Antioxidant Potential of Sesame (Sesamum Indicum) And Flax Seed (Linium Usitatissimum) And Assessement of Genoprotective Role of Sesame Seed Extract Against H_20_2 Induced Genotoxicity in Cultured Human Lymphocyte

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***Abstract*—Flaxseeds (*Linum usitatissimum*) belong to the family Linaceae. It is an annual or biennial plant and one of the most useful crops, playing an important role in human health due to its chemical composition. Sesame seeds (*Sesamum indicum*) belong to the family Pedaliaceae. It is one of the most ancient crops and is also known as benniseed. The color of sesame seeds varies from cream-white to charcoal black, with white and black being the typical seed colors. Both seeds were subjected to ethanol and aqueous extraction followed by phytochemical tests and antioxidant assays, which showed the presence of phytochemicals such as phenolics and flavonoids. DPPH, FRAP, TAC, phenolic, and flavonoid content assays showed the highest antioxidant potential for raw sesame aqueous extract, with values of 13.95±1.40, 0.95±0.014, 13.95±1.30, 579.61, and 737.21, respectively.**

Raw sesame, which showed the highest antioxidant property in all assays, was selected for the genotoxicity assay at two different concentrations (20 µg/mL and 100 µg/mL), and it showed a decrease in chromosomal damage when exposed to hydrogen peroxide compared to the control. The micronucleus frequency of the control was found to be 0.02, while the MN frequency of the extract at 20 µg/mL was 0.009 and at 100 µg/mL was 0.007.

I. INTRODUCTION

Free radicals play an important role in the etiology of cancer, cardiovascular disease, diabetes mellitus. Antioxidants scavenge free radicals and reactive oxygen species and they inhibit oxidative mechanisms that lead to degenerative diseases. They are not always harmful to the body, provided they are kept under adequate control. Many plants, including fruits and vegetables, are the natural antioxidants owing to the presence of phenolic and flavonoid compounds that will exert antioxidant capacity. Phenolic compounds such as flavonoids which have many benefits including antioxidant, antibacterial, and antidiabetic activities.

Flaxseeds (*L.usitatissimum)* belong to Linaceae family. It is an annual or biannual plant, and also one of the most useful crops, that has been cultivated as a commercial plant in all over the world. It has been cultivated for oil and fibre. Flaxseed is rich in fat, and it contain 30 to 40% of oil, 20 to 25% protein, 20 to 28% total dietary fibre, 4 to 8% moisture and 3 to 4% ash. The oil contains vitamins A, B, D and E, minerals and amino acids, and that may provide health benefits beyond basic nutrition.

Flaxseed is one of the key sources of phytochemicals such as phenolic acids, cinnamic acids, flavonoids and lignins which are antioxidants and affect the cell growth and viability. Daily consumption of flaxseed improves the glycaemic control in obese men and women with pre-diabetes. This is because of the protein content present in flaxseed which has anti-diabetic and antihypertensive activity.

Flaxseeds plays an important role in human health due to their chemical composition.

The antidiabetic effects of lignans and polyphenols found in flaxseed extract have been widely reported. Flaxseed consumption leads to favorable changes in body weight, food and water intake, glycosylated

hemoglobin, and blood glucose levels in diabetic individuals. It also contributes to a moderate reduction in glucose levels and hyperlipidemia, along with improvement in impaired organ function.

Sesame (*S. indicum*) belongs to the family Pedaliaceae and is one of the most ancient crops, also known as benniseed. The fiber content in sesame is associated with antidiabetic, antitumor, antiulcer, cancer-preventive, and cardioprotective properties. Sesame seed oil is rich in lignans and tocopherols and is well known for its beneficial effects on human health. Water-soluble fractions purified from defatted sesame seed flour also exhibit antioxidant activity in vitro

Sesame seeds are good sources of oil, protein, carbohydrates, and minerals for human nutrition. Colour of the sesame seed vary from cream-white to charcoal black, whereas white and black are the typical skin color. Black sesame seed is superior to white one. It has been reported that the seed colors of sesame affect the phytochemical contents and their biological activities too.

Genotoxicity is an important toxicological end point that is assessed adequately throughout the safety assessment process. genotoxicity of various chemical agents is assessed by in vitro or in vivo assays. In vitro tests are suitable for primary genotoxicity and in vivo provide information about secondary genotoxicity (inflammation, oxidative stress)

Variety of environmental and internal cellular factors challenges the genomic integrity of living cells. Environmental stresses, such as drought, salinity, ultraviolet (UV), ionizing radiation, xenobiotic toxicity, heavy metals, and mutagenic chemicals damages DNA and also affect its stability. DNA damage can also be caused by cellular respiration, recombination errors, and reactive oxygen species resulting as a byproduct of metabolism as DNA damage response (DDR) is a cells reaction to genotoxic stress starts with cell cycle arrest and, in the case of plants, endoreplication. several DNA repair mechanisms are active to ensure the repair of variety of different types of lesions and constitute the DNA repair phase of DDR.

With this background, our study aims to evaluate antioxidant potential of flax seeds and sesame seeds andassessment of Geno protective role of sesame seed extract against hydrogen peroxide induced genotoxicity in cultured human lymphocytes.

Scientific Classification

Figure 1 SESAME SEEDS

Kingdom: Plantae
Division: Angiosperms
Order:Lamiales
Family: Pedaliaceae
Genus: Sesamum
Species: indicum.

Scientific Classification

Figure 2 FLAX SEEDS

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Malpighiales
Family: Linaceae
Genus: Linum
Species: usitatissimum
Botanical Name: Linumusitatissimum

II. REVIEW OF LITERATURE

According to Rubeena M et al (2020) their study focused on the genotoxic efficiency of higher concentrations of methanolic extract of Cissus latifolia Lam. and also its Geno protective role at lower concentrations against the induced cytogenetic

aberrations by 2% hydrogen peroxide [H_2O_2] on the Allium cepa plant model. Results showed the toxic effect of extract of C. latifolia which induced major chromosomal abnormalities, these aberrations were found to be unaltered and showed the potent clastogenic nature of plant extract. As exposure time and concentration of the plant extract increased, the frequency of occurrence of cytogenetic aberrations also increased. Then, the potential genotoxicity of plant measured in terms of mitotic index reduction and also confirms its significant role in inducing mutagenicity.

Free radical production is a continuous process which occurs as part of normal cellular function, so the excessive production of these free radicals is strongly implicated in many diseases. The function of antioxidants is to prevent adverse effects caused by free radicals. The final result showed that Sesame seeds possess significant antioxidant activity and they also contain significant amounts of phenolics, flavonoids, nutrients and minerals and suggests that dietary uptake of sesame seeds could be potentially protective against diverse diseases (Emmanuel ElikemDravieet.al (2019).

According to Alachaher, F. Z., et.al (2018), flaxseed plays an important role in the field of diet and disease due to its potential health benefits. This study indicates the potential of flaxseed as antidiabetic and cardioprotective activity. The result showed that phytochemical analysis of flaxseed extracts exhibits the presence of flavonoids, phenolic compounds, glycosides, alkaloids, terpenoids, proteins and carbohydrates by simple qualitative phytochemical screening methods. Plant food contains a variety of biological activities, non-nutritive compounds called phytochemicals, which impart health benefits such as antioxidant activity, antimicrobial effect, and antidiabetic effect.

Sesame seed extracts possess antimicrobial, antimutagenic properties with prospective applications in the food preservative and nutraceutical industries. It showed that the sesame seeds exhibit a preventive role against the many disorders like atherosclerosis, low density lipoprotein oxidation, cancer, platelet aggregation and various cardiovascular diseases. And the higher oxidative stability of this seed reveals its possession of a high amount of antioxidants (Syed ammar Hussain et.al 2018).

The ethanol and aqueous extracts of flaxseeds (L. usitatissimum) exhibited potent in various types of phytochemicals. Plant extract is the best source of phytochemicals, and it is the natural medicine for many illnesses. phytochemicals are effective in combining or preventing disease due to their antioxidant property. The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites. Among these variety of secondary metabolites in flaxseeds: tannins and terpenoids are predominant antioxidant compounds present which have anti-inflammatory and hyperglycemic activities. In this study, it showed that, ten different chemical compounds have been identified from ethanol extract of flaxseeds. The presence of phytoactive compounds proves the use of flaxseeds (L. usitatissimum) for various disorders. Therefore, it is recommended as a source of phytochemical significance. (ThamilmaraiSelvi B, et.al 2018).

According to Lin Zhou et.al (2016) Sesame seeds are good sources of oil, protein, carbohydrates, and minerals for human nutrition. The common nutritional evaluations of sesame seeds are based on the contents of proteins, oils, and lignans. It showed thatthe sesame seeds, that contain free phenolic contents were higher than the bound phenolic contents. The antioxidant activities were associated with the contents of total phenolics and flavonoids. 179 The study was performed to investigate the in vitro antioxidant and cytoprotective effect of fermented sesame sauce against hydrogen peroxide induced oxidative damage in renal proximal tubule LLC-PK1 cells and it was found that the FSeS is an effective radical scavenger and protects against hydrogen peroxide induced oxidative damage in LLC-PK1 cells. (Jia-le-song 2014).

According to Rangarajan Narasimhan et.al (2012) the extract of Sesamum indicum reveals the presence of significant phytochemicals which have important therapeutic applications. Lignans and lignan glycosides present in sesame appear to be the important functional components. The main sesame lignans are sesamin and sesamolin which are found in sesame oil and the Carbohydrates in sesame seeds are composed of 3.2% glucose, 2.6% fructose and 0.2%

sucrose and the remaining quantities seem to be dietary fibers. And it showed that the presence of several secondary metabolites mainly, polyphenols, alkaloids, flavanoids, terpenoids, and glycosides. The chemical screening further showed the presence of various amino acids.

Antioxidants in the herbaceous plants can protect against DNA damage. This study evaluates the antioxidant substances, antioxidant activity, and the protection of DNA from oxidative damage in human lymphocytes which induced by hydrogen peroxide (H2O2). methods used acidic methanol and water extractions from six herbaceous plants, including Bidens alba (BA), Lyciumchinense (LC), Mentha arvensis (MA), Plantago asiatica (PA), Houttuynia cordata (HC), and Centella asiatica (CA). antioxidative capacities for protecting human lymphocyte DNA from H2O2-induced strand breaks was evaluated by comet assay and the study concluded that Quercetin and morin are important for preventing peroxidation and also oxidative damage to DNA, and the leaves of MA and HC extracts have excellent potential as functional ingredients representing potential sources of natural antioxidants. (Kuan-Hung Lin,et al,2013)

According to Yasir Hasan Siddique et.al (2009) Apigenin is a member of the flavone family of flavonoids and it possesses anti-inflammatory, free radical scavenging and also anti-carcinogenic properties. Hydrogen peroxide, generated during oxidative stress, is known to damage proteins, nucleic acids and cell membranes and also implicated in cancer, ageing and several chronic neurogenerative diseases. The present study focuses on the protective effect of apigenin against genotoxic doses of hydrogen peroxide (H2O2) using sister chromatid exchanges (SCEs) and cytokinesis blocked micronucleus (CBMN) assay. The results of the investigation conclude that apigenin reduce the genotoxic damage caused by H2O2 on cultured human peripheral blood lymphocytes.

III. OBJECTIVES

- To determine the presence of preliminary phytochemicals and antioxidant potential of aqueous and ethanol extracts of flax seed and sesame seed.
- To evaluate the genoprotective role of sesame seed against hydrogen peroxide induced genotoxicity in cultured human lymphocytes.

IV. MATERIALS AND METHODS

Chemicals and reagents: Acetic anhydride, Conc. sulphuric acid, glacial acetic acid, ferric chloride, bromine water, sodium bicarbonate, Hager's reagent, Wagner's reagent, sodium hydroxide, dilute hydrochloric acid, lead acetate (10%), con hydrochloric acid, magnesium ribbon, gelatin, 0.1% copper sulphate, 0.2% Ninhydrin, benedict's reagent, DNPH, acetone. DPPH solution, 99% ethanol, ascorbic acid, potassium hexacyanoferrate, TCA, ferric chloride, Porcine pancreatic α-amylase (PPA), 3,5-dinitrosalicylic acid (DNS colour reagent), potassium phosphate buffer solution (PBS), p-Nitrophenyl α-D glucopyranoside (pNPG), α-glucosidase, and acarbose were purchased, soluble starch potato, sodium potassium tartrate, sodium chloride, disodium hydrogen phosphate and sodium hydroxide.

Sample Preparation –
Aqueous extract: 50g of ground sample (sesame and flax seeds) was soaked in 500mL distilled water. Then the mixture was filtered through Whatman filter paper. The filtrate was evaporated to dryness using rotary flash evaporator and the resulting extract was stored in refrigerator until further analysis.

Ethanol extraction (Soxhlet extraction): The raw and roasted sesame and flax seeds powdered using mixer grinder, further subjected to soxhlet extraction with 99% ethanol for 48 hrs. The resulted mixture was evaporated to dryness using a rotary flash evaporator and the condensed extract was stored in refrigerator which was then used for further analysis.

Phytochemical analysis of plant extract (seeds): -
The aqueous and ethanol extract of *L.usitatissimum*and *S.indicum*was subjected to phytochemical analysis.
The phytochemical analysis, 2.5mg of sample was diluted with 25 ml distilled water.
PHYTOCHEMICAL TEST & METHOD
1)Test for Steroids and Triterpenoids –

Liebermann Burchard Test: -
Crude extract was mixed with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was then added from the sides of the test tube and observed for the formation of a brown ring at the junction of two layers. Green coloration of the upper layer and the formation of deep red color in the lower layer would indicate a positive test for steroids and triterpenoids respectively.

2)Test for Glycosides –
Keller Killiani Test: -
Test solution was treated with few drops of glacial acetic acid and Ferric chloride solution and mixed. Concentrated sulphuric acid was added, and observed for the formation of two layers. Lower reddish-brown layer and upper acetic acid layer which turns bluish green would indicate a positive test for glycosides.

Bromine water Test: -
Test solution was dissolved in bromine water and observed for the formation of yellow precipitate to show a positive result for the presence of glycosides.

3)Test for Saponins –
Foam Test:
Test solution was mixed with water and shaken and observed for the formation of froth, which is stable for 15 minutes for a positive result.

Froth Test:
To 5 mL of extract of the drug added a drop of sodium bicarbonate solution. Shaken the mixture vigorously and left for 3 minutes. Honey comb like froth is formed.

4)Test for Alkaloids –
Hager's Test:
Test solution was treated with few drops of Hager's reagent (saturated picric acid solution). Formation of yellow precipitate would show a positive result for the presence of alkaloids.

Wagner's Test (Iodine in Potassium iodide): -
The acid layer was treated with few drops of Wagner's reagent. Formation of reddish-brown precipitate indicates the presence of alkaloids.
5)Test for Flavonoids –
Ferric chloride Test: -
Test solution when treated with few drops of Ferric chloride solution would result in the formation of blackish red color indicating the presence of flavonoids.

Alkaline reagent Test: -
Test solution when treated with sodium hydroxide solution, shows increase in the intensity of yellow color which would become colorless on addition of few drops of dilute Hydrochloric acid, indicates the presence of flavonoids.

Lead acetate solution Test: -
Test solution when treated with few drops of lead acetate (10%) solution would result in the formation of yellow precipitate.

Shinoda test: -
To the alcoholic solution of extract a few fragments of magnesium ribbon and concentrated hydrochloric acid was added. Appearance of red to pink color after few minutes indicates the presence of Flavonoids.

6)Test for Tannins –
Gelatin Test: -
Test solution when treated with gelatin solution would give white precipitate indicating the presence of tannins.

7)Test for Proteins –
Biuret Test: -
Test solution was treated with 10% sodium hydroxide solution and two drops of 0.1% copper sulphate solution and observed for the formation of violet/pink color.

8)Test for Free Amino Acids –
Ninhydrin Test: -
Test solution when boiled with 0.2% solution of Ninhydrin, would result in the formation of purple color suggesting the presence of free amino acids.

9)Test for Carbohydrates –
Benedict's Test: -
Test solution was mixed with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) and boiled in water bath, observed for the formation of reddish-brown precipitate to show a positive result for the presence of carbohydrate.

10)Test for Resins
The extract was dissolved in acetone and then was added to distilled water. Turbidity indicates the presence of resins.

Assessment of Invitro antioxidant activity of sesame and flax seed extract: -
The invitro antioxidant potential of Linum usitatissimum and Sesamum indicum aqueous and ethanol extract was assessed using DPPH radical scavenging, Ferric reducing antioxidant power, total antioxidant capacity assays, Total phenolic by FC method and Total flavonoid content.

Total Phenolics by FC method: -
The experiment was performed to determine the total phenolic content of the extracts. Different concentrations of the standard and sample solutions were taken and the volume of each is made up to 1ml with distilled water. 2.5ml of freshly prepared folin-ciocalteu reagent, 1.5ml of sodium carbonate and 2.8ml of distilled water was added to all the test tubes. Control tube is maintained without adding the extract. The reaction mixture was incubated in dark at 37ºC for 90 minutes and the optical density was measured at 765nm against an appropriate blank solution. Gallic acid was used as reference standard. All tests were performed in duplicates. (Dr. Suchethakumari. N – 2010)

Total Flavonoid Content: -
To determine the total flavonoid content of the extracts, this experiment was performed. Different concentrations of the standard and sample solutions were taken and the volume of each is made up to 1ml with methanol. 0.1ml of freshly prepared aluminum chloride, 0.1ml of potassium acetate and 2.8ml of distilled water was added to all the test tubes. Control tube is maintained without adding the extract. The reaction mixture was incubated in dark at 37ºC for 30 minutes and the optical density was read at 435nm against an appropriate blank solution. Quercetin was used as reference standard. All tests were performed in duplicates. (Dr. Suchethakumari. N – 2010)

DPPH Radical Scavenging assay: -
The free radical scavenging capacity of sesame and flax seeds was determined using DPPH assay. Freshly prepared DPPH solution (0.004% w/v) in 95% ethanol was added to test solutions (0-100µg/ml) and then incubated at room temperature in dark for 20 minutes. Following incubation, the mixture was vortexed and the optical density was measured at 517nm using a spectrophotometer. 99% ethanol was used as blank. Control sample was prepared containing the same volume without any extract. Ascorbic acid (AA) was used as a standard. All tests were performed in duplicates. Percentage scavenging of the DPPH free radical was calculated using the following equation (Dr.Suchethakumari.N – 2010)

DPPH radical scavenging activity (%) = (A control – A test)/A control X 100

Where ‘A’ control indicated the absorbance of the control reaction and ‘A’ test indicated the absorbance in the presence of the extracts or standard. Lower absorbance of the reaction mixture suggested higher free radical scavenging activity.

Total Antioxidant capacity: -
The total antioxidant capacity of plant extract was determined using Phosphomolybdenum method. Different concentrations of the sample solutions (0-100µg/ml) were taken and the volume of each is made up to 2ml with distilled water. Trichloro acetic acid (0.5mL) reagent was added to all the tubes to precipitate out the proteins if any. The mixture was then allowed to stand for about 5 minutes. 100µL of the clear supernatant was transferred into a clean test tube to which 1mL of total antioxidant capacity (TAC) reagent was added and the mixture was incubated in water bath at 90ºC for 90 minutes. After incubation, the mixture was cooled, which give greenish to blue color and the optical density was read at 695nm. Ascorbic acid was used as reference standard. All the test tubes were performed in duplicates. Increased absorbance of the reaction mixture indicated the increased total antioxidant capacity. (Dr.Suchethakumari.N – 2010)

Ferric reducing antioxidant power assay: -
This experiment was performed to determine the Fe3+ reducing power of the ethanol and aqueous extracts. Different concentrations of the sample solutions were taken and the volume of each is made up to 1ml with distilled water. 1% of 0.5mL of potassium hexacyanoferrate was added and incubated at 50ºC in

water bath for 20 minutes. Control tube was maintained without adding the extract. After incubation, 0.5mL of 10% TCA was added to terminate the reaction. The upper portion of the solution was mixed with 1mL of distilled water and 0.1mL of ferric chloride solution (0.1%) was added. The reaction mixture was left for 10 minutes at room temperature and the optical density was measured at 700nm against an appropriate blank solution. Ascorbic acid was used as a reference standard. All tests are performed in duplicates. Increased absorbance of the reaction mixture indicated stronger reducing power. (Dr.Suchethakumari.N – 2010)

MICRONUCLEUS ASSAY

1. Isolation of lymphocytes

To isolate the lymphocytes, 6 ml of blood was added to 6 ml of histopaque and centrifuged at 2000 rpm for 10 minutes. White buffy coat at the junction of RBC and Histopaque was isolated. 1–2 ml of PBS was added and centrifuged at 2000 rpm for 10 minutes. (Samples – RWSA (20 and 100µg/ml) and RWSE (20 and 100 µg/ml))

2.Treatment with Hydrogen peroxide

Cells were centrifuged at 1000 rpm for 10 minutes and 200 mM H_2O_2 was added, incubated for appropriate time in the incubator and again centrifuged at 1000 rpm for 10 minutes.

3.Culture initiation

To 0.5 ml of cell suspension, 4.5 ml of RPMI media with FBS and 150 µl of PHA was added and kept in the incubator for 44 hours. After 44 hours of incubation 300 µl of Cytochalasin B was added to the culture.

4.Harvest

Cells from the culture tubes were transferred to the falkan tube and centrifuged at 800 rpm for 10 minutes. 5 ml of hypotonic solution was added to the pellet and again centrifuged, and the pellet was resuspended in 5 ml of fixative (1:5 Acetic acid: methanol).

5.Slide preparation

Washed the suspension with fixative and pellet was suspended with appropriate volume of fixative. Sample was dropped with small height and kept for airdry at overnight.

6.Staining

Stain was prepared by adding 4 ml of giemsa, 4 ml of NaH_2PO_4 and 4 ml of Na_2HPO_4. And the volume was made upto 50 ml and filtered. The slide was stained for 10 min and destained with distilled water for 45 seconds at coupling jar. Airdried and observed under microscope.

MN frequency was calculated by formula,

MN frequency = no of MN cells counted / no of BN cells

V. STATISTICAL ANALYSIS:

The statistical analysis was done using prism software. The retention for statistical significance was set at $p < 0.05$. The results were analysed using ANOVA and for multiple comparison between different extract (raw and roasted) Tukey's multiple comparison test was used.

VI. RESULTS AND DISCUSSION: -

Phytochemical analysis

The aqueous and ethanolic extracts of S indicum and L usitatissimum revealed the presence of glycosides, phenolics and flavonoids, aminoacids, alkaloids, saponins and steroids. Results of preliminary phytochemical screening are shown in table 2 a and b.

a. sesame and flax seed aqueous extract

Table 1a: Phytochemical analysis sesame and flax seed aqueous extract

Test	Raw sesame aqueous	Raw flax aqueous	Roasted sesame aqueous	Roasted flax aqueous
Test for Steroids	-	-	+	-
Test for glycosides	+	+	+	-

Test	Raw sesame aqueous	Raw flax aqueous	Roasted sesame aqueous	Roasted flax aqueous
Test for Saponins	-	-	+	-
Test for Alkaloids	-	-	-	-
Test for Flavonoids	+	+	+	+
Test for tannins	-	-	-	-
Test for Proteins	+	+	+	-
Test for free Amino acids	+	+	-	-
Test for Carbohydrate	-	-	-	-
Test for Resins	-	-	-	-

b. sesame and flax seed ethanol extract

Table 1 b: Phytochemical analysis ofsesame and flax seed ethanol extract

Test	Raw sesame ethanol	Raw flax ethanol	Roasted sesame ethanol	Roasted flax ethanol
Test for Steroids	+	+	+	+
Test for glycosides	+	+	+	+
Test for Saponins	+	+	+	+
Test for Alkaloids	+	+	+	+
Test for Flavonoids	+	+	+	+
Test for tannins	+	+	+	+
Test for Proteins	-	-	-	-
Test for free Amino acids	-	-	-	-
Test for Carbohydrate	-	-	-	-
Test for Resins	-	-	+	+

RWSA – raw sesame aqueous
RWFA-raw flax aqueous
RWSE- raw sesame ethanolRWFE-raw flax ethanol
RSA-roasted sesame aqueous
RFA-roasted flax aqueous
RSE-roasted sesame ethanol
RFE-roasted flax ethanol

1.Total phenolic content: -
The total phenolic content of the extract was determined by Folin-Ciocalteu reagent method and measured in terms of GAE and the concentration ranges from 0 to 100 µg/mL.Raw sesame aqueous extractshowed that the maximum phenolic content which was found to be 579.61µg gallic acidequivalents /mg of extract equivalents at a concentration of 100 µg/mL.
The total phenolic content of all the extracts is given below,

Table 2 phenolic of extract Total content

Samples at 100 µg/mL	Standard (Gallic acid)	RWSA	RWFA	RSA	RFA	RWSE	RWFE	RSE	RFE
Gallic acid equivalence	831.46	579.61	325.18	267.15	257.11	295.95	278.11	395.77	198.55

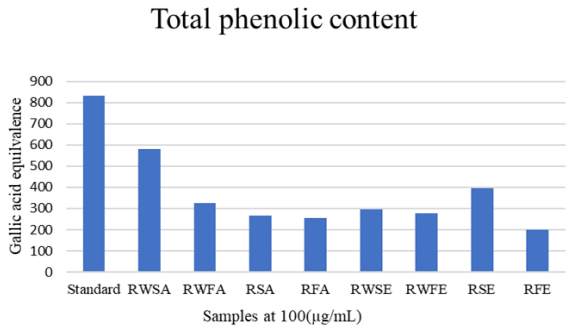

Figure 3: The total phenolic content of sesame and flax seed in both aqueous and ethanol extract. Data are represented as gallic acid equivalence.

2.Total flavonoid content: -

The total flavonoid content measured in terms of QE and concentration ranges from 0 to 100 µg/mL. In total flavonoid content it showed the maximum flavonoid content in Raw sesame aqueous extract (737.2 µg quercetin equivalents/mg of extract) at a concentration of 100 µg/mL, as well as minimum in Raw sesame ethanol extract (252.744 µg quercetin equivalents/mg of extract) at a concentration of 100 µg/mL. hence the aqueous extract showed high value total flavonoid content than the ethanol extract and the better antioxidant activity than the other extracts.

Table 3 Total flavonoid content of extracts

Sample at 100µg/mL	Standard (Quercetin)	RWSA	RWFA	RSA	RFA	RWSE	RWFE	RSE	RFE
Quercetin equivalence	930.51	737.21	470.51	396.52	258.41	252.74	390.47	328.54	552.51

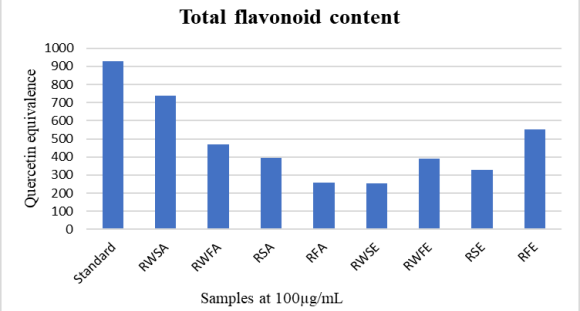

Figure 4. The total flavonoid content of sesame and flax seed in both aqueous and ethanol extract. Data are represented as quercetin equivalence.

3.DPPH Radical Scavenging assay: -

DPPH radical scavenging effect of the extracts at different concentrations is summarized in figure 1 and the percentage inhibition of each extract are given in table 5. It showed that maximum radical scavenging activity for Raw sesame aqueous extract which was found to be 33.12±1.40 and it was exhibited at 100 µg/mL concentration. Overall, the aqueous extract showed higher scavenging ability compared to ethanol extract. The extracts RWSA vs RSA, RWSA vs RWFA, RWSA vs RFA are statistically significant ($p < 0.01$). The aqueous extracts, RSA vs RWFA, RSA vs RFA, RWFA vs RFA are not statistically significant ($p > 0.05$). The ethanol extracts, RWSE vs RFE, RSE vs RFE, RWFE vs RFE showed statistically significant difference ($p < 0.001$). No statistical differences were observed in the extracts, RWSE vs RSE, RWSE vs RWFE, RSE vs RWFE ($p > 0.05$).

Table 4 Radical scavenging activity of extracts

Samples at 100µg/mL	% DPPH radical scavenging potential
Standard (ascorbic acid)	25.56±0.62
RWSA	33.12±1.40
RWFA	22.15±0.77
RSA	22.15±0.71
RFA	25.67±0.71
RWSE	21.95±0.71
RWFE	21.17±0.24
RSE	21.73±0.39
RFE	7.779±0.70

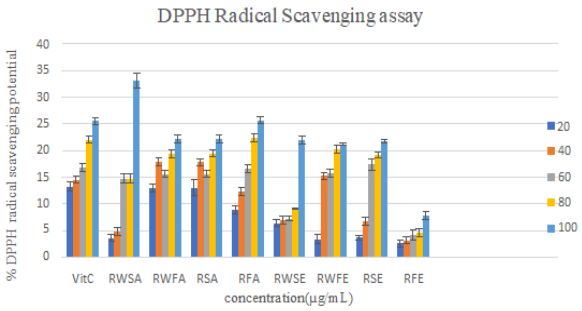

Figure 5. DPPH radical scavenging potential of sesame and flax seed in both aqueous and ethanol extract. Data are represented as mean ± standard deviation

4.Total antioxidant capacity

Total antioxidant effect of extracts at different concentrations is summarized in figure 3 and the Ascorbic acid efficiency of each extract given in the table 6. It showed that maximum antioxidant capacity for Raw sesame aqueous which was found to be 13.95±1.30 and exhibited at the concentration of 100 µg/ml. Overall, aqueous extract showed higher antioxidant capacity compared to ethanol extract.

The extracts RFE vs RWSE, RFE vs RSE, RSE vs RWSE, RSE vs RWFE and RWFE vs RWSE showed no statistical significance ($p > 0.05$). The aqueous extracts, RFA vs RWSA, RFA vs RSA, RSA vs RWSA, RSA vs RWFA and RWFA vs RWSA showed no statistical significance.

Table 5 Total antioxidant capacity of extracts

Samples at 100µg/mL	Ascorbic acid efficiency
Standard (ascorbic acid)	94.71±1.34
RWSA	13.95±1.30
RWFA	9.98±1.25
RSA	11.00±1.22
RFA	12.13±1.21
RWSE	3.81±0.24
RWFE	3.65±0.29
RSE	3.63±0.34
RFE	3.03±0.28

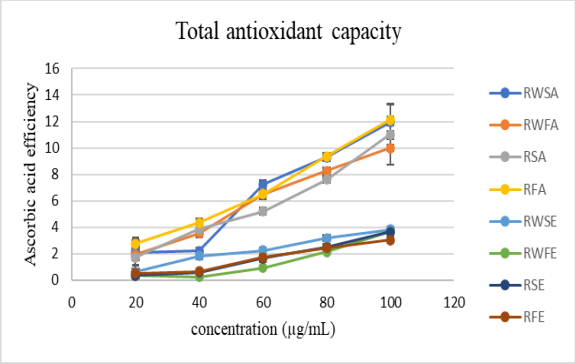

Figure 6. Total antioxidant capacity of sesame and flax seed in both in both aqueous and ethanol extract. Data are represented as mean ±standard deviation

5.Ferric reducing antioxidant power assay

Ferric reducing power of the extracts at different concentration was shown in figure 2. And the ferric reducing power of each extract given in table 7. It showed that highest ferric reducing antioxidant power for Raw sesame aqueous which was found to be 0.95±0.014 exhibited at the concentration of 100 µg/mL. Overall, aqueous extract showed higher antioxidant power compared to ethanol extract. The extracts RWSE vs RSE, RWSE vs RWFE, RSE vs RFE, RWFE vs RFE showed statistically significant difference ($p < 0.001$). The extracts RSE vs RFE, RWFE vs RFE showed no statistically significant ($p > 0.05$). The aqueous extracts of RWSA vs RSA, RWSA vs RWFA, RSA vs RFA, RWFA vs RFA showed statistically significant ($p < 0.001$). The extracts RSA vs RFA, RWFA vs RFA showed no statistically significant ($p > 0.05$).

Table 6 Ferric reducing capacity of extracts

Samples at 100µg/mL	Ferric reducing capacity
Standard (ascorbic acid)	0.75±0.035
RWSA	0.95±0.014
RWFA	0.91±0.007
RSA	0.88±0.001
RFA	0.88±0.007
RWSE	0.36±0.002
RWFE	0.40±0.002
RSE	0.38±0.01
RFE	0.35±0.007

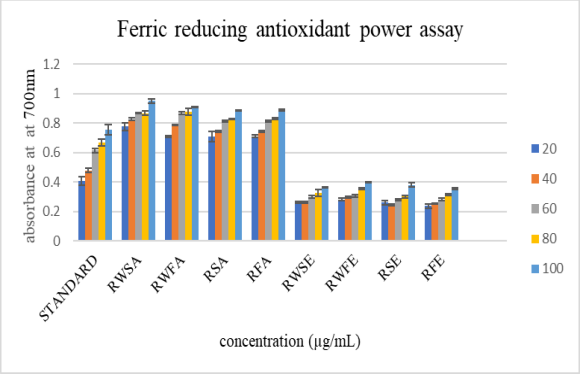

Figure 7 Ferric reducing capacity of sesame and flax seed in both in both aqueous and 556 ethanol extract. Data are represented as mean ±standard deviation

MICRONUCLEUS ASSAY

Table 7 Micronucleus frequency

No of trials	positive control	RWSA (20µg)	RWSA (100µg)
1	0.02	0.009	0.01

2	0.03	0.008	0.006
3	0.02	0.01	0.007
MEAN	0.02	0.009	0.007
STDV	0.005	0.001	0.002

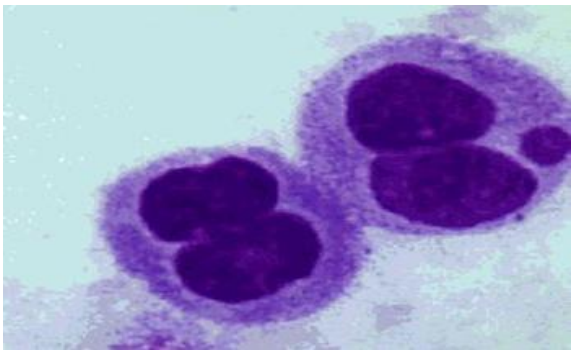

Figure 8 *Binucleated cell with micronucleus*

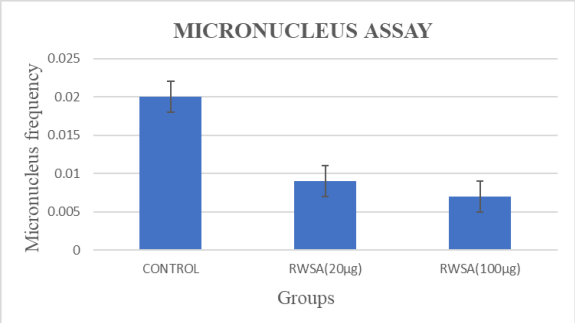

Figure 9

Micronucleus frequencies in various groups, Represents the P- value < 0.05 According to Figure a significant increase in the micronucleus frequency was observed in the Positive control which was shows increased damage due to the exposure to hydrogen peroxide. In the treatment groups, a decrease in the same was observed, but the results were not statistically significant.

VII. DISCUSSION

According to this study, the extract RWSA showed high antioxidant property which was chosen for the DNA damage studies in two different concentrations (20 and 100 µg/mL). The results were statistically significant in the positive control group because of the increase in the micronucleus frequency. The treatment with different extract showed a decreasing micronucleus frequency but of not statistically significant. The studies showed that the plant extracts successfully protected the DNA from damage caused by free radicals. overall methanol and aqueous methanol extracts showed great promise and should therefore be used further for fractionation and isolation of pure compound responsible for antioxidant activities. (vinaykumar et.al 2013). Noroozi et.al showed that, in addition to quercetin, kaempferol could also inhibits H2O2-induced DNA strand breaks in human lymphocytes. According to Zhu and Lofueous, the extracts of cooked and autolysed Brusselssprouts decreased DNA strand breaks in human lymphocytes, with the maximum inhibition of 38 and 39% at cooked and autolysed extract levels of 10 µg/mL and 5 µg/mL, respectively, with the inhibition effect decreasing at increasing concentrations up to 100 µg/mL. Javad Behravan et.al demonstrated that all concentrations of hydrogen peroxide (50, 100, and 200 µM) caused dose-dependent damage to DNA of lymphocytes. They also reported that neither aqueous nor ethanolic extracts of P. oleracea caused DNA damage in human lymphocytes, which was supported by other studies examining the genotoxic activity of P. oleracea extract. The aqueous extract of P. oleracea displayed protective activities against H2O2-induced chromosomal damage, while the ethanolic extract did not.

In my study, treatment with different extracts with two different concentrations [20 and 100 µg/mL] showed no statistical significance. Hence, we can say that these extracts have antioxidant properties and has genoprotective role but of no statistically significant and further studies have to be carried out with different concentration to of the extracts to know the further potential of extracts in decreasing damage due to the exposure to hydrogen peroxide.

VIII. CONCLUSION

This study suggests that sesame and flax seeds possess significant antioxidant activity. They also contain significant amounts of phenolics, flavonoids, nutrients and minerals. This suggests that dietary uptake of sesame and flax seeds could be potentially protective against diverse disease. The sesame and flax seed have tremendous potential to be used as human food, animal feed, and good quality fiber. This study showed that Raw sesame aqueous exhibit high antioxidant property at 100 µg/ml in DPPH, FRAP, TAC, Total flavonoid content and total phenolic content assays. DPPH, FRAP, TAC, phenolic and flavonoid content assays

showed the highest antioxidant potential for Raw sesame aqueous which was found to be 13.95±1.40, 0.95±0.014, 13.95±1.30, 579.61, 737.21 respectively. From the above discussion, RWSA showed high antioxidant properties in the assays which was chosen for DNA damage studies at two different concentrations [20 and 100 µg/mL]. Significant increase in the Micronucleus frequency in control indicates the increased Chromosomal damage, which may be due to the exposure of Hydrogen peroxide to human lymphocytes. The treatment with the extracts showed a decreasing micronucleus frequency but of not statistically significant. Micronucleus frequency of control was found to be 0.02, MN frequency of extract at 20 µg/mL was found to be 0.009 and 100 µg/mL was found to be 0.007. Thus, we can conclude that further extensive studies have to be carried out using different concentrations of the extracts to know the further potential of extracts in decreasing damage due to the exposure to hydrogen peroxide.

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