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The Efficiency of Flaxseed Against Cancer and Bacterial Infection

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Abstract:
Flaxseed (Linum usitatissimum) has garnered attention for its potential health benefits , particularly its role in combating colon cancer and bacterial infections . This study aims to elucidate the mechanisms by which flaxseed exerts its effects and evaluate its overall efficacy. A comprehensive review of existing literature was conducted , alongside laboratory involving in vitro models . The anticancer effects were evaluated using human colon cancer cell lines, while antibacterial properties were tested against a spectrum of pathogenic bacteria. Flaxseed exhibited significant antiproliferative effects on colon cancer cells ,primarily through the induction of apoptosis and inhibition of cell cycle progression . Key bioactive compounds, such as lignans , omega-3fatty acids, and fiber, were identified as major contributors to these effects. In antibacterial assays, flaxseed demonstrated broad-spectrum activity, inhibiting the growth of several clinically relevant bacteria strains. The presence of lignans and omega-3fatty acids played a crucial role in disrupting bacterial cell walls and interfering with metabolic processes. The findings suggest that flaxseed holds substantial promise as a natural therapeutic agent against colon cancer and bacterial infections. Its dual action , mediated by a complex interplay of bioactive compounds , highlights its potential for inclusion in dietary strategies and development into functional foods or complementary therapies.

Keywords: Flaxseed, colon cancer, bacterial infections, lignans, omega-3fatty acids, natural therapeutics.

Introduction:

Flaxseed or linseed (*Linum usitatissimum*) is an ancient crop that has been used for food and fiber. In North America, flaxseed is the preferred term for flax used in human consumption whereas Europeans use the term linseed for edible flax (Vaisey-Genser and Morris, 2003). Historical records indicate that flaxseed dates back to around 9000–8000 BC in Turkey (van Zeiste, 1972), Iran (Helbaek, 1969), Jordon (Hopf 1983, Rollefson 1985), and a (Hillman 1975, Hillman 1989). Although the evidence does not clearly show that flaxseed was cultivated, the seeds have been found alongside domesticated wheat and barley (Zohary and Hopf, 2000).

Flaxseed is grown in approximately 50 countries most of which are in the Northern Hemisphere. In 2002, Canada was the largest producer of flaxseed accounting for approximately 33%, of the 2 million metric tons produced, followed by China (20%), the United States (16%), and India (11%) (Berglund, 2002). In general, the world supply of flaxseed has remained constant. Flaxseed acreage in the United States reached

516,000 harvested acres in 2004. Ninety-four percent of the flaxseed was grown in North Dakota followed by Montana (4%), and South Dakota (2%) (National Agricultural Statistics Service, 2005). Production in North Dakota totaled 10.5 million bushels and the yield per acre was 20.3 bushels (National Agricultural Statistics Service, 2005). The estimated harvest acreage for 2005 in North Dakota was approximately 955,000 acres (National Agricultural Statistics Service, 2006). Flaxseed acreage in the Canada totaled 1.9 million with a production of approximately 42 million bushels in 2004 (Agriculture and Agri-Food Canada, 2006).

History shows that flaxseed has been used as an ingredient in breakfast cereals and breads; however, since the 1990s, a number of products containing flaxseed have been developed primarily for the health food market. The renewed interest in flaxseed as a food source is due to findings that suggest that flaxseed can provide a variety of health benefits (Thompson and Cunnane, 2003). The components that contribute the health benefits include lignans (secoisolariciresinol diglucoside [SDG] being the predominant form), α-linolenic acid (ALA), and nonstarch polysaccharides (i.e., gum or fiber).

Flaxseed is an oilseed that contains roughly 38–45% oil. ALA, a polyunsaturated lipid, accounts for 52% of the fatty acids in the oil. Flaxseed is also a rich source of plant lignans (up to 13 mg/g flaxseed). The interest in ALA and lignans as food ingredients has opened opportunities for the utilization of flaxseed in foods. In contrast, the same level of interest has not been observed for other flaxseed components, such as protein and dietary fiber, which account for 20% and 28% of the flaxseed, respectively (Carter, 1993). This chapter will provid e a general overview of flaxseed research completed over the past 50 years with the major focus being on data from 1990 to 2006. It will highlight the basic composition, health benefits, and finally the processing and application of flaxseed. f flax (about 38% of total production) .

The important flaxseed growing countries include India, China, United States, and Ethiopia4-5,.In India flaxseed is mainly cultivated in Madhya Pradesh, Maharashtra, Chattisgarh and Bihar. It is interesting to know that flaxseed was native of India and was a staple food crop. In India, flaxseed is still being consumed as food and as well as for medicinal purposes . It enjoys a good status among oilseeds because of its versatile uses. It has emerged as an attractive nutritional food because of its exceptionally high content of alpha-linolenic acid (ALA), dietary fiber, high quality protein and phytoestrogens. Flaxseeds contain about 55% ALA, 28– 30% protein and 35% fiber.

Flaxseed is establishing importance in the world's food chain as a functional food. Functional food can be defined as the food or food ingredients that may provide physiological benefits and helps in preventing and/ or curing of diseases. Presently, flaxseed has new prospects as functional food because of consumer's growing interest for food with superb health benefits. Owing to its excellent nutritional profile and potential health benefits, it has become an attractive ingredient in the diets specially designed for specific health benefits. ALA is one of the essential polyunsaturated fatty acid and reported to exhibit anti-inflammatory, anti-thrombotic and anti-arrhythmic properties. Nutritionists all over the world suggest incorporation of omega 3 fatty acid sources in the diet. Flaxseed serves as the best omega-3 fatty acid source to the non-fish eaters. Edible flaxseed products include the whole flaxseed, ground meal and extracted oil or mucilage. The lignan content in flaxseed differs between varieties, but has also been shown to depend on growing location and year25-26. The basic structure of lignans consisting of two interlinked phenylpropanoid molecules, secoisolariciresinol diglucoside,a dibenzylbutane lignan, csesamin, a furanofuran lignan. Flaxseed is the richest source of plant lignans . Secoisolariciresinol diglycoside (SDG) is the predominant lignan in flaxseed with minor. Therefore, the seed may be an alternative for supplying this fatty acid to populations concentrated in regions of the world where there is

not large access to marine foods, which are the best sources of n-3 fatty acids. The tissue's fatty acid composition is not homogeneous. The linolenic acid contents in embryos, testa, and endosperm are all higher than that in the embryo axis. ALA is classified as an omega- 3 fatty acid, a group that also includes long-chain metabolites of ALA. Researchers are investigating whether omega-3 fatty acids contained in flaxseed may help protect against certain infections and in treating conditions including ulcers, migraine headaches, attention deficit/hyperactivity disorder, eating disorders, preterm labor, emphysema, psoriasis, glaucoma, Lyme disease, lupus, and panic attacks. Evaluated the anti-ulcer activity of the oil and mucilage obtained from flaxseed in a rat model of ethanol-induced gastric ulcer. Even if flaxseed oil was found to have a higher capacity in reducing the number of ulcers, both flaxseed oil and mucilage were pointed to provide a cytoprotective effect against ethanol-induced gastric ulcers in rats. Same results about antiulcer and anti-secretory properties of flaxseed oil were obtained . The oil also exhibited significant inhibitory effect on gastric secretion/total acidity and on aspirin-induced gastric ulceration in rats. The influence of flaxseed fatty acids on lupus nephritis disease. Plasma lipids and serum viscosity were unaltered by the flaxseed supplementation whereas serum creatinine in the compliant patients declined. Flaxseed appeared to be reno-protective in lupus nephritis, but authors suggested that their interpretation was affected by under powering due to poor adherence of patients. Investigated the anti-atherogenic capacity of flaxseed in an animal model that represents the human atherosclerotic condition. Supplementation of the cholesterol enriched diet with ground flaxseed lowered plasma cholesterol and saturated fatty acids, increased plasma content of ALA and inhibited plaque formation in the aorta and aortic sinus compared with mice fed a diet supplemented with only dietary cholesterol. Authors demonstrated that dietary flaxseed can inhibit atherosclerosis through a reduction of circulating cholesterol levels and, at a cellular level, via anti-proliferative and anti-inflammatory actions. Although direct studies on flaxseed and blood pressure are limited (and mostly confined to flaxseed oil versus ground flaxseed), numerous studies have shown the ability of increased omega-3 fatty acid intake to help regulate and reduce blood pressure in persons who have been diagnosed with hypertension. Furthermore, a diet low in saturated fats and rich in monounsaturated and polyunsaturated fats, including omega-3 fatty acids from flaxseed, can reduce heart disease. Preventing the occurrence of cardiovascular disease with nutritional interventions is a strategy that is widely focusing attention of researchers. Analysing epidemiological investigations and experimental studies suggested that ALA intake from flaxseed has been demonstrated to combat cardiovascular disease. Focused on flaxseed consumption and blood pressure in patients with hypertension. The objective was to examine whether flaxseed consumption altered plasma oxylipins in a manner that influenced blood pressure. After the clinical trial, authors concluded that α-linolenic acid in flaxseed may have inhibited soluble epoxide hydrolase, which altered oxylipin concentrations that contributed to the antihypertensive effects in Proteins in flaxseed and health benefits Like all vegetables, flaxseed proteins have techno-functional properties that affect their behaviour in a food system through interaction with other ingredients.

These properties are mainly dependent on their hydration mechanisms for solubility and water ⁄oil retention capacity. The amino acid pattern of flax protein is similar to that of soybean protein, which is viewed as one of the most nutritious of the plant proteins . Flaxseeds are not considered a source of complete proteins because the lack of some essential amino acids, necessarily introduced from the diet. Essential amino acids contained in flaxseed. Flaxseed grain and flaxseed paste contain about 21% and 34% protein respectively and may varies with the genetic and environmental factors. Cool climates usually result in high oil and low protein content in the seeds. Flaxseed has two major storage proteins, a

predominant salt soluble fraction with high molecular weight (11-12S; globulin; 18.6 % nitrogen) and a water soluble basic component with low molecular weight (1.6- 2S; albumin; 17.7 % nitrogen). Flaxseed has a favourable ratio of amino acids with Lysine, Threonine and Tyrosine as the limiting amino acids. Furthermore, it is a good source of sulfur amino acids (Metionine and Cysteine) and of branched chain amino acids (BCAA; Isoleucine, Leucine and Valine). Flaxseed is reach in essential amino acids of great importance in the synthesis of proteins that have the role of maintenance and repair of cells, tissues and organs. Carbohydrates in flaxseed and health benefits Flax is low in carbohydrates (sugars and starches), providing only 1 gram (g) per 100 g. For this reason, flax contributes little to total carbohydrate intake; it's recommended for people with specific disease. Flaxseed polysaccharide is composed of two major fractions: a neutral arabinoxylan (75%) and an acidic rhamnogalacturonan (25%). The arabinoxylan is composed mainly of xylose, arabinose, and galactose and the rhamnogalacturonan consists of L-rhamnose, D-galactose, D-galacturonic and L-fucose acid. Considerable and significant variations exist in monosaccharide composition, carbohydrate yield and quality among accessions from the world collection of flaxseed. Micronutrients in flaxseed and health benefits

Materials and Methods

SAMPLE COLLECTION

Flaxseed were collected from commercial store. The flaxseeds were dried in the Hot air oven 40 degree Celsius to 70 degree Celsius [104 degree to 158 degree] .Flaxseeds are first ground into a fine powder to increase the surface area for extraction.

PACKING

The powdered flaxseed is packed into a thimble which is then placed in the extraction chamber of the Soxhlet apparatus.

SOLVENT EXTRACTION

A suitable solvent, such as aqueous is heated in the round-bottom flask of the apparatus as the solvent vaporizes, it rises into the condenser. where it condenses and drips down onto the flaxseed sample. The condensed solvent dissolves the desired compounds from the flaxseed as it completed 5 cycles for 24 hours through the Soxhlet apparatus. This continuous extraction process helps ensure thorough extraction of the target compounds. The extracted solution collects in the round-bottom flask over time. Once a sufficient amount of extraction has occurred, the solution is removed from the flask and concentrated using techniques such as evaporation or rotary evaporation to yield the flaxseed extract.

QUALITATIVE ANALYSIS OF PHYTOCHEMICAL ANALYSIS

Steroids

An aliquot of the seed extract (1ml) was dissolved in 10 ml of chloroform and equal volume of concentrated sulfuric acid was added by sides of the test tube. The upper layer turns red and sulfuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids.

Terpenoids

An aliquot of the seed extract (2ml) was added to 2ml of acetic anhydride and concentrated H2SO4. The formations of blue green ring indicate the presence of terpenoids. Tannins: An aliquot of the seed extract

(2ml) was added to few drops of 1% lead acetate, and the yellowish precipitate indicated the presence of tannins

Saponins

An aliquot of the seed extract (5ml) was mixed with 20ml of distilled water and then agitated in agraduated cylinder for 15 minutes. Formation of foam indicates the presence of Saponins.

Anthocyanins

An aliquot of the seed extract (2ml) was added to 2ml of 2 NHCl and ammonia. The appearance of pink-red which turns to blue-violet indicates the presence of anthocyanins.

Glycosides

Concentrate H2SO4 Test:2ml. glacial acetic acid, one drop of5% FeCl3 and conc.H2SO4 were added into 5ml extract, the appearance of brown ring indicates the presence of glycosides.

Emodin

Two ml of NH4OH and 3 ml of Benzene were added to the extract. Appearance of red color indicates the presence of emodins .

Alkaloids [Mayer's test]

To the acidic solution, Mayer's regent (Potassiummercuric iodide solution) was added. Cream colored precipitate indicates the presence of alkaloids.

Phenols

Half ml of FeCl3 (w/v) solution was added into 2 ml of test solution, formation of an intense colour indicates the presence of phenols.

Flavonoids

An aliquot of the seed extract (2-3ml) and few drops of sodium hydroxide solution were added into a test tube. Formation of intense yellow color that became colour lesson addition of few drops of dilute HCl indicates the presence of flavonoids.

ANTIBACTERIAL ACTIVITY

Stock cultures were maintained at 4° C on slopes of nutrient agar and Muller Hinton agar. Active culture for experiments were prepared by transferring a loop full of cells from stock cultures to test tubes of 50ml nutrient broth bacterial cultures were incubated with agitation for 24 hours and at 37°c on shaking incubator and bacterial cultures were incubated at 37°c for 24 hrs. Each suspension of test organism was subsequently stroke out on nutrient agar media and Muller Hinton agar. A single colony was transferred to nutrient agar media slants were incubated at 37°c for 24 hours. These stock cultures were kept at 4°c. For use in experiments, a loop of each test organism was transferred into 50ml nutrient broth and incubated separately at 37°c for 18-20 hours for bacterial culture.

Well Diffusion method

The antibacterial activity of crude extract extracts was determined by Well Diffusion method (Bauer et al., 1996). MHA plates were prepared by pouring 20ml of molten media into sterile petri plates. After solidification of media, 20-25µl suspension of bacterial inoculums was swabbed uniformly. The sterile paper discs were dipped into required solvents then placed in agar plates. Then 10-50 µl of plant extract was poured into the wells. After that, the plates were incubated at 37°C for 24 hours. Assay was carried into triplicates and control plates were also maintained. Zone of inhibition was measured from the edge of the well to the zone in mm. The tested cell suspension was spread on Muller-Hinton agar plate. Well were put into the agar medium using sterile forceps. plant extract were poured on to wells. Then plates

were incubated at 37°c for about 24 hours and control was also maintained. Zone of inhibition was measured from the clear zone in mm.

Antibacterial activity was performed by agar diffusion method. Van der Watt et al., 2001. The stock culture of bacteria (*E.coli* and *Staphylococcus aureus*, *Salmonella typhi*) were received by inoculating in nutrient broth media and grown at 37 % for 18 hours. The agar plates of the above media were prepared. Each plates was inoculated with 18 hours old cultures the bacteria were swab in the sterile plates. Placed the extract treated cloth and untreated cloths were placed. All the plates were incubated at 37oC for 24 hours and the diameter of inhibition zone was noted in mm.

Agar well diffusion method has been used to determine the antimicrobial activities and minimum inhibitory concentrations or plant extracts against gram positive, gram negative bacteria. The extracts exhibited antibacterial activities against tested microorganisms.

FIGURE-1 FLAXSEED

FIGURE - 2 FLAXSEED POWDER

FIGURE - 3 FLAXSEED EXTRACT IN SOXHLET APPRATUS

FIGURE - 4 FLAXSEED EXTRACT STORED IN BROWN BOTTLE

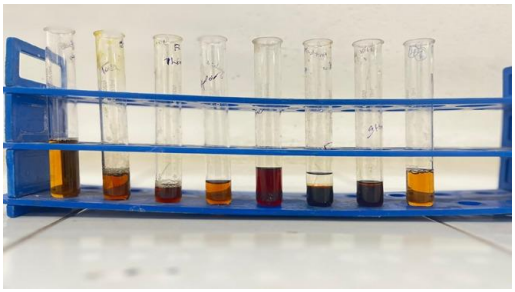


FIGURE - 5 PHYTOCHEMICAL ANALYSIS

PHYTOCHEMICAL ANALYSIS

TANNIN	(-)
SAPONIN	(-)
TERPENOIDS	(+)
ALKALOIDS	(-)
EMODINS	(+)
STEROIDS	(+)
GLYCOSIDES	(-)
PHENOLS	(+)
FLAVONOIDS	(+)

TABLE - 1

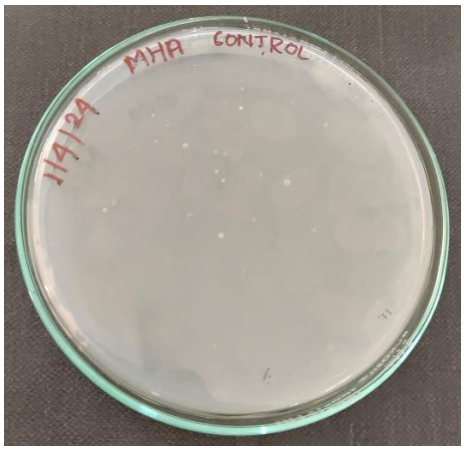


FIGURE - 6 CONTROL

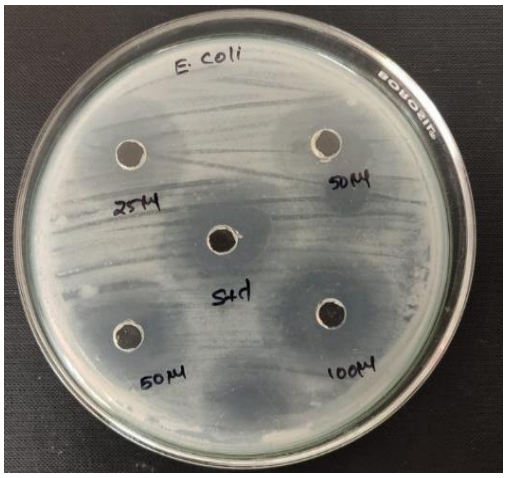


FIGURE - 7 ANTIBACTERIAL ACTIVITY AGAINST *E.coli*

With the results of the antibacterial activity of *E.coli*, on the comparative studies it is observed that in the 100µl of flaxseed solution the inhibitory activity is higher than the 50µl of flaxseed solution.

FIGURE - 8 ANTIBACTERIAL ACTIVITY AGAINST OF *Staphylococcus aureus*

With the results of the antibacterial activity of *Staphylococcus aureus*, On the comparative studies it is observed that on both the 75µl and 50µl of flaxseed solution possess the equal rate of inhibitory activity.

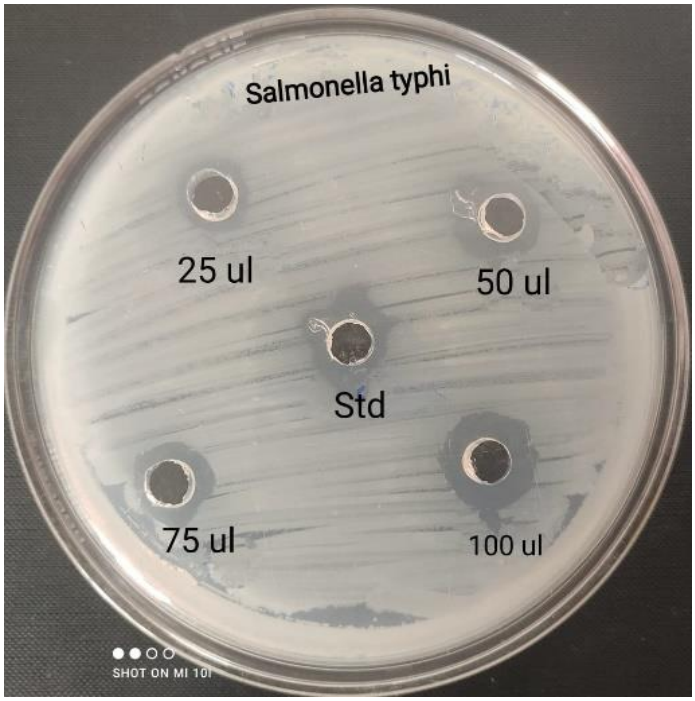


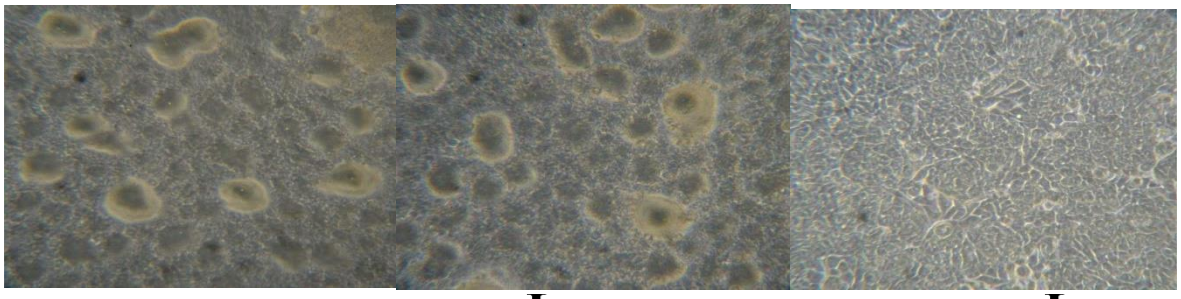
FIGURE - 9 ANTIBACTERIAL ACTIVITY AGAINST *Salmonella typhi*

With the results of the antibacterial activity of Salmonella typhi, on the comparative studies it is observed that on 100 µl of flaxseed solution the inhibitory activity is higher than the 75 µl of flaxseed solution.

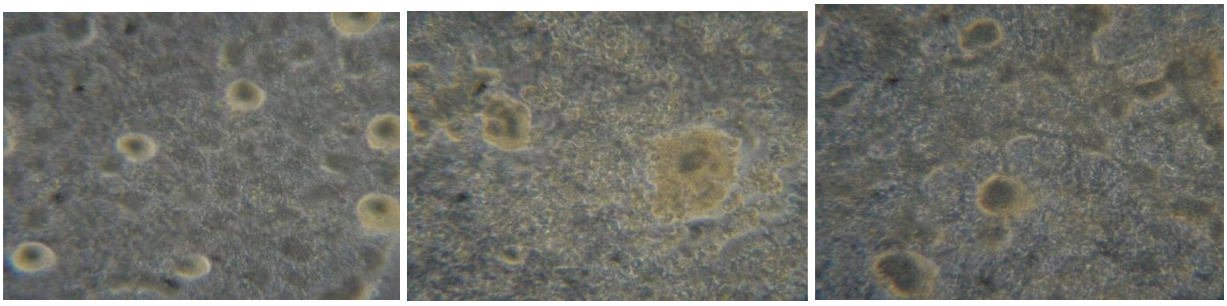
Organisms	***E.coli***	***Staphylococcus aureus***	***Salmonella typhi***
25 µl	0.5mm	0.7mm	0.1mm
50 µl	0.7mm	0.8mm	0.2mm
75 µl	0.8mm	0.9mm	0.3mm
100 µl	1.2mm	1.3mm	0.4mm
Standard (Bacteria-Chloramphenicol)	1.0mm	1.0mm	0.5mm

TABLE -2

COLON ANTICANCER ACTIVITY WITH IC50-MTT METHOD
CELL LINE: HT-29
SAMPLE PARTICULARS: FLAX SEED EXTRACT

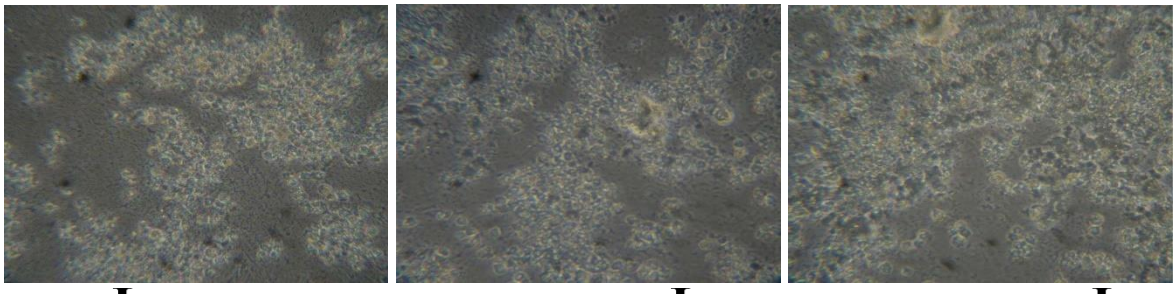


CONTROL **10 µL** **20µL**

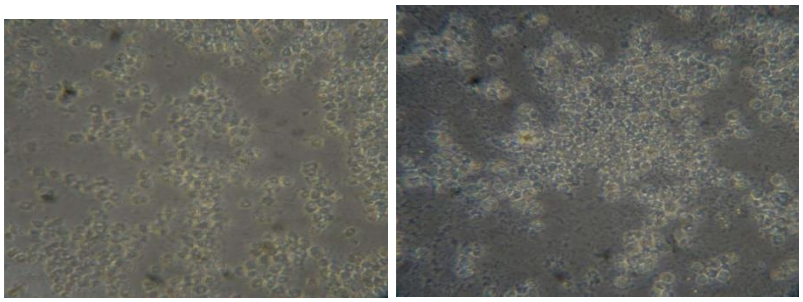


30µL **40µL** **50µL**

COLON ANTICANCER ACTIVITY WITH IC50-MTT METHOD
CELL LINE: HT-29
SAMPLE PARTICULARS: FLAX SEED EXTRACT



60µL **70µL** **80µL**



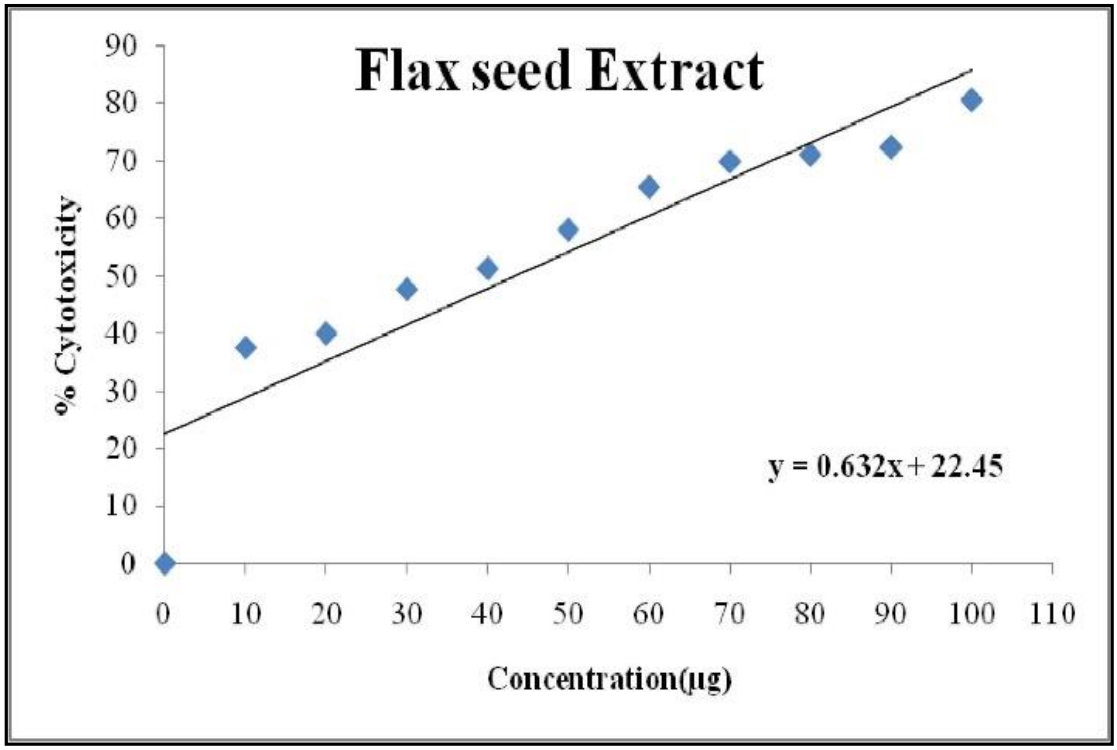
90µL 100µL

m- refers to the column
C- refers to the row
IC- refers to the Individual cell

in cell HT-29
M- Column (HT)
C- Row (29)
IC- Individual Cell (HT-29)

In this examination of flaxseed extract, at 100µl the cells began to break down due to their oily condition. Thus, at 100 µl it finally began to degrade, allowing us to begin consuming flaxseed in the precise amount that we needed each day.

m	0.632
C	22.45
IC50	(50-C)/m
	27.55
IC50	43.6



Discussion

The use of traditional treatments has received attention in recent years. The aim of the present work was to provide an overview of medicinal plants effective on colon cancer with special emphasis on bioactive components and underlying mechanism of action. The anti-cancer activity of flaxseed extract against colon cancer using HT-29 cell line, the given sample flaxseed extract showed mild to severe cytotoxicity HT-29 cells after 24 hrs . Green Tea extract polyphenols have shown inhibitory effects on colon cancer cell growth by modulating cell cycle progression and inducing apoptosis. Curcumin (Turmeric Extract) Curcumin has been reported to possess anti-inflammatory and anti-cancer properties by targeting multiple signalling pathways involved in colon cancer development and progression. Grape Seed Extract Rich in proanthocyanidins, grape seed extract has demonstrated anti-cancer effects by inhibiting cell proliferation and inducing apoptosis in colon cancer cells. Comparison and Insights Compared to these feed extracts, flaxseed extract stands out due to its multifaceted anti-cancer properties attributed to its unique composition of bioactive compounds. While each feed extract has shown promising anti-cancer effects against colon cancer, the mechanisms of action and efficacy can vary. While other seed feed extracts like green tea, curcumin and grapes seed extract have also shown anti-cancer effects , flaxseed's unique composition offers a comprehensive approach to colon cancer prevention. In fact, purification of herbal compounds and demonstration of their efficacy

Summary

The study aimed to investigate the anti-cancer activity of flaxseed, a natural source rich in lignans,omega-3 fatty acids, and dietary fiber. The results demonstrated that flaxseed extract exhibited significant anti-cancer cell lines, including breast, prostate, and colon cancer cells. The anti-cancer effects were attributed to the presence of bioactive compounds like lignans. The bioactive compounds present in flaxseed have shown promising results in inhibiting cancer cell growth, inducing apoptosis, and reducing tumor growth. These results imply that flaxseed may be a useful dietary supplement or medicinal medication for the preservation and treatment of cancer. The flaxseeds have preventive benefits against colon cancer due to its high concentration of bioactive compounds and the human colon adenocarcinoma derived HT-29 cell line have shown the effect on the apoptosis cell proliferation and cancer related molecular pathway. These results imply that flaxseed maybe useful as a dietary supplement or medication for the prevention and treatment of cancer.

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