# PHYTOCHEMICAL EVALUATION AND IN-VITRO ANTIOXIDANT, ANTI-INFLAMMATORY, AND ANTI-DIABETIC ACTIVITY ASSESSMENT OF *LINUM USITATISSIMUM* L. SEED EXTRACT

## ABSTRACT

**Objectives**: *Linum usitatisimum* (L.) is a rich source of different types of phytochemicals and possesses nutritive and therapeutic value. The study was carried out to evaluate phytochemicals, antioxidant, anti-inflammatory and anti-diabetic of flaxseed extract.
**Methods**: The seed was defatted using hexane and defatted powder was then extracted using 80% ethanol. Phytochemical evaluation was performed using standard screening method and Fourier transform infrared (FTIR) spectroscopy. The total phenolic content was determined using Folin-ciocalteu method. The antioxidant activity of extract was determined by free radical scavenging activity using 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. The anti-inflammatory activity was determined by human RBC membrane stabilization assay. The anti-diabetic activity of extract was determined by α-amylase and α-glucosidase enzyme inhibition assay.
**Results**: Qualitative phytochemical analysis of ethanolic extract confirmed the presence of alkaloids, phenols, flavonoids, tannin, phytosterols, carbohydrates, glycosides. FTIR analysis of the extract demonstrated presence of compounds phenols, flavonoids and tannins. The total phenolic content was found to be 90.98 mg Gallic acid equivalent / g of dry extract. The median inhibitory concentration (IC50) value of ethanol extract for anti-oxidant activity was found to be 297.39 µg/ml. The IC50 values of the extract in RBC membrane stabilization assay was found to 197.312 µg/ml. The results obtained demonstrate that extract can dose dependently inhibit RBC hemolysis. The IC50 value of extract against amylase was found to be 8.54 mg/ml. The plant extract showed an inhibition of 31.52 % at concentration of 10 mg/ml against alpha-glucosidase.
**Conclusion**: These results suggest that flaxseed has promising antioxidant, anti-inflammatory and antidiabetic properties, making it a valuable candidate for further research and therapeutic applications.


**Keywords:** *Linum Usitatissimum* L., Ethanolic Extract, Phytochemical Screening, Antioxidant activity, Anti-Inflammatory activity, Anti-diabetic activity.

## 1. INTRODUCTION

Since ancient times, herbs and spices have been used for the treatment of various ailments. Modern medicines are discovered and developed using phytochemicals that are extracted from plant.[1]It is estimated that more than 25% of modern medicines are derived directly or indirectly from plants.[2,3] Due to their pharmacological activity, low toxicity, and cost-effectiveness when compared to synthetic drugs, medicinal plants are becoming more and more popular worldwide as a viable option for treating chronic illnesses. Diabetes mellitus is a growing global health concern, affecting over 536 million people worldwide and projected to affect 643 million by 2030 and 783 million by 2045. [4] It is a chronic disease characterized by hyperglycemia and carbohydrate, protein, fat metabolism disturbances caused either due to the insufficient production of insulin or body's inability to use insulin effectively. Medicinal plants remain vital for diabetes treatment, especially in developing countries where majority of people lack access to modern medicines and have limited financial resources. Anti-diabetic medications like acarbose and voglibose come with gastrointestinal side effects highlighting the need for safer and more effective alternatives. One promising therapeutic approach involves inhibiting α-amylase and α-glucosidase, two enzymes responsible for carbohydrate metabolism, for managing blood glucose levels in individuals with type 2 diabetes. Inflammation is a biological response of body tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. However, chronic inflammation can lead to tissue damage. Conventional anti-inflammatory drugs, like steroids and NSAIDs, have side effects which has led to growing interest in medicinal plants with anti-inflammatory properties, which may not only suppress inflammation but also address various diseases where inflammation exacerbates the condition.

*Linum usitatissimum*, commonly known as flaxseed or linseed, is a valuable herb belonging to family Linaeceae. It is an annual herb and may grow to heights of 60-120 cm. The fruits are capsular that consist of five cells with a pair of seeds in each. The seed are ovate, flattened and obliquely pointed at one end, about 4-6 mm long and 2-2.5 mm broad. Flaxseed consists of bioactive compounds like alkaloids, flavonoids, polyphenols, tannins, lignans (notably secoisolariciresinol diglucoside), omega-3 fatty acids, alpha-linolenic acid, dietary fibers, sterols, protein, and glycosides. These compounds have demonstrated wide range of biological and pharmacological activity against various chronic diseases such as cardiovascular disorders, cancer, arthritis etc.[5–7] Anti-oxidant, anti-diabetic, anticancer, antimicrobial, anti-obesity,

anti-inflammatory effects of flaxseed extract have also been reported on various animal models.[8–13] Flaxseed has gathered significant attention in recent years as a potential nutraceutical, due to its high content of omega-3 fatty acids, alpha-linolenic acid, dietary fiber, and lignans.

Despite the widespread recognition of flaxseed's nutritional and medicinal properties, there are few in depth studies on their phytochemical, antidiabetic and anti-inflammatory potential. This gap is particularly important to address given the rising prevalence of various health issues and illnesses, many of which can be linked to lifestyle factors such as diet and obesity. Although many research studies have been carried out on flaxseed; particularly in countries like Nepal, where altitude and climatic variations could influence its properties are yet to be explored. This study aims to investigate and evaluate the phytochemical constituents of flaxseed and to assess its various activities, including antioxidant, antidiabetic, and anti-inflammatory properties. By providing scientific evidence supporting it traditional uses, this research seeks to explore flaxseed's potential as a source of novel therapeutic agent.

## 2. MATERIAL AND METHODS

### 2.1 Plant material

*Linum usitatisiumum* seeds were bought from local market in Asan, Kathmandu. The flaxseed was then washed with water to remove any dirt if present and left to shade dry for 3 days. The dry seeds were then grinded to fine powder using a grinder. The powder was passed through mesh no.22. Finally, it was stored in air-tight container for further use. The plant material was authenticated from National Herbarium and Plant Laboratories, Godavari.

### 2.2 Extraction

About 50gm of flaxseed powder was defatted using 300ml of n-hexane (1:6 w/v) for around 16 hours. The defatted flaxseed powder was then filtered using Whatman filter paper no. 41 and left to air dry. About 35gm of the air dried powder was weighed and macerated in 350 ml of 80% ethanol for around 48 hours. The extract was then filtered and concentrated in rotary evaporator. The concentrated extract was then stored in the refrigerator with proper labelling and used for further research purpose.

## 2.3 Determination of physiochemical parameters

2.3.1 Flow property

2.3.1.1 Tapped and bulk density

- For bulk density, 30gm of the powder was taken and kept in a graduated measuring cylinder. The volume that it covered was noted and the bulk density was calculated using formula:

  Bulk density = (weight of the powder taken ÷ volume covered by powder).

- For tapped density, the measuring cylinder was then tapped on the surface until the volume that it occupied was constant.

  Tapped density =(weight of the powder taken ÷ volume covered after tapping)

2.3.1.2 Hausner ratio

HR = Tapped density ÷ Bulk density

2.3.1.3 Carr's index

C.I = (Tapped Density-Bulk density) ÷ Bulk density ×100 %

2.3.2 Loss on drying

The hot air oven was switched on and left to heat up. The petri plate was cleaned and left inside the hot air oven for about 30 minutes. About 10gm of flaxseed powder was weighed and put in the petri plate and inside the hot air oven at 105℃ for about an hour. An hour later, the petri plate was taken out from the hot air oven, left to cool in desiccator and the weight of the powder was taken.

LOD = (weight loss ÷ weight of sample) × 100

2.3.3 Swelling index

It is the volume in ml taken up by swelling of 1g of plant material under specified condition. 1g of plant material was taken in 50ml graduated measuring cylinder and 25ml of distilled water was added to it. The mixture was shaken thoroughly every 10 minutes for an hour and was allowed to stand for 3 hours. The volume occupied with the plant material was noted.

2.3.4 Extractive value

It is used for the estimation of constituents extracted with solvent used for extraction. 100 gm of flaxseed was defatted using 600 ml of hexane. The defatted powder was air-dried and about 72.5 gm of dried powder was macerated in 720 ml ethanol for 2 days. The extract was filtered and concentrated using rotary evaporator. Finally yield value was calculated using the following formula:

% yield = (weight of dried extract ÷ weight of dried plant sample) ×100 %

### 2.4 Phytochemical screening

The qualitative phytochemical screening for the various constituents present in plant extract was performed according to the standard method described by Junaid R Shaikh and MK Patil.[14]

### 2.5 Total phenolic content assay

The total phenolic content in ethanol extract was determined by Folin-Ciocalteu reagent method as described by Singleton and Rossi [15] with minor modifications. 1 mL of plant extract was treated with 5ml of Folin-Ciocalteu reagent and allowed to stand for 5 minutes. 4ml of sodium carbonate solution was added to above mixture. The reaction mixture was allowed to stand in the dark for about 1 hour and then the absorbance was measured at 765 nm using methanol as blank. Gallic acid was used as standard. The calibration curve was plotted with standard of solution of gallic acid in the range of 25-500 mcg/ml. The total phenolic content in the sample was determined as milligram of Gallic acid equivalent by using the following equation:

$$TPC = C\times (V\div M)$$

Where C = concentration of Gallic acid from curve (mg/mL)

V = volume of extract (mL)

M = weight of plant extract (g)

### 2.6 Total flavonoid content

The total flavonoid content in the extract was estimated by the aluminum chloride colorimetric method. 200µl of aluminum chloride solution (10% w/v) was added to 1 ml of extract and 200µl of potassium acetate (0.1M) and finally 4ml of distilled water was added to the mixture. After vortex mixing, the solution was stored in dark for 45 minutes. Finally, the absorbance of the mixture was measured at 415 nm. Quercetin was used as standard. The total flavonoid content in the sample was calculated as milligram of quercetin equivalent by using the following equation:

$$TFC= C\times (V \div M)$$

Where C = concentration of quercetin from curve (µg/mL)

V = volume of extract (ml)

M = weight of plant extract (g)

### 2.7 FTIR analysis

The dried ethanol extract of flaxseed was subjected to FTIR analysis for the detection of various compounds and functional groups. The IR spectra was measured using FTIR spectrophotometer (IRPrestige-21Shimadzu). The spectra were captured at a resolution of 1 $cm^{-1}$ in the range of 4500–500 $cm^{-1}$.

### 2.8 Anti-oxidant activity:

The anti-oxidant activity of plant extract was determined by 1, 1- diphenyl-2-picryl hydrazyl radical (DPPH) scavenging method according to Alacher et al. [16] 1mM DPPH Solution was prepared and 150 µl of this solution was added to 50 µl of extract at different concentrations (15.625, 31.25,62.5, 125, 250, 500 µg/ml). Then the mixture was allowed to stand in dark at room temperature for 30 minutes. The absorbance was then measured at 517nm using microplate reader. Ascorbic acid was used as standard. All the tests were carried out in triplicates. Then finally a calibration curve was plotted.

The percentage of radical scavenging activity was determined using the formula below:

Percentage scavenging = $(A_o\text{-}A_T)/A_T \times 100$ %

Where, $A_o$ = Absorbance of control (DPPH only).

$A_T$ = Absorbance of sample.

IC50 value is defined as the effective concentration of the sample required to scavenge 50% of the DPPH free radicals. IC50 values were determined by plotting the concentration of extract versus the corresponding scavenging effect. Lower the IC50 value, higher the antioxidant activity.

### 2.9 In –vitro anti-inflammatory activity

The anti-inflammatory activity of the extract was studied using human RBC membrane stabilization assay. [17] The principle behind this method is stabilization of human red blood cell membrane by hypo tonicity induced membrane lysis. This assay is based on the principle that the erythrocyte membrane resembles lysosomal membrane and its stabilization can be an indicator of the anti-inflammatory activity of substance being treated.

Blood sample was taken from healthy volunteer student who had not taken any NSAIDS since two weeks and distributed into tubes containing Ethylene diamine tetra acetic acid(EDTA) (1%) solution using a 3 ml syringe. The blood was centrifuged at 3000 rpm in 10 minutes and serum was discarded. The blood samples were mixed with the same amount of Alsever's solution, (containing, 0.8 g sodium citrate, 2 g of dextrose, 0.05 g citric acid, and 0.42 g of

sodium chloride, dissolved in 100 mL of distilled water). The blood solution was centrifuged at 3000 rpm for 10 minutes. Then the supernatant was discarded, and pellet was washed again three times with isotonic saline. A 10% v/v suspension of RBC was made in normal saline and kept at 4 °C undistributed before use.

Different concentrations (62.5, 125, 250, 500, 1000 µg/ml) of extracts were prepared. Test samples were prepared using 1 ml phosphate buffer, 2 ml hypotonic saline, 0.5 ml of the plant extract, and 0.5 ml of RBC suspension. The assay mixtures were then incubated at $37^0$C for 30 minutes. After incubation, the assay mixtures were centrifuged at 3000 rpm for 10 minutes and carefully handled without disturbing the pellet. Then absorbance of the supernatant was measured at 560 nm wavelength. The whole test procedure was done in triplicates. Diclofenac sodium was used as standard and control sample was prepared by omitting extracts.

% Protection (stabilization) of the RBC membrane was measured by using following formula:

% Protection = 100 – [Absorbance of the sample ÷Absorbance of the control] ×100.

**2.10 In vitro antidiabetic activity:**

1. α-glucosidase inhibitory assay

   α-glucosidase inhibition was measured using p-nitrophenyl-α-D- glucopyranoside (p-NPG) as the substrate.[18] Different concentrations of linseed extract, ranging from 1 to 10 mg/mL were prepared (dilution in phosphate buffer). 40 µl of extract was incubated with the solution containing 20 µL of α-glucosidase (1 U/mL) for 30 min at 37 °C. 40µl of 2.5mM para-nitrophenyl-α-D-glucopyranoside (PNPG) in 0.1 M potassium phosphate buffer was added to initiate the reaction, and the mixture was further incubated for 15 min. Finally, the absorbance of the mixture was recorded at 405 nm. Acarbose was utilized as the positive control and each concentration was tested in triplicate. The results were presented as % inhibition of enzyme activity and calculated using the following formula:

   $$\% \text{ Inhibition} = [(X_A - X_B)/X_A] \times 100.$$

   $X_A$ is the absorbance of the control (100% enzyme activity) and $X_B$ is the absorbance of the sample.

2. α-amylase inhibitory assay

   α-amylase inhibition was measured using 2-Chloro-4-Nitrophenyl-α-Maltotrioside ($CNPG_3$) as the substrate. Different concentrations of linseed extract, ranging from 1 to 10 mg/mL were prepared (dilution in phosphate buffer). 20 µl of extract was incubated with the solution containing 80 µl of α-amylase (2 U/mL) for 30 min at 37 °C. 100µl of

1 mM $CNPG_3$ in 0.1 M potassium phosphate buffer (6.9) was added to initiate the reaction, and the mixture was further incubated for 15 min. Finally, the absorbance of the mixture was recorded at 405 nm. Acarbose was utilized as the positive control and each concentration was tested in triplicate. The control was prepared by adding deionized water instead of plant extract. The results were presented as % inhibition of enzyme activity and calculated using the following formula:

$$\% \text{ Inhibition} = [(X_A - X_B)/X_A] \times 100.$$

$X_A$ is the absorbance of the control (100% enzyme activity) and $X_B$ is the absorbance of the sample.

## 3. RESULTS

### 3.1 Physiochemical parameters

3.1.1 Flow property

3.1.1.1 Bulk density = weight of the powder taken ÷volume covered by powder

= 30÷70.7 = 0.424 gm/cm³

3.1.1.2 Tapped density = weight of the powder taken ÷ volume covered after tapping

= 30÷57 = 0.526 gm/cm³

3.1.2 Hausner ratio

HR = Tapped density÷ Bulk density

= 0.526÷0.424

=1.24

3.1.3 Carr's index

C.I = (Tapped Density-Bulk density) ÷ Bulk density ×100 %

= [(0.526-0.424)÷0.424] * 100%

= 24.06%

3.1.4 Loss on drying

LOD = (weight loss ÷ weight of sample) × 100

= (10.001 - 9.066 )÷10.001× 100%

= 9.349%

3.1.2 Swelling index = [(0.7-0.4) ÷0.4] ×100% = 75%

3.1.3 Extractive value

% yield = (weight of dried extract ÷ weight of dried plant sample) ×100 %

= (1.863÷72.5) ×100 % =2.569 %.

### 3.2 Phytochemical screening analysis

The results of phytochemical screening of flaxseed are shown below:

Table 1: Phytochemical screening of flaxseed ethanol extract

| S.N. | Chemical constituents | Phytochemical test | Observation | Result |
|---|---|---|---|---|
| 1. | **Flavonoids** | Lead acetate test<br>Shinoda test<br>$FeCl_3$ test<br>Conc. $H_2SO_4$ test | Yellow precipitate<br>Crimson red color<br>Green precipitate<br>Light orange color` | +ve<br>+ve<br>+ve<br>+ve |
| 2. | Phenols | $FeCl_3$ test<br>$K_2Cr_2O_7$ test<br>Iodine test | Dark green precipitate<br>Light dark color<br>Red color | +ve<br>+ve<br>+ve |
| 3. | Alkaloids | Wagners test<br>Hagers test<br>Mayers test | White precipitate<br>Brown precipitate<br>No change in color | +ve<br>+ve<br>-ve |
| 4. | Phytosterols | Salkowski test | Ring at the junction and red color in lower layer | +ve |
| 5. | Carbohydrates | Molisch test | Violet ring | +ve |
| 6. | Cardiac glycosides | Keller Killani test | Bluish black color | +ve |
| 7. | Saponin | Froth test | Frothing present | +ve |

### 3.3 Total phenolic content analysis:

The total phenolic content was determined as a milligram of Gallic acid equivalent using the calibration curve of Gallic acid. The absorbance of each solution was measured and noted as follows:

Table 2: Absorbance of Gallic acid

| Concentration(mcg/ml) | Average absorbance |
|---|---|
| 25 | 0.115 |
| 50 | 0.179 |
| 100 | 0.239 |
| 250 | 0.461 |
| 500 | 0.733 |

Figure 1: Calibration curve of gallic acid for total phenolic content determination

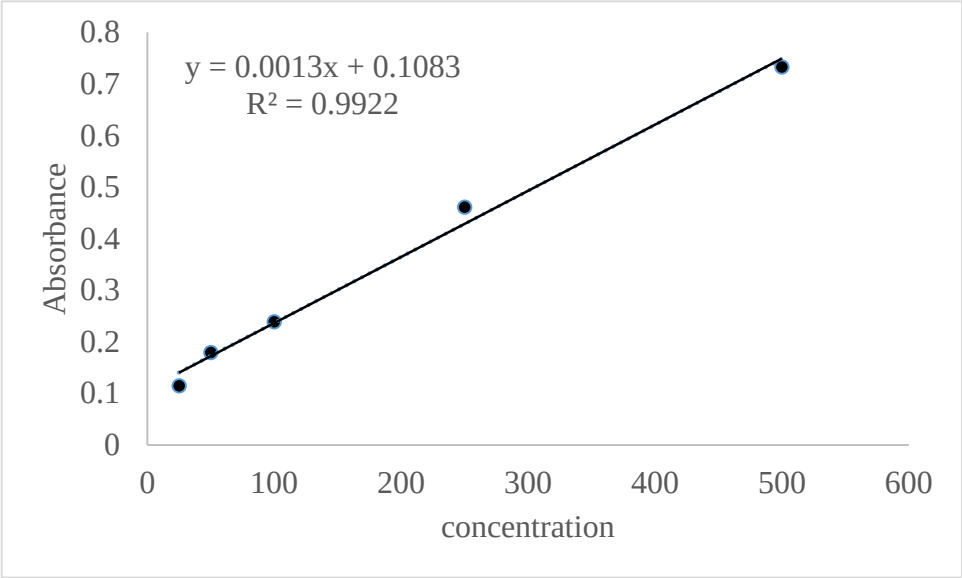



Table 3: Total phenolic content in ethanol extract of flaxseed

| **Sample concentration** | **Average absorbance** | **Concentration mg/ml** | **TPC as GAE (mg/g)** |
|---|---|---|---|
| 48 mg/ml | 0.474 | 0.281 | 90.98 |

**3.4 Total flavonoid content analysis:**

TFC is expressed as mg of quercetin equivalents per gram of dried sample. The absorbance of each solution was measured and noted as follows:

Table 4: Absorbance of quercetin

| Concentration (µg/ml) | Average |
|---|---|
| 1 | 0.07503 |
| 5 | 0.1038 |
| 10 | 0.195 |
| 20 | 0.2645 |
| 40 | 0.3968 |

Figure 2: Calibration curve of quercetin for total flavonoid content determination

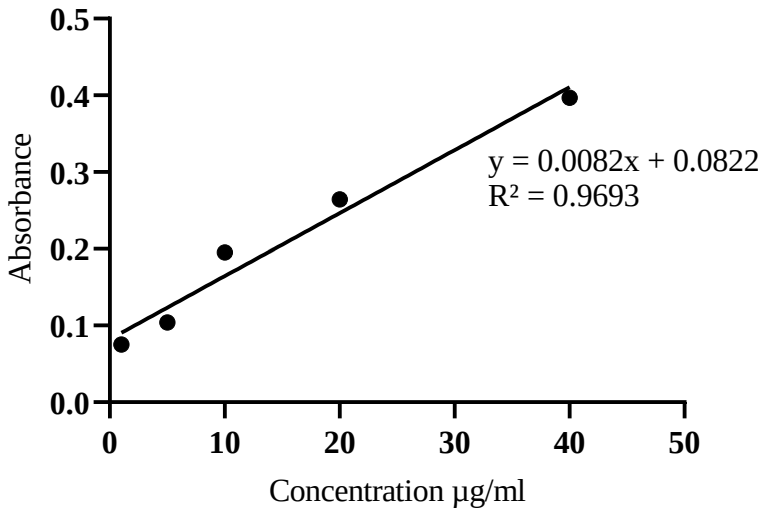



Table 5: Total flavonoid content in ethanol extract of flaxseed

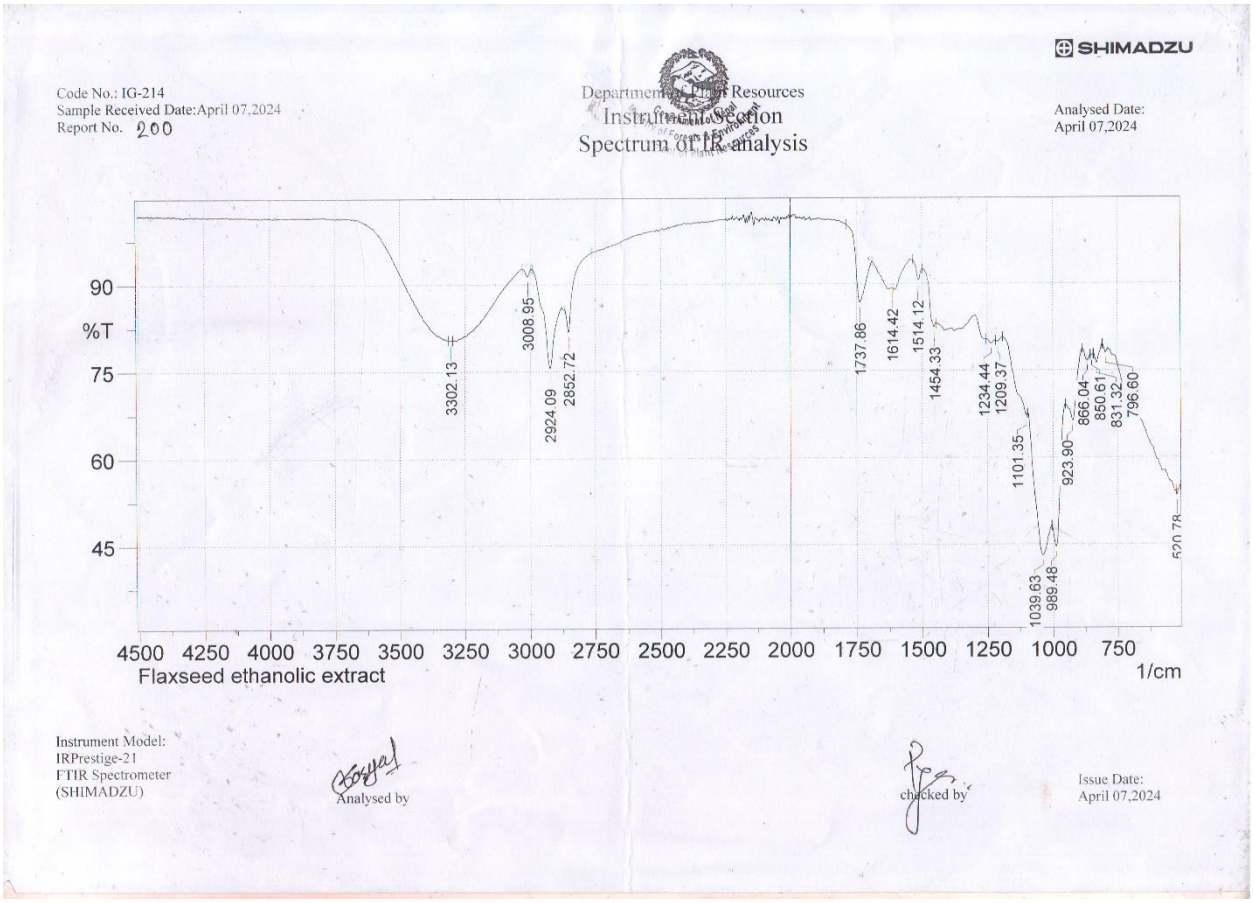
| Sample concentration | Average absorbance | Concentration mg/ml | TFC as QE (mg/g) |
|---|---|---|---|
| 20 mg/ml | 0.2796 | 0.024073 | 1.20 |

### 3.5 FTIR analysis

FTIR spectra of the flaxseed extract revealed a variety of distinctive IR absorption peaks at wave numbers in the aromatic and fingerprint region of IR band. The broad peak at 3400 $cm^{-1}$ results from -OH groups (alcohol and phenols), the 3008 $cm^{-1}$ peak from aromatic C-H bonds. The absorption band at 2924 and 2852 can be related to –CH stretching vibrations. The 1737 $cm^{-1}$wave-number peaks result from the presence of C = O (carbonyl groups), functional group arising for flavonoids and tannins derivatives. 1039$cm^{-1}$, 1101$cm^{-1}$, 989 $cm^{-1}$ wave-

number peaks result from C-O stretching vibrations of alcohols, carboxylic acids, ester, or ethers of biomolecules found in the flaxseed. The absorption peaks at 1614 $cm^{-1}$ could be assigned for C=C stretching of alkene or aromatic compounds. The absorption peaks at 1514 $cm^{-1}$ could be assigned for the presence of N–O stretching in nitro. The small peaks between 890-796 $cm^{-1}$ correspond to chloro alkane (C-Cl) and = C-H bending aromatic C -H out of plane bend.

Figure 3: FTIR spectra of ethanol extract



### 3.6 Anti-oxidant analysis:

The percentage of DPPH radical scavenging increased with higher concentrations of the extract, demonstrating a dose-dependent effect as shown in following table:

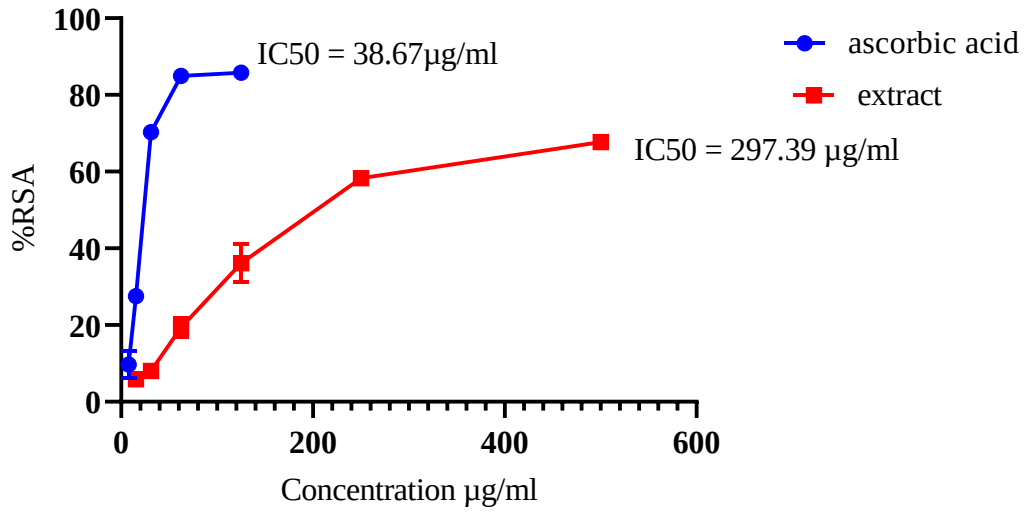
Table 6: DPPH scavenging activity of ethanol extract

| Concentration µg/ml | % RSA (mean ± S.E.M) |
|---|---|
| 15.625 | 6.707 ± 1.59 |
| 31.25 | 8.076 ± 0.79 |
| 62.5 | 19.37 ± 1.51 |
| 125 | 36.13 ± 2.91 |
| 250 | 58.31 ± 0.77 |
| 500 | 67.69 ± 1.07 |

Table 7: DPPH scavenging activity of ascorbic acid

| Concentration mcg/ml | % RSA (mean ± S.E.M) |
|---|---|
| 7.8125 | 2.737 ± 0.59 |
| 15.625 | 9.034 ± 1.48 |
| 31.25 | 29.08 ± 1.85 |
| 62.5 | 70.294 ± 1.00 |
| 125 | 84.94 ± 0.18 |

Figure 4: DPPH scavenging activity of extract and ascorbic acid



### 3.7 In-vitro anti-inflammatory analysis:

The percentage protection of RBC was relatively lower in samples treated with the extract compared to diclofenac as shown in following table:

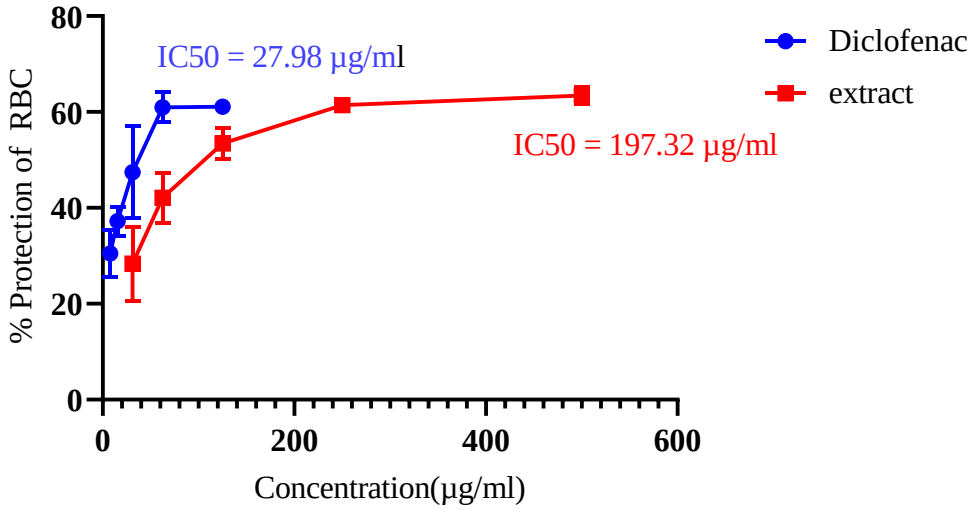
Table 8: Anti Inflammatory Activity of diclofenac by HRBC Method

| **Concentration (mcg/ml)** | **% Protection (mean ± S.E.M)** |
|---|---|
| 7.8125 | 30.53 ± 2.03 |
| 15.625 | 37.21 ±1.23 |
| 31.25 | 47.43 ± 3.93 |
| 62.5 | 60.93 ± 1.30 |
| 125 | 61.13 ± 0.34 |
| 250 | 61.43 ± 0.61 |
| 500 | 63.53 ± 1.33 |

Table 9: Anti Inflammatory Activity of extract by HRBC Method

| Concentration(mcg/ml) | % Protection (mean ± S.E.M) |
|---|---|
| 31.25 | 28.395 ± 3.19 |
| 62.5 | 42.121 ± 2.15 |
| 125 | 53.421 ± 1.38 |
| 250 | 61.456 ± 0.64 |
| 500 | 63.465 ± 0.74 |

Figure 5: Anti Inflammatory Activity of extract and diclofenac by HRBC Method



**3.8 Anti-diabetic activity:**

3.8.1 Alpha – glucosidase assay:

The inhibitory activity of the extract and acarbose against α-glucosidase were measured and presented in the following table:

Table 10: α-glucosidase inhibitory activity of flaxseed extract

| Concentration mg/ml | % inhibition (mean ± SEM) |
|---|---|
| 1 | 7.903 ± 0.28 |
| 2 | 9.778 ± 1.78 |
| 4 | 13.77 ± 2.88 |
| 6 | 16.40 ± 2.28 |
| 8 | 25.71 ± 3.48 |
| 10 | 31.52 ± 3.98 |

Table 11: α-glucosidase inhibitory activity of acarbose

| Concentration mcg/ml | % inhibition (mean ± SEM) |
|---|---|
| 100 | 8.538 ± 0.59 |
| 200 | 20.689 ± 0.49 |
| 300 | 48.440 ± 0.32 |
| 350 | 54.187 ± 0.28 |
| 400 | 64.203 ± 0.59 |
| 450 | 77.504 ± 1.07 |

Figure 6: α-glucosidase inhibitory activity of flaxseed extract

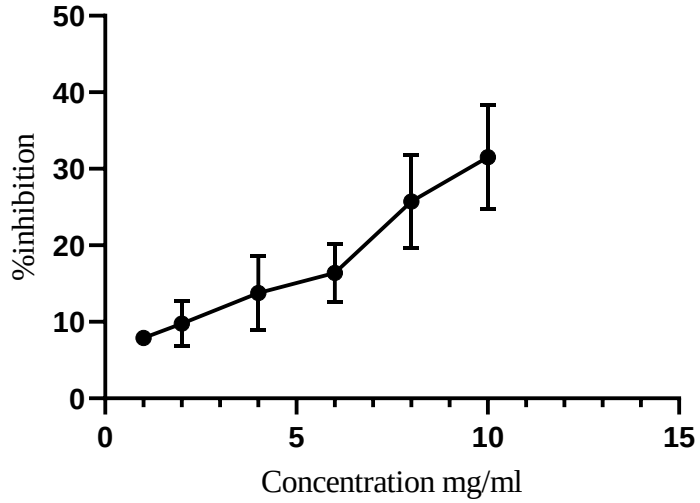
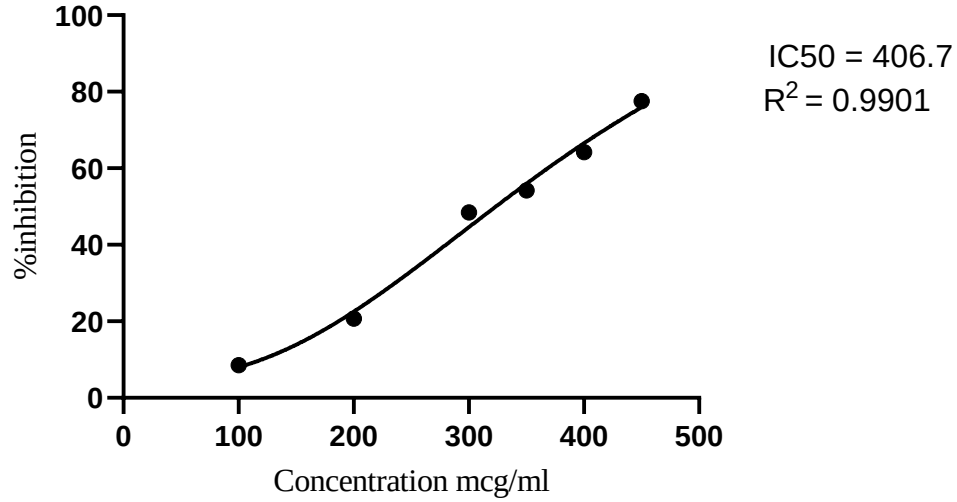


Figure 7: α-glucosidase inhibitory activity of acarbose




3.8.2 Alpha amylase assay:

The inhibitory activity of the extract and acarbose against α-amylase were measured and presented in following table:

Table 12: α-amylase inhibitory activity of flaxseed extract

| Concentration mg/ml | % inhibition (mean ± SEM) |
|---|---|
| 1 | 2.954545455 |
| 2 | 15.22727273 |
| 4 | 20.07575758 |
| 6 | 39.92424242 |
| 8 | 45.22727273 |
| 10 | 58.56060606 |

Table 13: α-amylase inhibitory activity of acarbose

| Concentration mcg/ml | % inhibition (mean ± SEM) |
|---|---|
| 100 | 67.68 ± 0.50 |
| 200 | 70.30 ± 1.46 |
| 300 | 83.57 ± 0.11 |
| 350 | 89.59 ± 0.10 |
| 400 | 92.65 ± 0.13 |
| 450 | 93.71 ± 0.03 |

Figure 9: α-amylase inhibitory activity of flaxseed extract

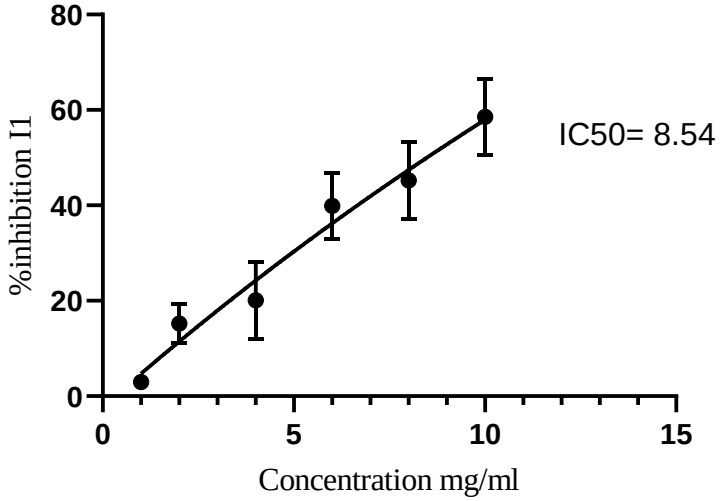
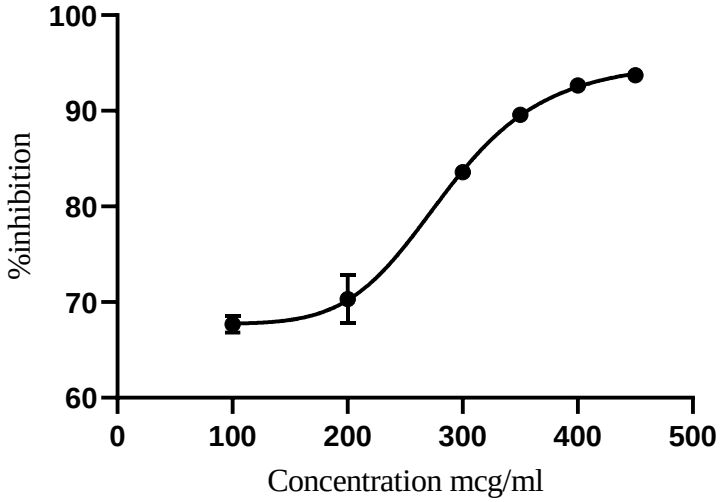



Figure 10: α-amylase inhibitory activity of acarbose




## 4. DISCUSSION

Physicochemical evaluation is essential for detecting adulteration or mishandling since a drug's efficacy depends on its physical and chemical properties. This assessment ensures the authenticity and purity of the material. The bulk density and tapped density were found to be 0.424 and 0.526 gm/cm$^3$. Carr's index was found to be 24.60%, suggesting moderate flow ability. The loss on drying was found to be 9.34%. This value is slightly higher than the value reported by the Canadian Grain Commission (2009) where moisture content was found to be 7.7 %,7.2 % reported by Eggie (2010), and 7.72 % as reported by Khan et al. The swelling

index of 75% indicates that the volume of the material increased by 75% of its original size where it absorbs the particular solvent. The extractive value was found to be 2.5 %.

The phytochemical analysis of L. usitatissimum ethanol extract showed the presence of flavonoids, phenolic compounds, glycosides, alkaloids, terpenoids, tannins, phytosterols, proteins and carbohydrates. These phytochemicals are responsible for versatile medicinal properties of extract like anti-oxidant, anti-inflammatory, antidiabetic, and antimicrobial activity. Amin and Thakur (2014) reported that phytochemical analysis of ethanol extract of flaxseeds contained tannins, flavonoids, terpenoids, phenols and proteins and amino acids were present while it did not contain saponins, sterols, and glycosides. FTIR analysis of the extract demonstrated compounds like phenols, flavonoids and tannins.

The total phenolic content in the flaxseed was found to be 90.98 mg Gallic acid equivalent / g of dry extract. The value of TPC was found to be higher in comparison to study performed by Hanaa M. H. et al (15.5 mg/g GAE) [11] and Alachahe et al (47.2 mg/g GAE). Phenolic compounds have a diverse set of physiological benefits that include reducing inflammation, protecting against oxidative stress, anti-cancer, cardio protective activity, and altering gene expression.The total flavonoid content in the flaxseed was found to be 1.20 mg quercetin equivalent / g of dry extract. The TFC value we obtained was similar to the one reported by Hanaa M. H. et al, where it was found to be 2.5 mg/g QE.

DPPH method is one of the reliable and widely used method for evaluating antioxidant activity. The antioxidant potential shows inverse relationship to the $IC_{50}$ value. The $IC_{50}$ value of extract was found to be 297.306 µg/ml. This result is in line with Amin T. and Thakur M. (2014) where the IC50 value of ethanolic extract of flaxseeds was found to be 256.313 µg/ml. The antioxidant activity may be due to the presence of phenols, lignans and flavonoids. The findings suggest that incorporating flaxseed extracts into functional foods may enhance their health benefits and contribute to the prevention of oxidative stress-related diseases.

The anti-inflammatory activity was studied by using RBC membrane stabilization assay. The results demonstrated that the extract effectively stabilized the RBC membranes, indicating anti-inflammatory activity. The $IC_{50}$ value of extract was found to be 197.312 µg /ml The results also represent that extract can dose-dependently inhibit RBC hemolysis. Similar results were obtained from the study of Alawlaqi et al. (2023) where hemolysis inhibition (%) increased with increasing linseed extract concentration in the range of 100 up to 1000 µg/ml. These findings support previous research on the role of plant extracts in mitigating inflammation.

Alpha-amylase and alpha-glucosidase are the carbohydrate metabolizing enzymes. Alpha-amylase catalyzes the breakdown of polysaccharides like starch into maltose and dextrin, whereas alpha glucosidase further breaks down these oligosaccharides into monosaccharides like glucose. The inhibition of these enzymes increases the carbohydrate digestion time, reduces glucose absorption, and finally blood glucose levels. There was a dose-dependent increase in the percentage inhibitory activity of the extract against α-amylase and α-glucosidase enzymes. The plant extract showed a percentage inhibition of 2.95 % and 58.56 % against α-amylase at concentrations of 1 mg/ml and 10 mg/ml, respectively. The IC50 value of the extract against amylase was found to be 8.54 mg/ml. This value is higher than the IC50 value of 300 mcg/ml as reported by Mechchate et al. (2021). This may be due to the use of crude extract rather than isolated compounds. The plant extract showed an inhibition of 31.52 % at 10 mg/ml concentration against alpha-glucosidase. Mohamed M. Alawlaqi et al. (2023) reported the IC50 value of methanol extract of linseed against alpha-glucosidase as 177.75 µg/ml. The predominance of alpha-amylase activity suggests that flaxseed may be more effective for breaking down larger polysaccharides rather than aiding in the final steps of carbohydrate digestion. The inhibitory activity may be due to the presence of phenols, lignans, carbohydrates and dietary fibers.

## 5. CONCLUSION

Flaxseed (*Linum usitatissimum L.*) is a multi-purpose crop and its consumption has significant health benefits. From the result of this study, it can be concluded that various phytochemicals, including alkaloids, phenols, flavonoids, saponins, proteins, glycosides, and phytosterols are present in flaxseed which are responsible for various activities of extract. The present study demonstrated that the flaxseed extract exhibits potential antioxidant, anti-inflammatory and antidiabetic activity in an in-vitro model. So, further isolation, purification and in vivo studies are essential to explore its full therapeutic potential.

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