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IN-VITRO EVALUATION OF ANTIOXIDANT AND ANTIULCER ACTIVITIES OF ETHANOLIC EXTRACT OF *BRASSICA OLERACEA VAR. GONGYLODES*

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ABSTRACT

Brassica oleracea var. gongylodes is a nutrient-rich cruciferous vegetable known for its health-promoting properties, yet its antioxidant and antiulcer activities have not been systematically evaluated. The present study aimed to investigate the phytochemical profile, *in vitro* antioxidant potential, and antiulcer activitity of its ethanolic extract. Qualitative phytochemical screening revealed the presence of carbohydrates, proteins and amino acids, flavonoids, phenolic compounds, and saponins. Antioxidant activity was assessed using DPPH radical scavenging, ferric reducing power, and phosphomolybdate assays, along with determination of total phenolic content. The extract demonstrated significant antioxidant potential, with a IC_{50} of 57.2 µg/ml in DPPH assay, in Ferric reducing power 100 µg/ml was equivalent to 74.4 µg/ml of ascorbic acid, in Phosphomolybdenum activity 30 µg/ml extract was equivalent to 23.37 µg/ml of ascorbic acid, and total phenolic content of 79.2 µg/ml gallic acid equivalents per 100 µg/ml of extract. In addition, antiulcer activity was evaluated through *in vitro* pepsin inhibition and protein denaturation inhibition assays. The extract exhibited strong inhibitory effects with a pepsin inhibition IC_{50} of 10.33 µg/ml and protein denaturation inhibition IC_{50} of 96.12 µg/ml, indicating protective potential against ulcer-related mechanisms. These findings suggest that kohlrabi extract possesses notable antioxidant and antiulcer activities, largely attributable to its phytoconstituents and highlighting its potential as a natural therapeutic agent.

1.INTRODUCTION:

Brassica oleracea var. gongylodes (kohlrabi) is a nutrient-dense cruciferous vegetable belonging to the family Brassicaceae. Its primary centre of origin is believed to be Northern Europe. In India, it is commonly cultivated in regions such as Jammu-Kashmir and West Bengal [(1)]. Similar to other cruciferous vegetables, kohlrabi is rich in vitamins, minerals, dietary fiber, and bioactive phytochemicals including glucosinolates, flavonoids, and phenolic acids, which contribute to its strong antioxidant and disease-preventive properties [(2)].

Oxidative stress occurs when the production of reactive oxygen species (ROS) exceeds the body's antioxidant defense capacity. This imbalance can damage vital biomolecules such as DNA, proteins, and lipids, contributing to aging and the development of various diseases, including cancer, diabetes, and cardiovascular disorders. Therefore, maintaining equilibrium between ROS and antioxidants is essential for overall health. Antioxidants are compounds that protect the body by preventing or minimizing ROS-induced

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damage to lipids, proteins, carbohydrates, and DNA [3].

An ulcer is a break in the gastrointestinal lining that develops when aggressive factors such as gastric acid, pepsin, and Helicobacter pylori overpower the natural defenses like mucus, bicarbonate, prostaglandins, and mucosal blood flow. The most common type, known as peptic ulcer, occurs either in the stomach as a gastric ulcer or in the duodenum as a duodenal ulcer. Hence, antiulcer agents act by reducing gastric acid secretion, inhibiting pepsin activity, neutralizing existing acid, or enhancing the protective mechanisms of the mucosa. These include proton pump inhibitors, histamine H_2 receptor antagonists, antacids, mucosal protective agents, prostaglandin analogs, and herbal preparations with gastroprotective activity [4].

Although cruciferous vegetables are well recognized for their antioxidant properties, scientific evidence regarding the antiulcer potential of kohlrabi remains limited. Considering its rich phytochemical composition, kohlrabi may provide combined antioxidant and antiulcer benefits. The present study was designed to evaluate the *in vitro* antioxidant and antiulcer activities of kohlrabi extracts. Antioxidant potential was examined using DPPH, ferric reducing power, phosphomolybdenum, and total phenolic content assays, while antiulcer activity was assessed through pepsin enzyme inhibition, protein denaturation inhibition and acid neutralization assays. The outcomes aim to generate preliminary evidence supporting the therapeutic role of kohlrabi in managing oxidative stress–induced gastric disorders.

2.MATERIALS AND METHODOLOGY:

2.1 Collection and Authentication of Kohlrabi: The fresh Kohlrabi was procured from the Bharathinagar (K. M. Doddi) Market, Maddur Taluk, Mandya District, Karnataka, India. The collected material was identified and authenticated by Dr. Thejesh Kumar M. P., Coordinator, Department of Botany, Postgraduate and Research Centre, Bharathi College, Bharathinagar, Mandya District, Karnataka, and was used for further experimental studies.

2.2 Preparation of Extract [5]: The collected kohlrabi was washed, sliced, shade-dried at room temperature, and coarsely powdered using a mixer grinder. About 65 g of coarse powder was macerated with hydroalcoholic solvent (80% ethanol:20% water) in a 1:10 ratio (w/v) for 48 h with intermittent shaking and magnetic stirring. The mixture was filtered, and the filtrate was dried at room temperature to yield a semi-solid extract.

2.3 Solubility analysis: The solubility of the extract was tested in various solvents, including water, DMSO, chloroform, ethanol, methanol, ethyl acetate, petroleum ether, butanol, toluene, carbon tetrachloride, acetone, and benzene to determine the most suitable vehicle for *in-vitro* experiments.

2.4 Phytochemical screening [6,7]: Preliminary phytochemical screening of the extract was carried out to identify the presence of alkaloids, glycosides, flavonoids, carbohydrates, proteins, amino acids, saponins, and phenolic compounds.

2.5 Antioxidant activity:

2.5.1 DPPH Free Radical Scavenging Assay [8]: The antioxidant activity of the kohlrabi extract was assessed by the DPPH method, which is based on the reduction of the stable free radical 2,2-diphenyl-2-picrylhydrazyl (DPPH). DPPH exhibits a deep violet color that turns yellow upon reduction to DPPH-H by hydrogen-donating antioxidants, with the decrease in absorbance indicating free radical scavenging potential. Different concentrations of the extract (20–100 µg/ml), prepared in water and the final volume was adjusted to 3 ml with DPPH. After thorough mixing, the reaction mixtures were incubated in the dark at room temperature for 20 minutes, and absorbance was measured at 517 nm using a UV–visible spectrophotometer. The IC_{50} value of the extract was calculated.

2.5.2 Ferric Reducing Power Assay [9]: The reducing power of the kohlrabi extract was evaluated based on its ability to reduce the Fe^{3+} /ferricyanide complex to Fe^{2+} , which subsequently forms Prussian blue upon reaction with ferric chloride, showing

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maximum absorbance at 700 nm. Different concentrations of the extract (20-100 µg/ml) and standard were mixed with 2.5 ml of phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide, followed by incubation at 50 °C for 20 minutes. After cooling, 2.5 ml of 10% trichloroacetic acid was added, and the mixture was centrifuged at 3000 rpm for 10 minutes. From the supernatant, 5 ml was combined with 5 ml of distilled water and 1 ml of freshly prepared 0.1% ferric chloride solution. The absorbance was measured at 700 nm using a UV–visible spectrophotometer, and the reducing power was expressed as the ascorbic acid equivalent concentration.

2.5.3 Phosphomolybdenum Assay [10]: The total antioxidant capacity of the kohlrabi extract was determined by the phosphomolybdenum method, which is based on the reduction of Mo(VI) to Mo(V) by antioxidants, forming a green-colored phosphomolybdenum complex under acidic conditions with maximum absorbance at 695 nm. Different concentrations of the extract (6-30 µg/ml) and standard were mixed with 3 ml of phosphomolybdenum reagent, and the tubes were sealed and incubated in a water bath at 95°C for 90 minutes. After cooling to room temperature, absorbance was measured at 695 nm against a reagent blank using a UV–visible spectrophotometer. The antioxidant activity was calculated from a standard calibration curve of ascorbic acid and expressed as ascorbic acid equivalent (AAE) per microgram of extract.

2.5.4 Estimation of Total Phenolic Content (Folin–Ciocalteu Method) [11]: The total phenolic content of the kohlrabi extract was determined using the Folin–Ciocalteu method, which is based on the reduction of the Folin–Ciocalteu reagent by phenolic compounds to form a blue-colored complex. The intensity of the color, measured at 765 nm, is directly proportional to the phenolic content. Different concentrations of the extract (20-100 µg/ml) and standard (gallic acid) were mixed with 5 ml of 10% Folin–Ciocalteu reagent and allowed to stand for 5 minutes at room temperature. Then, 4 ml of 7% sodium bicarbonate was added, and the mixtures were incubated in the dark for 30 minutes. Absorbance was recorded at 765 nm using a UV–visible spectrophotometer. The total phenolic content was calculated from a gallic acid standard calibration curve and expressed as gallic acid equivalents (GAE) per microgram of extract.

2.6 Antiulcer activity:

2.6.1 Pepsin enzyme Inhibition Assay [12,13]: The pepsin inhibition assay is based on the ability of the test extract to inhibit the proteolytic activity of pepsin, which normally hydrolyses casein into smaller peptides and amino acids under acidic conditions. The extent of protein hydrolysis can be measured spectrophotometrically at 280 nm. In the presence of an inhibitor, the breakdown of casein is reduced, resulting in lower absorbance. Different concentrations of the kohlrabi extract (5–25 µg/ml) were made up to a final volume of 1 ml using 0.06 N HCl and incubated with 1 ml of 0.5% casein solution and 1 ml of pepsin (1 mg/ml) at 37 °C for 20 minutes. The reaction was terminated by adding 2 ml of 5% trichloroacetic acid, and the mixture was filtered to remove precipitated proteins. The absorbance of the clear supernatant was measured at 280 nm against a blank, and the percentage inhibition of pepsin activity was calculated by comparing the absorbance with that of the control.

2.6.2 Protein Denaturation Assay [14]: The protein denaturation assay evaluates the ability of test compounds to prevent heat-induced denaturation of proteins, which is one of the factors contributing to inflammation and ulceration. Egg albumin from egg flakes was used as the protein source, and the protective effect of the kohlrabi extract was measured by the reduction in turbidity at 660 nm. Different concentrations of the kohlrabi extract (20–100 µg/ml) were prepared in water and made up to a final volume of 1 ml. Each was mixed with 1 ml of 1% egg albumin, and the pH was adjusted to 6.4. The mixtures were incubated at room temperature for 20 minutes, followed by heating at 55 °C for 30 minutes in a water bath. After cooling, the absorbance of the reaction mixture was measured at 660 nm using a spectrophotometer. A mixture of egg albumin without extract served as the

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control, and the percentage inhibition of protein denaturation was calculated relative to the control.

2.6.3 Acid Neutralization Assay [15]: The acid neutralization assay assesses the ability of a test sample to neutralize gastric acid, an important mechanism for preventing or treating ulcers. Substances with anti-ulcer potential reduce acidity by neutralizing hydrogen ions, thereby protecting the gastric mucosa. 100 mg of Kohlrabi extract was mixed with 30 ml of 0.1 N hydrochloric acid and incubated at room temperature for a fixed period. After incubation, the mixture was titrated against 0.1 N sodium hydroxide using phenolphthalein as an indicator until a persistent light pink color appeared. The volume of sodium hydroxide required to neutralize the acid in the presence of the extract was recorded, and the percentage of acid neutralization was calculated relative to the control (acid without extract). Higher neutralization values indicate stronger anti-ulcer potential of the test sample.

3. RESULTS:

3.1 Solubility analysis: The extract exhibited good solubility in water, ethanol, butanol, acetic acid and DMSO. This evaluation facilitated the identification of appropriate solvents for *in vitro* studies. Among these, water was selected as the vehicle for conducting the assays.

3.2 Phytochemical Investigation: Qualitative phytochemical screening of the ethanolic extract of *Brassica oleracea var. gongylodes* was carried out using standard methods. The results are presented table in 1.

3.3 Antioxidant activity:

3.3.1 DPPH Radical Scavenging Assay: The kohlrabi extract exhibited a concentration-dependent increase in radical scavenging activity. The IC_{50} value was determined to be 57.2 µg/ml. The DPPH radical scavenging activity of extract is presented in Table 2 and Figure 1.

3.3.2 Ferricyanide Reducing Power Assay (FRAP): The extract exhibited reducing power in a concentration-dependent manner. The reducing activity of the extract is compared to the standard ascorbic acid. The 100µg/ml extract showed activity equivalent to 74.4µg/ml of ascorbic acid. The ferric reducing activity of the extract is presented in Table 3 and Figure 2.

3.3.3 Phosphomolybdenum Assay: The phosphomolybdate ion reducing activity of both the extract and the standard (ascorbic acid) increased proportionally with concentration. At 30 µg/ml, the extract demonstrated antioxidant activity equivalent to 23.37 µg/ml of ascorbic acid. The results are presented in Table 4 and Figure 3.

3.3.4 Estimation of Total Phenolic Content: The total phenolic content of the kohlrabi extract was expressed as gallic acid equivalents. The phenolic content of 100 µg/ml of the extract was found to be equivalent to 79.2 µg/ml of gallic acid. The results are presented in Table 5 and Figure 4.

3.4 Antiulcer Activity:

3.4.1 Pepsin enzyme Inhibition Assay: The extract exhibited dose-dependent inhibition of pepsin activity. The IC_{50} value of the extract was determined to be 10.33 µg/ml. The pepsin inhibition potential of the kohlrabi extract is presented in Table 6 and Figure 5.

3.4.2 Protein Denaturation Assay: The extract exhibited concentration-dependent inhibition of protein denaturation. The IC_{50} value of the extract was 96.12 µg/ml. The protein denaturation inhibitory activity of the extract is presented in Table 7 and Figure 6.

3.4.2 Acid neutralization assay: The pH of the kohlrabi extract was initially measured using pH paper and found to be in the range of 6–7, indicating a slightly acidic nature. To further confirm this, an acid neutralisation assay was performed. In the standard titration, 30 ml of 0.1 N HCl required 24.6 ml of 0.1 N NaOH for neutralisation. When 100 mg of the extract was dissolved in 30 ml of 0.1 N HCl, it consumed 28.4 ml of NaOH for neutralisation. These results confirm that the kohlrabi extract is weakly acidic in nature. Since it does not possess significant gastric acid neutralisation capacity, it is unlikely to act through an antacid mechanism.

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Table 1: Preliminary phytochemical screening of the extract

S.no	Phytochemical compounds	Ethanolic extract of kohlrabi
1	Alkaloids	-
2	Glycosides	-
3	Flavonoids	+
4	Phenolic compounds	+
5	Saponins	+
6	Carbohydrates	+
7	Proteins and amino acids	+

Note: (+) indicates presence of phytochemicals, (-) indicates absence of phytochemicals

Table 2: Inhibitory effect of Kohlrabi extract on DPPH radical scavenging activity

S.no	Concentration(µg/ml)	% inhibition	IC50 value (µg/ml)
1	20	41.5	57.2
2	40	43	
3	60	51.1	
4	80	54.6	
5	100	62.3	

Figure 1: Scatter graph of DPPH radical scavenging activity of Kohlrabi extract

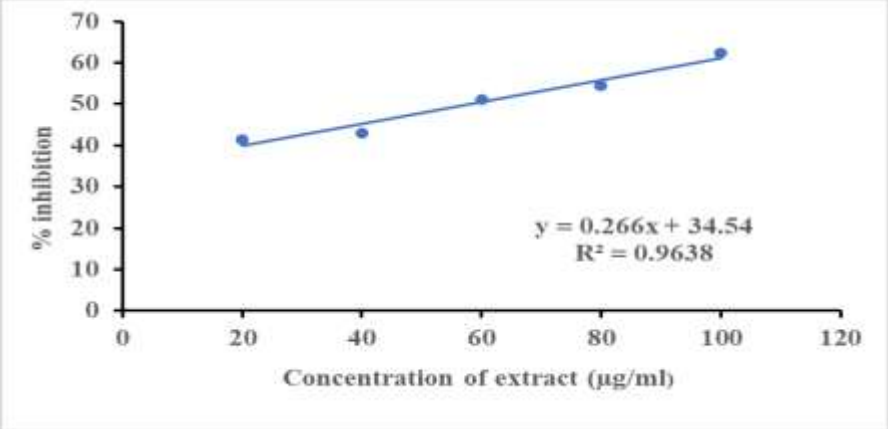

Table 3: Concentration and absorbance values of Ascorbic acid and Kohlrabi extract

Sample	Concentration(µg/ml)	Absorbance
Standard (Ascorbic acid)	20	0.340
	40	0.447
	60	0.542
	80	0.657
	100	0.667
Extract	20	0.281
	40	0.354
	60	0.429
	80	0.515
	100	0.609

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Figure 2: Scatter graph of FRAP assay of Ascorbic acid and Kohlrabi extract

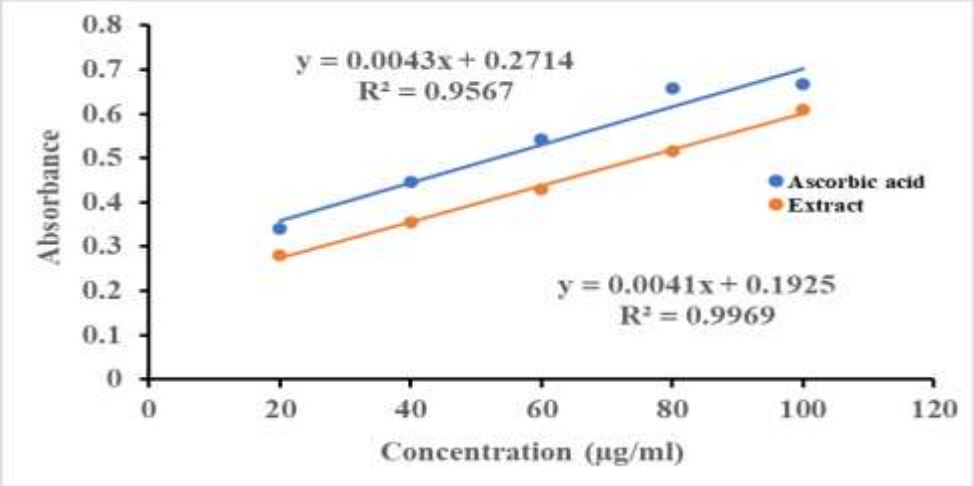

Table 4: Concentration and absorbance values of Ascorbic acid and Kohlrabi extract

Sample	Concentration(µg/ml)	Absorbance
Standard (Ascorbic acid)	6	0.348
	12	0.451
	18	0.562
	24	0.635
	30	0.734
Extract	30	0.619

Figure 3: Scatter graph of Phosphomolybdenum assay of Ascorbic acid

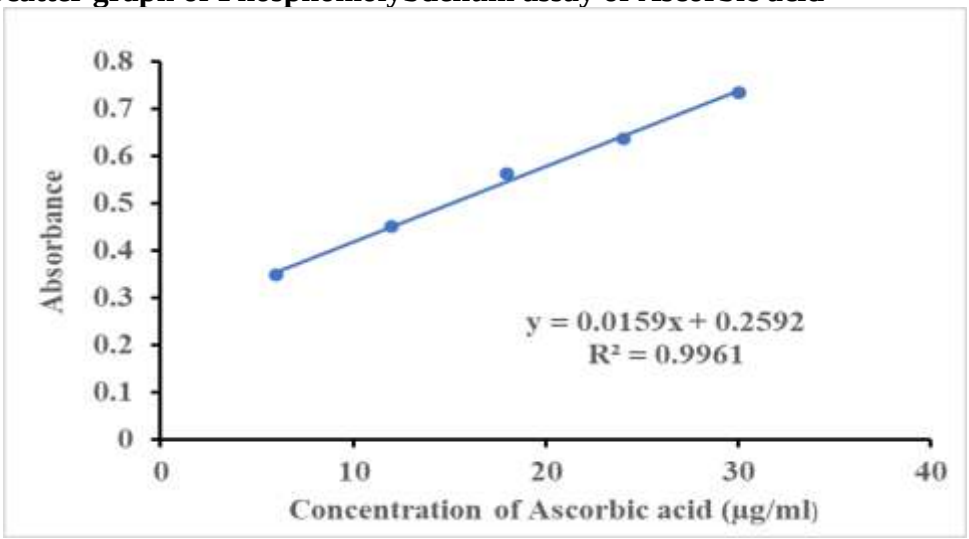

Table 5: Concentration and absorbance values of Gallic acid and Kohlrabi extract

Sample	Concentration(µg/ml)	Absorbance
Standard (Gallic acid)	20	0.176
	40	0.298
	60	0.352
	80	0.419
	100	0.537
Extract	100	0.416

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Figure 4: Scatter graph of Estimation of Total Phenolic Content of Gallic acid

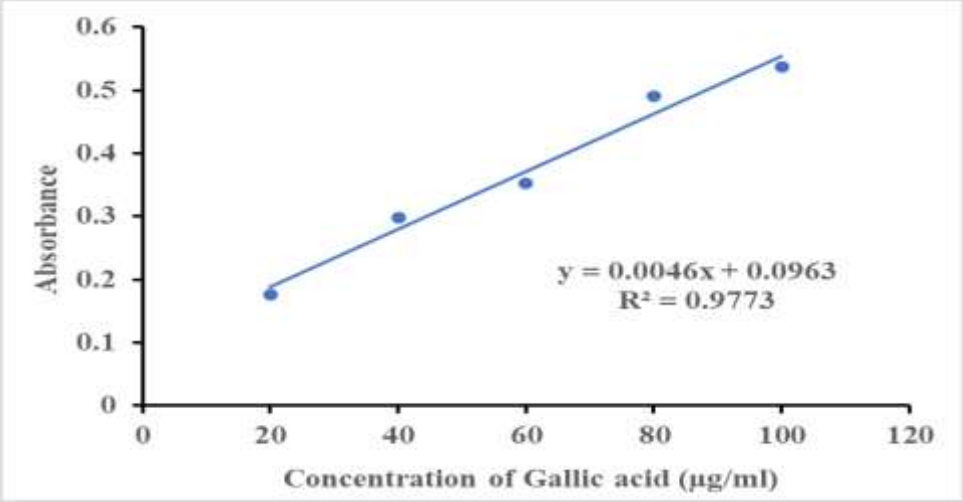

Table 6: Inhibitory effect of Kohlrabi extract on Pepsin enzyme activity

S. no	Concentration (µg/ml)	% inhibition	IC_{50} value (µg/ml)
1.	5	38.3	10.33
2.	10	49.9	
3.	15	51.4	
4.	20	54.1	
5.	25	54.9	

Figure 5: Scatter graph of Pepsin inhibition assay of Kohlrabi extract.

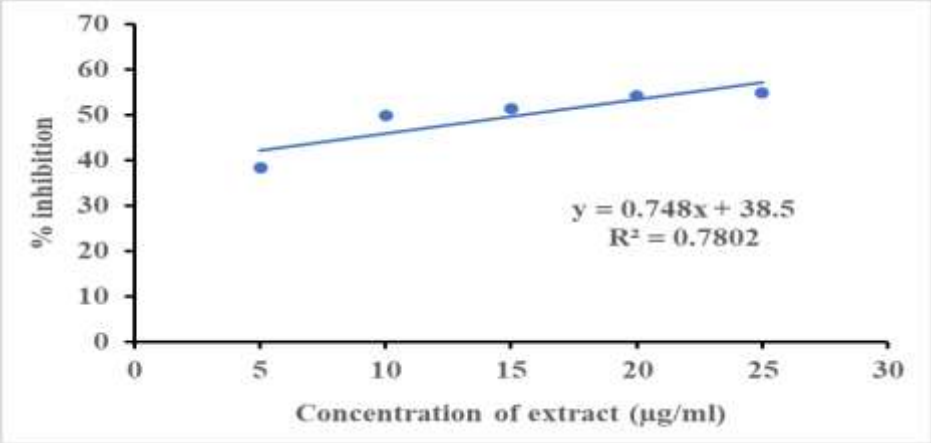

Table 7: Inhibitory effect of Kohlrabi extract on Protein denaturation

S. no	Concentration(µg/ml)	% inhibition	IC_{50} value (µg/ml)
1	20	15.0	96.12
2	40	29.6	
3	60	37.3	
4	80	45.1	
5	100	51.2	

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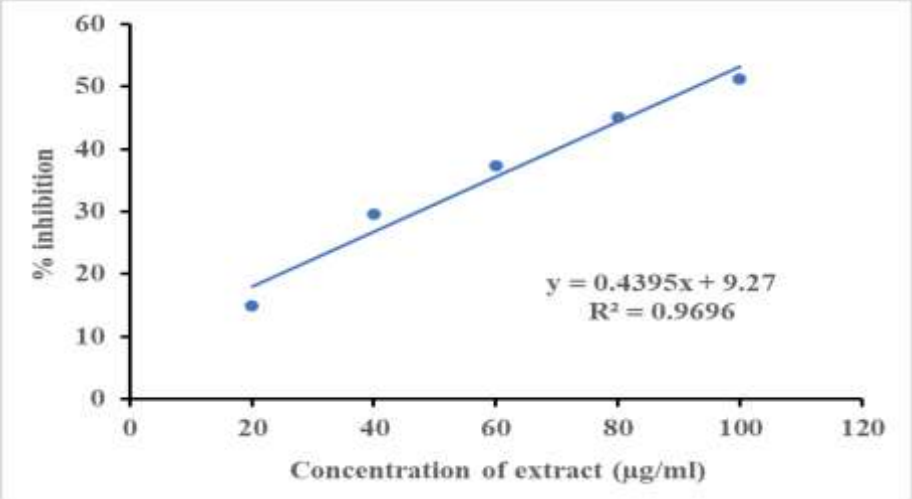
Figure 6: Scatter graph of Protein denaturation inhibition assay of Kohlrabi extract.



DISCUSSION:

The etiology of peptic ulcer is largely unknown, but it is attributed to an imbalance between mucosal defence and aggressive factors. Excessive gastric acid secretion, denaturation of proteins in the gastric mucosa, and increased proteolytic activity of pepsin can contribute to ulcer formation. Compounds that counteract these effects are considered potential antiulcer agents [16].

Qualitative phytochemical screening of the ethanolic extract of kohlrabi revealed the presence of carbohydrates, proteins and amino acids, flavonoids, phenolic compounds, and saponins. These phytoconstituents are known to play important roles in biological activities, particularly antioxidant and antiulcer effects.

The antioxidant activity of the extract was confirmed by multiple *in vitro* assays. The DPPH radical scavenging assay demonstrated a concentration-dependent activity with an IC_{50} of 57.2 µg/ml, indicating significant radical-quenching potential. This effect is likely attributed to the phenolic and flavonoid components, which can donate hydrogen atoms to stabilize free radicals. The ferric reducing power assay further substantiated the antioxidant nature of the extract, showing a strong electron-donating ability. At a concentration of 100 µg/ml, the extract exhibited activity equivalent to 74.4 µg/ml of ascorbic acid. Likewise, the phosphomolybdate assay confirmed its total antioxidant capacity, where 30 µg/ml of extract showed activity equivalent to 23.37 µg/ml of ascorbic acid. The high total phenolic content, expressed as 79.2 µg/ml gallic acid equivalents per 100 µg/ml of extract, emphasizes the central role of polyphenols in mediating these effects.

Beyond antioxidant activity, the extract also exhibited antiulcer potential. The pepsin inhibition assay showed a strong inhibitory effect with a low IC_{50} value of 10.33 µg/ml, suggesting that the extract can modulate proteolytic activity in the gastric environment. In addition, inhibition of protein denaturation with an IC_{50} of 96.12 µg/ml supports its ability to protect proteins from structural damage under stress conditions, a mechanism often implicated in ulcer formation. These findings are consistent with reports on other cruciferous vegetables, where secondary metabolites such as flavonoids and phenolics are known to exert gastroprotective effects through antioxidant and enzyme-modulating mechanisms.

CONCLUSION:

The present study demonstrated that *Brassica oleracea var. gongylodes* extracts exhibit significant *in vitro* antioxidant and antiulcer activities. Antioxidant effects, confirmed by DPPH radical scavenging, ferric reducing power, and phosphomolybdenum assay, estimation of total phenolic content. The antiulcer potential, evidenced by pepsin enzyme inhibition assay and protein denaturation inhibition assays, suggests a protective effect against gastric ulcer. These findings

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highlight kohlrabi as a promising source of natural therapeutic agents. However, further *in vivo* studies and clinical evaluations are required to validate its efficacy and support its potential therapeutic applications.

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CONFLICT OF INTEREST: The authors declare no conflict of interest.

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