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# A COMPARATIVE STUDY ON KINETICS OF LIPID OXIDATION (RATE OF MALONDIALDEHYDE FORMATION) OF ASSORTED INDIAN SPICES UNDER ACCELERATED OXIDATION CONDITION IN EDIBLE OILS SYSTEMS AND THEIR ANTIOXIDANT, ANTILIPIDEMIC AND ANALGESIC ACTIVITY

Banani De\*1 and Payel Mondal1

#### **ABSTRACT**

This study examines the lipid oxidation kinetics and evaluates the antioxidant, antilipidemic and analgesic properties of selected Indian spices-clove, cinnamon, and turmeric-combined with edible oils like mustard and sunflower oil. Lipid peroxidation was measured using the thiobarbituric acid (TBA) assay, with malondialdehyde (MDA) as an indicator of oxidative degradation. Antioxidant activity was assessed through DPPH and FRAPS assays, while analgesic potential was evaluated using the nitric oxide (NO) inhibition method. Among the tested samples, clove consistently showed greater ability to reduce lipid oxidation, free radical scavenging activity and NO production. Turmeric and cinnamon also demonstrated beneficial effects, though with comparatively lower activity. Mustard oil exhibited better oxidative stability than sunflower oil and 50-50 oil blend further improved stability. Overall, the incorporation of these spices into edible oils may provide a natural method to reduce oxidative stress, support lipid balance and assist in inflammation management. These findings suggest practical applications in the development of functional foods and natural therapeutic formulations.

#### **KEYWORDS**

Lipid peroxidation, Antioxidant, Thiobarbituric acid, Malondialdehyde, Analgesic activity and Spectrophotometer.

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#### INTRODUCTION

Lipids are vital nutrients for humans, playing a key role in numerous functional and regulatory processes in the body<sup>1</sup>. They are also essential for the structural development of human tissues. Plants are valuable source of fats and oils, commonly used in cooking and food preparation. For example, oils extracted from seeds, such as sunflower and

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mustard oils, are widely consumed for their culinary benefits<sup>2</sup>. These plant oils are rich in essential fatty acids, including linoleic, linolenic, oleic, palmitic, and stearic acids<sup>3</sup>.

Lipid oxidation in common cooking oils leads to the deterioration of quality, causing off-flavors, rancidity, and a reduction in nutritional value, especially during prolonged storage or high-temperature cooking<sup>4</sup>. Therefore, in the food industry, understanding the lipid oxidation process, slowing it down, and enhancing lipid stability are crucial for ensuring dietary safety and health, which has increasingly drawn the attention of scientists<sup>5</sup>.

Lipid peroxidation is initiated by the abstraction of a hydrogen atom from a polyunsaturated fatty acid by reactive oxygen species, forming a lipid radical, which reacts with molecular oxygen to form a lipid peroxyl radical. This propagates the chain reaction leading to the accumulation of lipid hydroperoxides, which decompose into secondary oxidation products such as malondialdehyde (MDA)<sup>6</sup>.

Malondialdehyde (MDA) is often used as an indicator for lipid peroxidation. The thiobarbituric acid (TBA) assay is commonly employed to quantify MDA levels through spectrophotometric analysis, reflecting the extent of oxidative damage. Due to MDA's reactivity with TBA, this reaction forms the basis of thiobarbituric acid reactive substances (TBARS) measurement, a standard method in oxidative stress studies<sup>7,8</sup>.

To counteract oxidative damage, antioxidants-both synthetic and natural-play a significant role. Natural antioxidants, especially from plant sources like spices, fruit are increasingly favoured due to concerns over the safety of synthetic compounds such as BHA (Butylated Hydroxyanisole) and BHT (Butylated Hydroxytoluene)<sup>1,9-11</sup>. Compounds such as tocopherols, flavonoids, and polyphenols can neutralize ROS, thus preventing lipid peroxidation and contributing to overall health<sup>12,13</sup>.

Beyond their antioxidant effects, natural compounds also show antilipidemic activity by lowering blood lipid levels, especially LDL cholesterol-a key risk factor for cardiovascular disease<sup>14</sup>. They may enhance lipid metabolism or inhibit absorption<sup>15</sup>.

Kinetic studies on lipid oxidation in the presence of

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antioxidants can offer insights into how these compounds slow down or inhibit oxidation reactions, indirectly suggesting their antilipidemic effects by preserving lipid integrity and preventing harmful by-product formation.

Since elevated NO levels are associated with inflammation and pain signalling, a reduction in nitrite concentration indicated by lower absorbance can suggest potential analgesic activity. A colorimetric assay used to detect nitrite, a stable end product of nitric oxide (NO) oxidation. It involves the reaction of nitrite with sulfanilic acid and 1-naphthylamine to form a pink azo dye, which can be measured spectrophotometrically. In experimental settings, this assay is used to evaluate the impact of test substances on NO production<sup>16</sup>. This method provides a useful way to test samples for their effects on nitric oxide (NO), a signalling molecule involved in inflammation and pain<sup>17</sup>.

This study investigates the lipid oxidation, antioxidant, antilipidemic, and analgesic properties of mustard oil, sunflower oil and their combinations with cinnamon, clove and turmeric. By applying kinetic modelling and spectrophotometric assays, this research aims to understand how these natural additives affect oxidative stability, LDL-associated lipid changes and pain modulation. The findings may provide valuable insight into the development of functional foods with therapeutic potential as both natural antioxidants and antilipidemic agents.

Samples used in the study are as follows:

#### Mustard oil (Brassica juncea)

Mustard oil is a popular cooking oil in many cultures, particularly in South Asia, known for its flavour and long shelf-life. Mustard oil is rich in monounsaturated fatty acids (MUFA) like oleic acid and erucic acid, as well as polyunsaturated fatty acids (PUFA) like linoleic acid and alpha-linolenic acid (omega-3). Inhibits microbial growth, supports cardiovascular health, and reduces inflammation<sup>18</sup>.

#### Sunflower oil (*Helianthus annuus*)

Sunflower oil is a popular edible oil derived from the seeds of the sunflower plant. It's rich in linoleic acid (a polyunsaturated fat) and oleic acid (a monounsaturated fat). Saturated fatty acids like palmitic and stearic acids are also present in

sunflower oil. Sunflower oil offers various benefits, including being a good source of vitamin E, particularly alpha-tocopherol, which acts as an antioxidant and is used in cooking, cosmetics and other applications <sup>19,3</sup>.

#### Clove (Syzygium aromaticum)

Clove is a spice and medicinal herb with a range of benefits due to its chemical composition. Key components include eugenol, eugenyl acetate and beta-caryophyllene<sup>20</sup>. These compounds contribute to clove's antioxidant, antimicrobial, anti-inflammatory and analgesic properties<sup>21,22</sup>.

#### Cinnamon (Cinnamomum verum)

The primary chemical component contributing to cinnamon's flavour and aroma is cinnamaldehyde, along with other compounds like eugenol and cinnamic acid<sup>23</sup>. Cinnamon offers various health benefits, including antioxidant, anti-inflammatory and antimicrobial properties. Cinnamon is a widely used flavouring agent. Cinnamon has a long history of use in traditional medicine systems like Ayurveda<sup>24</sup>.

#### Turmeric (Curcuma longa)

Turmeric, scientifically known as *Curcuma longa*, is a perennial herb from the ginger family used as a spice, preservative, and colouring agent. It's known for its numerous medicinal properties, including anti-inflammatory, antioxidant, and antimicrobial effects. Turmeric's chemical composition primarily includes curcuminoids, specifically curcumin, which is a key component responsible for many of its health benefits. Turmeric also contains essential oils, terpenes, and other phytochemicals<sup>25,26</sup>.

# MATERIAL AND METHODS COLLECTION OF SAMPLES AND CHEMICALS SAMPLES

Spices such as clove, cinnamon and turmeric were carefully sourced from local markets in Howrah. Cold-pressed mustard oil and sunflower oil were obtained from seeds that were processed in local factories and collected for research purposes.

#### **Chemicals**

Thiobarbituric Acid (TBA) and 2, 2-diphenyl-1picrylhydrazyl (DPPH) were purchased from Sisco Research Laboratories Private Limited. Glacial

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acetic acid, ethanol, sulphanilamide, 1-naphthylamine, hydrochloric Acid (HCl), ascorbic acid, potassium phosphate, potassium ferricyanide, ferric chloride, trichloroacetic acid, potassium dihydrogen phosphate, potassium chloride, distilled water. All the chemicals were purchased from Merck Specialties Private Limited.

#### **Apparatus**

SYSTRONICS Spectrophotometer 106 was used in this experiment.

#### **Preparation of samples**

For the preparation of clove (Syzygium aromaticum), cinnamon (Cinnamomum verum), and turmeric (Curcuma longa) extracts, solutions of 10mg/mL, 20mg/mL and 50mg/mL concentrations were prepared. For the aqueous extracts, 0.15g, 0.3g, and 0.75g of the respective materials were weighed and dissolved in 15mL of distilled water for each concentration. Similarly, for the alcoholic extracts, 0.25g, 0.5g and 1.25g of the materials were weighed and dissolved in 25mL of alcohol, maintaining the same concentrations (10mg/mL, 20mg/mL and 50mg/mL).

## EXPERIMENTAL PROCEDURE TBA (Thiobarbituric assay for lipid peroxidation)

The TBA assay was conducted to measure malondialdehyde (MDA), a key marker of lipid peroxidation. Fusion tubes were prepared for the experimental setup, with oils (mustard, sunflower and 1:1 blend) and spice powders thoroughly mixed to ensure homogeneity. Each fusion tube was labelled according to the experimental group, including oil-only controls and mixtures containing cinnamon, clove, or turmeric. The prepared tubes were incubated at 37°C for various time intervals (e.g., 30, 60, 90, 120 and 150 minutes). This step allowed the lipid peroxidation process to occur under controlled conditions, simulating oxidative stress.

At each designated interval, a beaker containing the fusion tubes for that time point was removed from the incubator, and the contents were allowed to cool to room temperature. From each fusion tube, 1 mL of the oil–spice mixture was aliquoted into fresh test

tubes for further analysis. Freshly prepared TBA reagent (4mM in glacial acetic acid) was then added to each test tube in equal volume, initiating the colorimetric reaction with MDA<sup>27-29</sup>.

The test tubes were placed in a boiling water bath at 90°C for 30 minutes to enable the formation of a pink chromogen due to the reaction between TBA and MDA. Following incubation, the tubes were cooled rapidly under running tap water to stabilize the chromogen. The absorbance of the supernatant was measured at 532 nm using a spectrophotometer, with higher absorbance values indicating greater lipid peroxidation<sup>31</sup>.

#### DPPH (2, 2-diphenyl-1-picrylhydrazyl) method:

For the DPPH assay, antioxidant activity was assessed by preparing stock solutions of DPPH (24mg in 100mL ethanol) and spice extracts at various concentrations (10mg/mL, 20mg/mL, and 50mg/mL). One millilitre of the spice extract was mixed with 1mL of DPPH solution in a clean test tube. After thorough mixing, the samples were incubated in darkness at room temperature for 30 minutes to prevent light-induced degradation. The absorbance was measured at 517nm using a spectrophotometer and antioxidant activity was calculated using the formula for DPPH radical inhibition. A blank (DPPH solution without the sample) and ascorbic acid as a positive control were included for comparison 17.

### Ferric Reducing Antioxidant Power (FRAP) Assav

The ferric reducing antioxidant power of clove, cinnamon and turmeric extracts was determined using ethanolic solutions at concentrations of 10mg/mL, 20mg/mL and 50mg/mL. One millilitre of each extract was mixed with 2.5mL of 0.2M sodium phosphate buffer (pH 6.6) and 2.5mL of 1% potassium ferricyanide solution. The mixture was incubated at 50°C for 20 minutes. Following this, 2.5mL of 10% trichloroacetic acid was added and the mixture was centrifuged at 3,000rpm for 10 minutes. The supernatant was collected and combined with 2.5mL of distilled water and 0.5mL of 0.1% ferric chloride solution 32,33. The absorbance was measured at 700nm. The FRAP values of the spice extracts were quantified using a standard

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curve generated with ascorbic acid, and the results were expressed as µmol AAE/mg.

## Nitric Oxide (NO) Assay (Greiss–Ilosvay Method for Analgesic Activity)

The nitric oxide assay was carried out using the Griess reaction to evaluate nitrite levels in the aqueous spice extracts. To prepare the reaction mixture, 1mL of the spice extract was mixed with 1mL of sulfanilic acid solution (0.5% w/v in 1 M HCl) in a test tube, followed by incubation at room temperature for 5 minutes. Then, 1mL of 1-naphthylamine solution (0.1% w/v in 1 M HCl) was added, and the mixture was further incubated for 10 minutes to develop a pink coloration, indicating the presence of nitrite. The absorbance was recorded at 520-540nm. Ascorbic acid was employed as a positive control for comparison.

#### **Statistical analysis**

All the experiments were conducted in triplicate and data from the experiments were subjected to two-way analysis of variance (ANOVA) (P<0.05).

#### RESULTS AND DISCUSSION

Based on the results of the malondialdehyde (MDA) formation for lipid peroxidation, sunflower oil consistently exhibited the highest MDA levels, indicating greater susceptibility to oxidative degradation compared to mustard oil and the "50:50" blend of sunflower and mustard oil. Among the spice treatments, clove powder demonstrated significantly (p<0.05) higher antioxidant activity, leading to the most substantial reduction in MDA levels across all oil samples.

At 50 minutes, sunflower oil mixed with turmeric powder recorded the highest MDA values (3.03µmol/mL) highlighting its susceptibility to lipid oxidation. In contrast, the "50:50" blend of mustard and sunflower oil mixed with clove powder showed the lowest MDA levels (0.31µmol/mL), indicating the strongest antioxidant effect among all tested samples. Across different time intervals, the presence of clove consistently resulted lower lipid peroxidation compared to cinnamon and turmeric treatments.

Mustard oil exhibited moderate lipid oxidation, with MDA values ranging from 0.78 to 1.86µmol/mL, on

the basis of spice used. The blend of sunflower and mustard oil generally showed lower MDA accumulation, highlighting the protective effect of mustard oil's natural antioxidants. Clove powder significantly (p<0.05) reduced lipid oxidation across all oils.

Antilipidemic activity is closely related to lipid oxidation kinetics, with slower malondialdehyde (MDA) formation indicating greater lipid stability. Sunflower oil showed the fastest oxidation, while mustard oil and the "50:50" blend exhibited lower MDA accumulation over time. The addition of clove, cinnamon and turmeric significantly (p<0.05) reduced oxidation rates, with clove demonstrating the strongest antilipidemic effect by delaying MDA formation the most. Cinnamon and turmeric also exhibited some protective effects, though with slightly faster oxidation kinetics. The "50:50" sunflower-mustard oil blends likely has improved oxidative stability due to mustard oil's antioxidants, dilution of oxidation-prone fatty acids and fatty acid interactions that slow lipid peroxidation.

These findings indicate that spices contribute to improved lipid stability and exhibit antilipidemic effects (p < 0.05).

Based on the DPPH radical scavenging activity, consistently demonstrated significantly (p<0.05) higher scavenging activity across all tested concentrations when compared to cinnamon and turmeric. At 50mg/mL, clove achieved a scavenging activity of 88.36%, whereas cinnamon and turmeric exhibited considerable scavenging activity of 83.25% and 81.48%, respectively. At 20 mg/mL, clove recorded a scavenging activity of 81.83%, which was remarkably higher than cinnamon (76.01%) and turmeric (74.07%). Similarly, at 10mg/mL, clove again showed the highest scavenging activity of 76.72%, while cinnamon and turmeric maintained comparable results of 70.55% and 71.25%, respectively.

These observations highlight clove's greater radical scavenging ability, likely due to its high phenolic content. The variations in scavenging activity were statistically significant (p<0.05) for clove across all tested concentrations, reinforcing its effectiveness as an antioxidant.

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Based on the results of the ferric reducing antioxidant power (FRAP) assay, clove consistently exhibited significantly (p<0.05) higher antioxidant activity compared to cinnamon and turmeric across all tested concentrations, as measured using the spectrophotometer. At 50mg/mL, clove demonstrated the highest activity with a FRAP value of 145.95, which was greater than the values for cinnamon (138.75) and turmeric (137.81).

A similar trend was observed at 20mg/mL, where clove recorded a FRAP value of 84.70, significantly (p<0.05) outperforming cinnamon (81.20) and turmeric (77.03). At the lowest concentration of 10mg/mL, clove maintained its lead with a value of 56.87, while cinnamon and turmeric showed lower activity (39.68 for cinnamon and 38.72 for turmeric).

The closely comparable FRAP values between cinnamon and turmeric suggest that these spices possess similar levels of reducing power, while clove's significantly (p<0.05) higher values across all concentrations highlight its better electrondonating ability and antioxidant potential. The consistent statistical significance (p < 0.05) of clove's performance across all measurements emphasizes its efficacy in neutralizing free radicals. Based on the results of the nitric oxide (NO) assay analgesic activity determination, clove demonstrated significantly (p<0.05)higher inhibition percentages across most concentrations compared to cinnamon and turmeric. At 50mg/mL, clove exhibited the highest inhibition percentage, recording 88.89% in the spectrophotometer. These values were significantly (p<0.05) greater than those for cinnamon (72.22%) and turmeric (86.11%), although turmeric showed activity comparable to clove at this concentration. At 20mg/mL, clove again showed inhibition, with value of 80.56%, higher than cinnamon (69.44%) and turmeric (75.00%). Turmeric's activity was closer to clove at this concentration, showing a trend of moderately similar inhibition. At the lowest concentration of 10mg/mL, clove maintained its lead with inhibition percentages of 52.78%, significantly (p<0.05) higher than cinnamon (22.22%). Turmeric showed moderately higher

inhibition than cinnamon at this concentration, with values of 38.89%, yet it remained significantly (p<0.05) lower than clove.

Overall, clove demonstrated the highest analgesic activity across all tested concentrations, with significant differences observed at most levels (p<0.05). Turmeric exhibited intermediate activity, showing but occasionally comparable inhibition to clove at specific concentrations, while cinnamon consistently exhibited the lowest inhibition percentages. This data underscores clove's effective potential for analgesic activity compared to the other tested spices.

**Table No.1: TBA (Thiobarbituric assay for lipid peroxidation)** 

	MDA values in µmol/ml						
S.No	Time in minutes						
	Sample	0 min	10 min	20 min	30 min	50 min	
1	Mustard oil + Clove	$0.78\pm0.02$	1.1±0.065	1.27±0.015	1.39±0.035	1.52±0.034	
2	Sunflower oil + Clove	1.44±0.025	1.46±0.012	1.59±0.026	1.64±0.036	1.97±0.033	
3	Blend oil + Clove	0.18±0.016	0.19±0.011	0.24±0.007	0.29±0.067	0.31±0.01	
4	Mustard oil + Cinnamon	0.9±0.01	1.22±0.06	1.5±0.034	1.53±0.076	1.76±0.013	
5	Sunflower oil + Cinnamon	1.86±0.056	2.08±0.055	2.09±0.013	2.1±0.045	2.21±0.1	
6	Blend oil + Cinnamon	0.26±0.014	$0.29\pm0.02$	0.33±0.01	0.38±0.02	$0.24\pm0.014$	
7	Mustard oil + Turmeric	1.53±0.01	1.62±0.043	1.65±0.047	1.83±0.02	1.86±0.019	
8	Sunflower oil + Turmeric	2.82±0.06	2.85±0.045	2.91±0.01	2.96±0.035	3.03±0.03	
9	Blend oil + Turmeric	0.31±0.04	0.37±0.033	$0.40\pm0.02$	0.45±0.024	$0.32\pm0.043$	

Table No.2: DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay

	in spectrophotometer					
S.No	% of scavenging activity					
	Sample name	50mg/ml	20mg/ml	10mg/ml		
1	Clove	88.36±0.60	81.83±0.75	76.72±0.70		
2	Cinnamon	83.25±0.32	76.01±0.71	70.55±0.40		
3	Turmeric	81.48±0.26	74.07±0.40	71.25±0.46		

Table No.3: Ferric reducing antioxidant power (FRAP) assay

	FRAP Values in µmol AAE/mg					
S.No	In spectrophotometer					
	Sample Name	50 mg/mL	20 mg/mL	10 mg/mL		
1	Clove	$145.95 \pm 0.15$	$84.70 \pm 0.06$	$56.87 \pm 0.17$		
2	Cinnamon	$138.75 \pm 0.23$	$81.20 \pm 0.12$	$39.68 \pm 0.06$		
3	Turmeric	$137.81 \pm 0.10$	$77.03 \pm 0.21$	$38.72 \pm 0.26$		

Table No.4: Nitric Oxide (NO) Assay (Greiss–Ilosvay Method for Analgesic Activity)

	Percentage of inhibition					
S.No	In spectrophotometer					
	Sample names	50 mg/ml	20 mg/ml	10 mg/ml		
1	Clove	88.89±0.06	80.56±0.07	52.78±0.11		
2	Cinnamon	72.22±0.10	69.44±0.21	22.22±0.23		
3	Turmeric	86.11±0.01	75.00±0.06	38.89±0.24		

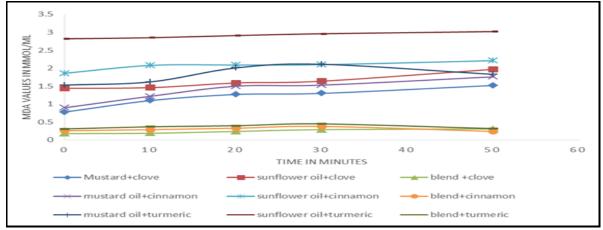


Figure No.1: MDA Levels over time in different Oil-Spice Combinations

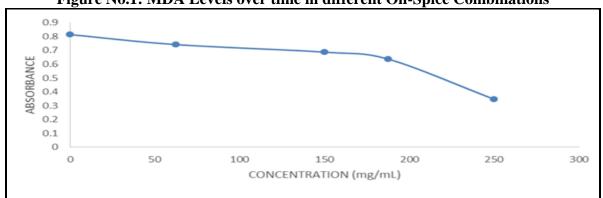


Figure No.2: Standard curve of Ascorbic acid in DPPH radical scavenging Assay

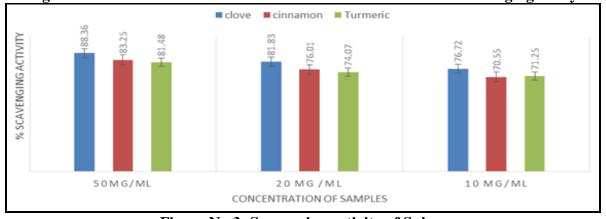


Figure No.3: Scavenging activity of Spices

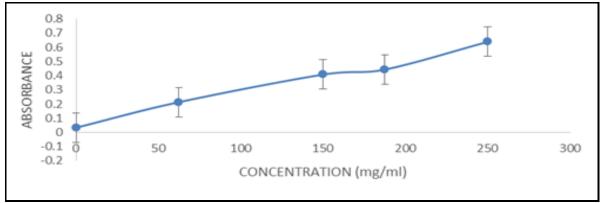


Figure No.4: Standard curve of Ascorbic acid in ferric reducing antioxidant power assay

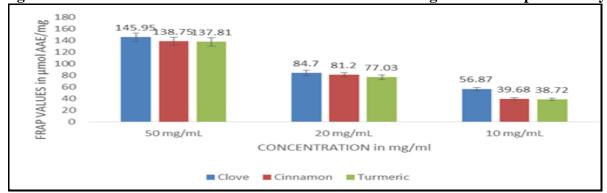


Figure No.5: Ferric reducing assay of spices

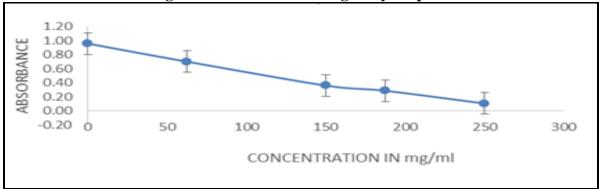


Figure No.6: Standard curve of ascorbic acid for nitric oxide assay

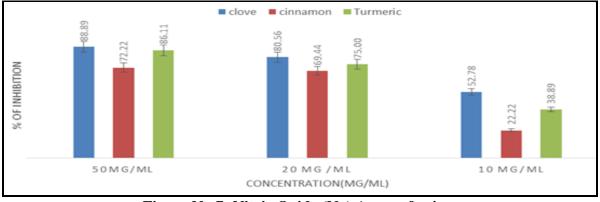


Figure No.7: Nitric Oxide (No) Assay of spices

#### CONCLUSION

From the result in this study, we can highlight the crucial role of spices-clove, cinnamon, and turmeric-in improving lipid stability and enhancing antioxidant and antilipidemic activity in edible oils. Sunflower oil exhibited the malondialdehyde (MDA) levels, confirming its greater susceptibility to lipid oxidation compared to mustard oil and the 50-50 blend. Mustard oil's natural antioxidant content contributed to lower oxidation rates, while the blended oil demonstrated better stability. Among the spice treatments, clove consistently demonstrated powder antioxidant and antilipidemic effects, leading to the most substantial reduction in MDA levels. Cinnamon and turmeric also exhibited protective effects, though their impact was comparatively similar in some instances. Kinetic analysis reinforced the antioxidant potential of these spices by tracking the rate of MDA accumulation over time, confirming that slower MDA formation is strongly associated with greater antilipidemic activity.

Additionally, antioxidant activity assessments through DPPH and FRAP assays further validated clove's efficient radical scavenging ability and reducing power compared to cinnamon and turmeric. In NO assay for analgesic activity, clove again exhibited greater inhibition, suggesting its promising role in pain modulation. Overall, the incorporation of spices into edible oils provides a natural and effective approach to delaying lipid compositions oxidation, stabilizing oil enhancing health benefits through their antilipidemic and analgesic properties. These results highlight the role of spices in promoting health management, food preservation, contributing to improved nutritional stability.

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#### CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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