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Antioxidant Activity and Molecular Docking Study of Isolated Bioactive Compound from *Linum usitatissimum*

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ABSTRACT

Flaxseed, or *Linum usitatissimum L.*, is a medicinal plant that is high in phytochemicals such as phenolic acids, flavonoids, and lignans, which give it strong antioxidant properties. In this study, a major antioxidant component of flaxseed was isolated and identified, and its interaction with the NF-κB protein was examined utilizing molecular docking. The Soxhlet device was used to obtain the ethanol extract, and column chromatography was used to isolate it. After FTIR, NMR, and LC-MS analyses, the chemically pure molecule was identified as p-coumaric acid. The DPPH & ABTS radical scavenging assays were used to determine its antioxidant capacity, and the results showed modest antioxidant activity with a TEAC of 8.05 µmol/mg and an RSA of 2.90% for DPPH. According to the ABTS assay, the TEAC was 4.698 µmol/mg, and the RSA was 1.606%. The Schrodinger suite molecular docking showed hydrogen bond interactions between TYR251, SER288, & LYS221 along with a high binding affinity of -5.299 to the NFκB protein (PDB ID:1IKN). The findings suggest that p-coumaric acid may operate as a natural inhibitor of the NFκB protein, hence influencing its antioxidant capacity. The study confirms the therapeutic efficacy of bioactives obtained from flaxseed and the use of computational methods to investigate molecular pathways.

Keywords: *Linum usitatissimum*, p-coumaric acid, DPPH, ABTS, Flavonoids, NF-κB, Molecular docking and Antioxidant activity.

INTRODUCTION

Often referred to as flaxseed or linseed, *Linum usitatissimum L.* is a significant annual herbaceous crop in the family Linaceae. Because it has been farmed on a vast scale for its fiber and oil seeds[1–4], it is a very nutritional and medicinal plant. The seeds have phytochemicals like phenolic acids, flavonoids, secoisolariciresinol diglucoside (SDG), & alpha-linolenic acid, an omega-3 fatty acid needed for a balanced diet. Extensive studies on the antioxidant, anti-inflammatory, anticancer, & lipid-lowering properties of these bioactives have shown the usefulness of flaxseed in controlling chronic diseases and promoting general well-being[5–8]. Flaxseed's low level of phenolic chemicals, well known for their powerful antioxidant properties, makes it unique. Including the nuclear factor kappa B(NFκB), a transcription factor essential for inflammation, immunological response, and

oxidative damage control, it plays a significant role in preventing free radicals and controlling pathways linked with oxidative stress[9,10]. Among the pathological diseases connected to NFκB dysregulation are diabetes, heart disease, and cancer. Consequently, dietary antioxidants from plants are turning into natural NFκB blockers for therapeutic application[9].Over the last few decades, molecular docking has developed into a vital computational tool in phytochemical research and drug discovery. It allows virtual screening for and prediction of target protein- ligand interactions, which helps to clarify prospective therapeutic pathways and improve lead compound optimization[9,11]. Against this context, the current work investigated the antioxidant capability of a pure Linum usitatissimum molecule and its possible method of action via molecular docking against NFκB. Using chromatographic and spectroscopic techniques, the primary bioactive component was purified and identified. Its antioxidant activity was evaluated using DPPH and ABTS assays, and its molecular interaction with NF-κB was examined using in silico docking simulations. This integrated approach attempts to make the compound's use as a naturally occurring antioxidant of possible medicinal interest relevant.

MATERIALS AND METHODS

Plant Authentication and Collection

An expert, Dr. Sunita Garg, Scientist F & Head Raw Material Herbarium & Museum (NIScPR), New Delhi, India, recognized fresh Linum usitatissimum seeds that were gathered in July from a botanical garden in Bareilly, Uttar Pradesh, India. For future use, a voucher specimen (NIScPR/RHMD/ Consult/2023/4520-21) has been retained at the department.

Drug and Chemical

Chemicals used in the study were purchased from Central Drug House Ltd., New Delhi, India.

Soxhlet Extraction *Linum usitatissimum*

Linum usitatissimumseeds (500g) were dried for 2-3 days, then crushed into powder form and defatted with petroleum ether and then extracted using the Soxhlet apparatus with ethanol. The extracted *Linum usitatissimum* seeds were filtered and evaporated under a rotary vacuum evaporator at 37°C. and yield value 39.15% stored airtight container for subsequent use[12].

Preliminary Phytochemical Screening

Linum usitatissimum extract was subjected to qualitative chemical testing for the presence as well as absence of phytochemicals including alkaloids, flavonoids glycosides, which tannins, saponin, while phenolic groups[7].

Thin Layer Chromatography

Linum usitatissimum extract was applied on a silica-coated aluminum plate and developed to obtain saturation. The plate was then put into the solvent system Ethanol: Ethyl acetate (90:10) ratio. Then dried the plate was dried and developed with an appropriate reagent followed by visualization under an iodine chamber. The R_f values were calculated further[13].

Determination of Total Flavonoid Content

TFC 1 mg of *Linum usitatissimum* extract to gauge the extract's concentration, it was dissolved in 2.8 milliliters of H_2O, 0.1 milligram of 10% $AlCl_3$, 0.1 ml of 1 M $C_2H_3KO_2$, or 1.5 milligrams of C_2H_5OH. This mix was incubated at 37°C for half an hour.A Systromic UV-Vis Double Beam Spectrophotometer was used to find the absorbance at 415 nanometers. Different quercetin levels (10-50 µg/mL) followed the same process. The TFC was calculated using Quercetin as a standard, and results were published as Quercetin equivalents (mg QE)//g of the sample[14–16].

Isolation and Characterization

After that, a silica gel (Merck Silica gel 60) was used for Column chromatography of theLinum usitatissimumextract. A solvent system was used to elute the column after it was packed onto a hydrodynamic column. The stationary phase was made up of this. The extract from *Linum usitatissimum* was then placed onto the silica gel as a thin band after being combined with a tiny quantity of the mobile phase. After adding the mobile phase at a steady flow rate, *Linum usitatissimum* extract was applied to the (silica gel 60). Using solvent mixtures made of (*n*-hexane and ethyl acetate), a gradient elution of increasing polarity was started, which involved the serial elution of many fractions under TLC monitoring[17]. The isolated compound was characterized by different spectroscopic techniques (that is, IR, NMR, and LC-MS)[11].

Molecular Docking Study

Ligand Preparation and Optimization

The structure of the isolated bioactive compound (identified as p-coumaric acid) was first drawn using ChemDraw and saved in.mol format. The compound was then subjected to geometric optimization using the LigPrep tool in the Schrodinger suite (version 2023-1). LigPrep processed the ligand by generating appropriate stereoisomers, tautomers, and ionization states at a physiological pH of 7.0±0.5, using the OPLS-2005 force field to diminish energy and ensure conformational stability.

Protein Preparation

The crystal structure of the human NF-κB protein (PDB ID: 1IKN) was retrieved from the PDB (Protein data bank). The structure was improved by adding hydrogen atoms, fixing missing side chains, and eliminating water molecules larger than 5 Å using the Protein Preparation Wizard in the Schrodinger suite. The optimization of hydrogen-bonding networks and energy minimization was carried out using the OPLS-2005 force field to stabilize the native protein conformation.

Grid Generation and Protocol of Docking

A receptor grid was created around the active site of NF-κB, centering on the co-crystallized ligand binding pocket. Molecular docking was performed using the Glide XP (extra precision) mode, which considers both polar and non-polar interactions, rotatable bonds, hydrogen bonding, van der Waals forces, and hydrophobic contacts. Docking results were evaluated based on Glide Score, XP GScore, Emodel energy, hydrogen bond interactions, and bonding distances.

Antioxidant Activity

DPPH

The assay was conducted as described. Briefly, 0.1 milliliter sample solution was added by 3.9 milliliter free radical resolution, and the absorption was measured after 30 min at 518 nanometers. A calibration curvature with six Trolox standards was plotted. Using the following formula, the inhibition of the DPPH scavenging effect (%) was determined using eqn.1:

$$\% \ Inhibition = \frac{A0 - A1}{A0} \times 100 \quad (1)$$

Where A_0 = The absorbance of control

A_1 = The absorbance of standard

The TEAC was calculated using eqn. 2

$$TEAC\ (\mu mol/mg) = \frac{(CTE \times 1/MTrolox)}{\text{Sample amoung (mg)}} \quad (2)$$

Isolated phytoconstituent was used for determining antioxidant evaluation DPPH radical scavenging capacity[18].

ABTS

The technique depends on the antioxidant molecules' capacity to neutralize the persistent ABTS radical. To make a stable stock solution of the ABTS radical, 2.45 mmol/L $K_2S_2O_8$ (final concentration) was mixed with 7 mmol/L ABTS aqueous solution. Before being used, the mixture was left to stand for 12 to 16 hours at 37°C in the dark. In order to establish an ABTS radical working solution at the beginning of the investigation, the stock solution was diluted in ethanol until it had an absorbance of 0.70±0.02 at 734 nm. Next, 1 mg of sample extract dissolved in 1 ml of methanol was mixed with 4.85 mL of diluted ABTSradical solution. After the first mixing, the absorbance at 734 nm was measured six minutes later. Various amounts of Trolox (10-50 µmol) were prepared, and they were treated with an equivalent volume of radical solution. Six minutes later, optical density was assessed in a control that included 4.85 milliliters of ABTS solution and 0.15 milliliters of 45% ethanol. Trolox's optical density was determined, & the percentage of ABTS radical scavenging was calculated using the using eqn. 3:

$$\%ABTS\ radical\ Scavenging = \frac{\text{Abs control} - \text{Abs sample}}{Abs\ Control} \times 100 \quad (3)$$

The %scavenging curve generated from Trolox standard was used to estimate the Trolox equivalent(mM/gm) for the isolated compound[19].

RESULTS

Extraction

The preliminary analysis of the *Linum usitatissimum* extract demonstrated significant extraction efficiency, with a percentage yield of 39.15%.

Phytochemical Screening

The results of the screening for phytochemicals revealed the presence of various bioactive components, which are brief in Table 1.

Table 1 Preliminary Phytochemical Components in the Seeds of *Linum usitatissimum* Extract

Sr.No	Test	Test name/ reagents	*Linum usitatissimum* extract
1	Alkaloids	Dragendroff's Mayer's	++
2	Saponins	Froth test Ferric chloride test	-+
3	Glycosides	Grignard test	+
4	Flavonoids	Free flavonoids Borntrager s test	++
5	Steroids	Salwaski test	-
6	Tannins	Phlobatannins	-
7	Phenolic group	Ethanolic $FeCL_3$ solution	+
8	Sugars	Molisch's test Fehling's	+-

(-) Negative result & Positive result (+) *Linum usitatissimum* extract

Total Flavonoid Content

The TFC of the isolated compound was ascertained using the $AlCl_3$ method. Using quercetin as a reference, the solution mixture's absorbance was measured at 415 nm (Fig. 1). The standardization curve of quercetin showed linearity in the variety of 10-40 micrograms per milliliter ($R^2 = 1$). The TFC of the isolated compound was calculated using the y = 0.001x obtained from the quercetin standard curvature and was stated as milligrams of quercetin equivalent (mg QE) per gram of the compound. The TFC of the isolated compound was found to be 2.1 mg QE/g of the compound. This result indicates the positive of a significant measure of flavonoids, suggesting its potential biological and pharmacological relevance.

Isolation of Phytoconstituents

The Linum usitatissimum extract using the following ratios100:0, 80:20, 60:40, 30:70, 20:80, n-Hexane: ethyl acetate and 0:100, the fraction was eluted with stepwise gradient polarity after being subjected to column chromatography on silica gel. For the various solvent system ratios to the specified seven fractions, 50 mL fractions were gathered. Two Fractions with a clear spot having the same R_f value were combined Table 2. The compound was characterized by FTIR, NMR, and LCMS spectra.

Table 2: Fractions collected in Column Chromatography of *Linum usitatissimum* extract

Solvent system	Ratio	Fractions number	Thin layer chromatography	Fractions code
n-H: E acetate	100::0	1	No spot	EE-1
n-H: E acetate	80:20	2	No spot	EE-2
n-H: E acetate	60:40	3,4	Clear	EE-3
n-H: E acetate	30:70	5	No spot	EE-4
n-H: E acetate	20:80	6	No spot	EE-5
n-H: E acetate	0:100	7	No spot	EE-6

n-H: E acetate (n-Hexane: Ethyl acetate)

Characterization of Isolated Compound

The thin-layer chromatography examination of the Linum usitatissimum extract was completed using a solvent system consisting of Ethanol: Ethyl acetate in a fraction of (90:10) v/v/v). A single spot was observed on the TLC plate, exhibiting a light brown color. The retention factor (R_f) value for this spot was calculated to be 0.52.

Figure 1 displayed many peaks in the FTIR of the isolated chemical LPCP. According to Fig. 2, the ^{1}H NMR (500 MHz, DMSO) δ values are 7.57 (2H d, *J* = 15.8 hertz, OH), 7.47 (2H, d, *J* = 8.8 Hz, CH), 6.83 (2H, d, *J* = 8.7 Hz, CH), and 6.32 (2H, d, *J* = 16.0 Hz, H). IR (KBr) Vmax: O-H stretching ranges from -3400-3100 cm^{-1}; O-H stretching 3339 cm^{-1}; C=O stretching 1634 cm^{-1}; C-O stretching 1153 cm^{-1}.

^{13}C NMR (126 MHz, DMSO) δ 168.68 (s), 160.08 (s), 144.81 (s), 130.51 (s), 125.86 (s), 116.35 (s), 115.81 (s), 39.50 (d, *J* = 21.0 Hz), 39.29 (s) (Figure 3).

Mass spectroscopy of active compound LUE had the chemical formula $C_9H_8O_3$ based on ES-MS data m/z 163.04 [M^+], and determined molecular weight was 164.04 (Fig. 4). Compound LUE was identified as p-Coumaric acid by comparison of the physical parameters, IR, Mass, The presented data along with ^{1}H-NMR and ^{13}C-NMR data (Figure 5).

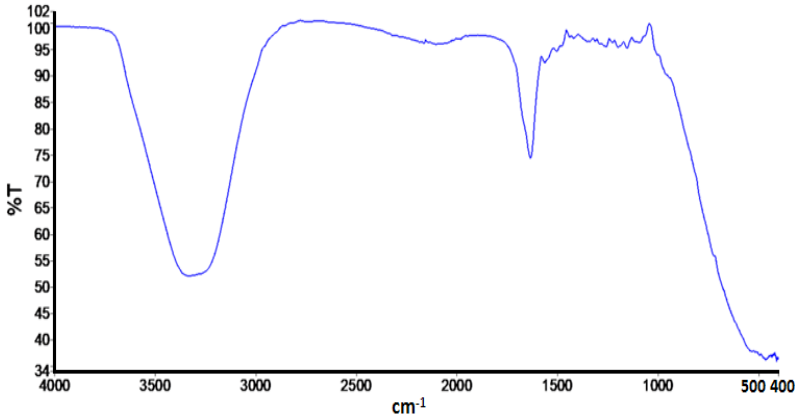

Fig. 1. FTIR spectrum of the isolated compound (p-coumaric acid) showing characteristic absorption peaks Major peaks include: A broad O–H stretching vibration at ~3400–3100 cm^{-1}; A sharp peak at 1634 cm^{-1} indicating C=O stretching; A distinct C–O stretching vibration at 1153 cm^{-1}; These signals confirm the presence of phenolic hydroxyl and carboxylic acid functional groups

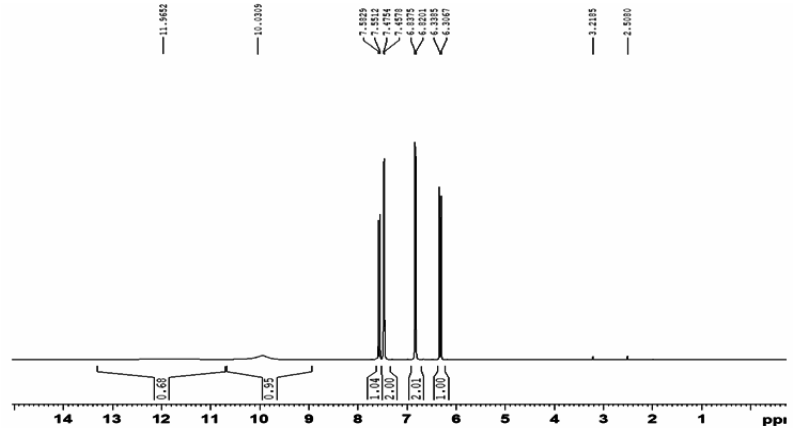

Fig. 2. ^{1}H NMR spectrum (500 MHz, DMSO) of the isolated compound (p-coumaric acid). Key signals include: δ 7.57 ppm (2H, d, *J* = 15.8 Hz, OH protons); δ 7.47 ppm (2H, d, *J* = 8.8 Hz, aromatic CH); – δ 6.83 ppm (2H, d, *J* = 8.7 Hz, aromatic CH); δ 6.32 ppm (2H, d, *J* = 16.0 Hz, vinylic CH); These signals correspond to the characteristic hydrogens of the coumaric acid backbone

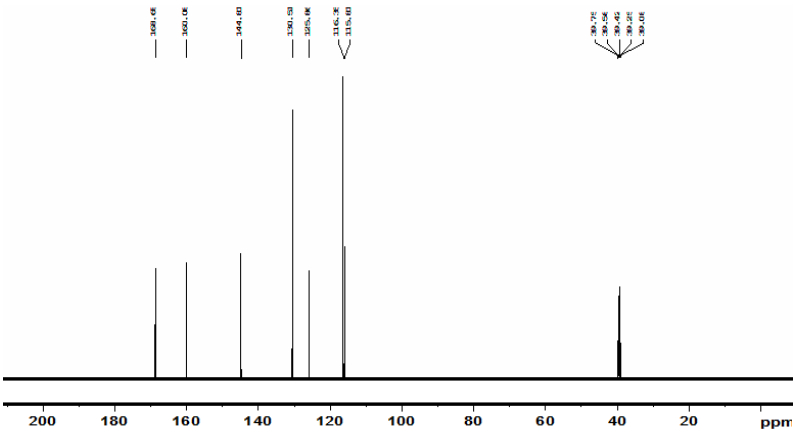

Fig. 3. ^{13}C NMR spectrum (126 MHz, DMSO) of the isolated compound (p-coumaric acid) Prominent carbon signals observed at: δ 168.68 (C=O carbon); δ 160.08, 144.81, 130.51, 125.86, 116.35, 115.81 (aromatic ring carbons); δ 39.50, 39.29 (aliphatic or side chain carbons); These values support the structure of p-coumaric acid

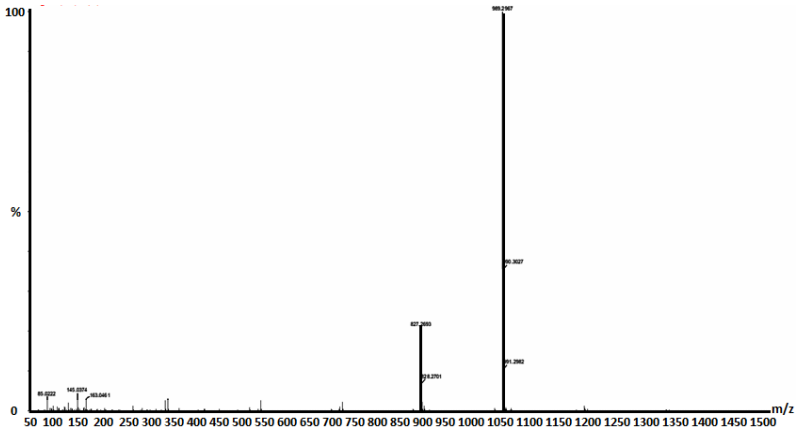

Fig. 4. LC-MS spectrum of the isolated compound (p-coumaric acid) LC-MS spectrum of the isolated compound (p-coumaric acid). The mass spectrum shows a molecular ion peak at m/z 163.04 $[M+H]^+$, which corresponds to the expected molecular weight of 164.04 g/mol. This confirms the molecular formula $C_9H_8O_3$, validating the compound's identity as p-coumaric acid based on molecular mass and fragmentation pattern

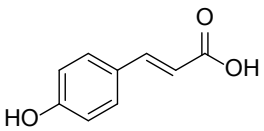

Fig. 5. Structure of isolated compound

Molecular Docking

The molecular docking simulation revealed that the isolated compound, p-coumaric acid, showed significant binding affinity toward the NF-κB protein. The docking score was calculated as –5.299, with an XP GScore of –5.299, indicating a strong and stable interaction within the binding pocket. The compound formed three hydrogen bonds with critical amino acid residues in the active site: TYR251, SER288, and LYS221, with bond lengths ranging from 1.87 Å to 2.67 Å.Additionally, the Emodel energy of –36.191 and the Glide energy of –35.624 further confirmed the ligand's stability and favorable binding conformation. These findings suggest that p-coumaric acid may act as a potential natural inhibitor of NF-κB, thereby contributing to its observed antioxidant activity. A two-dimensional interaction diagram revealed polar interactions and hydrogen bonding with the aforementioned residues, while the 3D binding pose visualized through Maestro confirmed the compound's snug fit within the receptor site, mimicking native ligand interactions (Fig. 6). Table 3 demonstrated the docking of ligands in the dataset along with amino acid interactions and count using glide grid sore.

Table 3: Molecular Docking score of isolated compounds

Compound	Dock score XP GScore	No of H-bonds	Interacting amino acids	Hydrogen bond lengths (Å)	Emodel energy	Glide energy
p-coumaric acid	-5.299	3	TYE 251 SER 288 LYS 221	2.11 1.87 2.67	- 36.191	-35.624

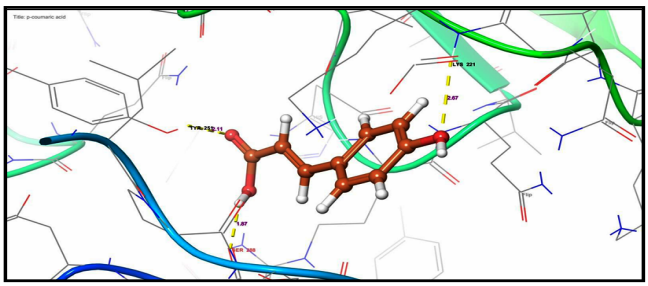

Fig. 6. Molecular docking interaction of p-coumaric acid with NF-κB (PDB ID: 1IKN) as visualized using Schrodinger Glide XP mode. The ligand forms three hydrogen bonds with active site residues: TYR251 (2.11 Å); SER288 (1.87 Å); LYS221 (2.67 Å). These interactions suggest stable binding and support the compound's potential as a natural NF-κB inhibitor

Antioxidant Evaluation

DPPH Free Radical Scavenging Assay

The antioxidant potential of the isolated compound was evaluated using 2,2-diphenyl-1-picrylhydrazyl test for scavenging free radicals (Fig. 7). The absorbance value recorded for the compound was 0.234, which was used to calculate its RSA and TEAC. The RSA was determined to be 2.90, indicating moderate free radical scavenging ability. Additionally, the concentration of Trolox Equivalent (CTE) was calculated to be 31.67 µg/mL, while the TEAC value was found to be 8.05 µmol/mg, reflecting the compound's antioxidant capacity relative to the standard antioxidant, Trolox (Table 4).

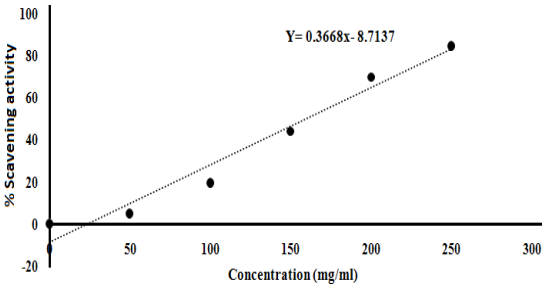

Fig. 7. DPPH free radical scavenging assay for Trolox

Table 4: Calculated RSA and TEAC of samples using recorded absorbance and trendline question of standard

Sr. No	Type	Sample	Absorbance	RAS(%)	CTE (µg/mL)	TEAC (µmol/mg)
1	Sample	LPCP	0.234	2.90	31.67	8.05

Trolox equivalent antioxidant capacity, Radical Scavenging Activity

ABTS Trolox Equivalent Antioxidant Capacity

The free radical activity potential of the isolated compound was evaluated by use of the ABTS test for free radical scavenging. The scavenging activity was calculated based on the absorbance values recorded at 734 nanometre and the trendline equation obtained from the standard (Trolox) (Fig. 8). The results are summarized in Table 5, where the RSA, TEAC, and CTE were determined for the isolated compound. The isolated compound exhibited an absorbance of 0.674, which corresponds to an RSA of 1.606%. The calculated CTE was 1.307µg/mL, and the TEAC value was found to be 4.698 µmol/mg, highlighting the compound's antioxidant capacity relative to the Trolox standard.

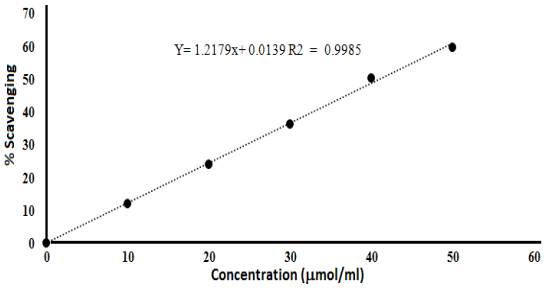

Fig. 8. ABTS free radical scavenging assay for Trolox

Table 5: Calculated RSA and TEAC of samples using recorded absorbances and trendline equation of standard

Sr.No	Type	Sample code	Absorbance	RAS (%)	CTE (µg/mL)	TEAC (µmol/mg)
1	Sample	LPCP	0.674	1.606	1.307	4.698

Reactive Scavenging Activity and Trolox equivalent capacity for antioxidants

DISCUSSION

The current work investigates the antioxidant potential of an isolated bioactive phytoconstituent, i.e., p-coumaric acid, from the seed of Linum usitatissimum.While the component was further clarified utilizing spectral approaches, including FTIR, NMR, and LC-MS, Soxhlet extraction with ethanol followed by extraction produced 39.15%. All of these analytical procedures verified the integrity and identification of the chemical in line with results from earlier studies confirming identical phytoconstituents present in flaxseed[11,20]. According to some studies, a wide range of bioactive chemicals, including phenolics, flavonoids, & lignans, accounts for most of flaxseed's antioxidant activity[5,8]. Reflecting a notable degree of flavonoid antioxidants, the molecule identified in this work had a total flavonoid content (TFC) of 2.1 mg QE/g. These findings are consistent with previous studies indicating that ethanol-extracted flaxseed, which is known to be an efficient solvent for phenolic compounds, has a high concentration of flavonoids and lignans[19,20]. The antioxidant activity of the isolated molecule was also evaluated using DPPH and ABTS radical scavenging tests. While the ABTS test produced a TEAC of 4.698 µmol/mg and an RSA of 1.606%, the DPPH test produced an RSA of 2.90% and a Trolox Equivalent Antioxidant Capacity (TEAC) of 8.05 µmol/mg.Though less potent than synthetic antioxidants or more complex

phytochemical combinations, these compounds' partial antioxidant activities show that theyhelp to reduce oxidative stress[20]. Molecular docking was done using the Schrodinger software suite in an effort to understand the molecular mechanism of its antioxidant activity. With a docking score of -5.299, the chemical exhibited good hydrogen bonding interactions with important amino acid residues such TYR251, SER288, and LYS221, as well as strong binding potential to the NFκB (PDB ID: 1IKN). These results suggest that by keeping p-coumaric acid in its latent phase, it may prevent NFκB activation, hence reducing the inflammatory and oxidative reaction. Further computational and experimental data in plant antioxidants aimed at the NF-kB pathway supports this outcome[9]. All things considered, the study supports the idea that via altering oxidative stress pathways at the molecular level and scavenging free radicals, flaxseed phenolics, especially p-coumaric acid, may be influencing antioxidant defense systems. Docketing information increases the relevance of the chemical by offering a mechanistic justification for its claimed antioxidant action. These results are in line with previous studies that have reported the high antioxidant and anti-inflammatory activities of flaxseed extracts, especially those that contain phenolic and flavonoid contents[5,7,20]. Soxhlet extraction, the extraction method utilized, was also efficient and produced a more concentrated bioactive than traditional maceration methods. As has been seen before in similar studies of oilseed crops, the high extraction rate is due to heat-assisted diffusion and improved penetration of the solvent[12,19].

CONCLUSION

In conclusion, this study confirms that p-coumaric acid, isolated from the seeds of *Linum usitatissimum*, exhibits moderate antioxidant potential and favorable binding affinity toward NF-κB, a central regulator of oxidative stress and inflammatory responses. The combined application of *in vitro* antioxidant assays and in silico molecular docking underscores its dual role as a free radical scavenger and a prospective modulator of inflammatory pathways. These findings highlight the therapeutic potential of flaxseed-derived phenolic compounds in the development of nutraceuticals and natural anti-inflammatory agents. Furthermore, the study reinforces the utility of computational tools in unraveling molecular mechanisms of bioactive compounds.

Future investigations will aim to assess the in vivo efficacy of p-coumaric acid in models of oxidative stress and inflammation. Additional studies will also focus on its pharmacokinetic profile, bioavailability, and the potential development of formulations for use as a dietary supplement or plant-based therapeutic agent.

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Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Authors Contributions

Sunaina: Original draft, Methodology, Investigation, Formal analysis. Phool Chandra:C onceptualization,Investigation, Formal analysis, Visualization, Software, Writing–review & editing, Supervision

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