oil has been very efficient toward several different kinds of cancer cells. The anti-proliferative effect of flaxseed oil on human prostatic cell lines PC3 was evaluated through MTT assay. MTT assays showed that the flaxseed oil at the concentration of 30 µg/mL for 48 h showed anti-proliferative effect on PC3 cells. The result of oil extracted through cold press were better as compared to hexane extracted. Cytotoxic (3T3 cell line) activity of flaxseed oil was also observed through MTT assay. Oil extracted by both method cold

press and hexane extracted were performed Cytotoxic (3T3 cell line) activity. MTT assays showed that the flaxseed oil at the concentration of 30 µg/mL for 48 h cytotoxic effect on 3T3 cell line. The result indicated the cytotoxic activity of flaxseed oil extracted through cold press was higher as compared to hexane extracted. It was also observed the effectiveness of flaxseed oil toward cancer treatment was dose dependent (Table 4).

Discussion

Plant-based drugs contain anticancer agents'vincristine, etoposide, vinblastine, irinotecan, paclitaxel, topotecan, and camptothecin. Many anticancer agents derived from fruits and vegetables use for the management of cancer therapy like capsaicin (red chili), genistein (soybean), resveratrol (peanuts, red grapes, and berries), allicin (garlic), diallyl sulfide, S-allyl cysteine (allium), lycopene present in tomato, ellagic acid present in pomegranate, diosgenin, 6-gingerol (ginger), ursolic acid present in apple, pears, silymarin in milk catechins, thistle, anethol, indole-3-carbinolbeta, carotene, eugenol, limonene and dietary fiber [21]. Nutraceuticals derived from 'Nutrition' and 'Pharmaceuticals'. Food or diet that give medical and health benefits also have the treatment and management of many diseases. Many studies show fiber-rich foods like fruits, vegetables, grains, and cereal have a protective effect on colorectal cancer in humans. The use of multivitamins regularly and moderate use of alcohol intake also helps in the management of diseases [22]. Cancer risk is reduced by the consumption of vegetables and fruits due to vitamin C concentration. Overall, the oral cavity, stomach, colorectum, and esophagus cancer risk decrease by the consumption of vegetables and fruits. The stomach cancer risk increases due to the consumption of salt-preserved foods and salt. Intake of hot beverages and foods consumed in many countries may increase the risk of cancers of the oral cavity, pharynx, and esophagus[23]. The use of flaxseed oil (ALA) decreases the growth and proliferation of breast cancer cells. It suppresses the size of the tumor and promotes cancer cell death. Flaxseed oil decreases the growth of cancer cells and promotes the apoptosis process [24,25].

Parallel results were also observed by Aslam *et al.*, [26] during investigation on physicochemical analysis of flaxseed and found the range of moisture contents 5.9-6.1%, crude protein 18.67-20.7%, total ash 6.98-7.02%, crude fiber 38.5-42.55% and crude fat 9.50-11.5%. Another parallel result observed by Ishag *et al.* [27] were also in well agreement with the current study results. They found the proximate composition of flaxseed as moisture 8.50 ± 0.49%, fat 43.17 ± 0.99%, protein 21.00 ± 0.74%, ash 1.96 ± 0.00%, and fiber 20.23 ± 3.47%. Similar results were also observed by Ishag *et al.*, [27]

during investigation on flaxseed oil and found the oil yield of flaxseed from solvent extraction $21.95 \pm 0.01\%$ method whereas $18 \pm 0.01\%$ yield observe from cold press method. Another parallel result observed by Kulkarni *et al.*, [28] was also in well agreement with the current study results. They found flaxseed oil from solvent extraction 41.53 ± 0.9 method whereas $25.50\% \pm 0.7$ yield observed from cold press method. The overall physiochemical results of current research work are justified by a number of related studies. Comparable outcomes were also observed by Ishag *et al.* [27] during investigation on physicochemical analysis of flaxseed oil extracted by solvent extraction and found the range of moisture contents $0.23 \pm 0.01\%$, refractive index 1.47 ± 0.00 , FFA $0.6 \pm 0.01\%$, peroxide value 4.67 ± 1.00 mg KOH/g and saponification value 185.61 ± 0.56 mg KOH/g. While the oil extracted through cold press method contained $0.4570.04\%$ moisture, FFA $0.6070.08\%$, peroxide value 1.24 ± 0.02 mg KOH/g, refractive index 1.305 ± 0.01 and saponification value 184.21 ± 0.55 mg KOH/g. Another parallel result observed by Shim *et al.*, [29] were also in well agreement with the current study results. They found the proximate composition of flaxseed oil moisture $0.25.04\%$, FFA $0.25 \pm 0.01\%$, peroxide value 0.9 ± 0.01 mg KOH/g, refractive index 1.295 ± 0.01 and saponification value 190 ± 0.3 mg KOH/g. Grajzer *et al.*, [30], Hernández-Salazar *et al.*, 2013, Herchi *et al.*, [31], Zhang *et al.*, [32], Grajzer *et al.*, [30], Ishag *et al.*, [27], Shim *et al.* 2015 [29], Khattab and Zietoun [33] and Danish and Nizami [34] also found the similar results in respect of composition and functional properties. Similar observation was also observed by Buckner *et al.*, [24] who also evaluated the anti-cancer effects of flaxseed oil through in vitro study through direct effects on the growth of cancer cell however, it was found to be dose-dependent. The anticancer potential of flaxseed oil might be due to the presence of omega 3 fatty acids in the oil. The results can be justified by the findings of Meng *et al.*, [35], who also observed that the omega 3 fatty acids showed anti-proliferative effect on human prostate cancer. These studies showed that flaxseed oil can specifically inhibit the growth of cancer cell and persuade the process of apoptosis.

In the current study, we explored role of flaxseed's oil in vitro anticancer activity as a natural therapy for the better management of cancer. The flaxseed powder contained $36.6 \pm 0.04\%$ oil contents with an average yield of $36.6 \pm 0.03\%$ by using hexane as solvent for extraction. Moreover, the oil contained polyunsaturated fatty acid with omega 3 fatty acid (alpha-linolenic acid) as a dominant content of the oil. In-vitro anticancer activity of flaxseed oil was observed by Cytotoxic (3T3 cell line) and Prostate Cancer (PC3 cell line) indicating that the oil possessed anticancer activity which was dose-dependent. On the basis of results it was concluded that

the flaxseed or its oil can be used for the management of cancer as a natural therapy by using on daily basis for a different types of cancer like breast and prostate.

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Conflict of interest

The authors have no conflict of interest.

Competing Interest

The authors declare that there is no conflict of interest.

Author Contributions

All authors have made a significant contribution to a journal article and share responsibility and accountability for the results. All authors have read and agreed to the published version of the manuscript.

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