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# A report on antimutagenic and antioxidant activities of Gallic Acid

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**Abstract**
Gallic acid (3,4,5-trihydroxybenzoic acid) is an organic acid and naturally occurring polyhydroxyphenolic compound abundantly found in various fruits and vegetables. This compound is widely used to cure various disorders and has anti-inflammatory, antibacterial, antifungal, antiviral, antidiabetic, antimalarial and antiallergic activities. Therefore, this study was planned to investigate its other medicinal values such as antimutagenic and antioxidant activities. The antimutagenic activity of gallic acid was checked by using Ames assay. Antioxidant activities was determined through various *in vitro* assays like DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging assay, lipid peroxidation, deoxyribose degradation (site-specific and non-site specific modes), and reducing power assay. In the present study, gallic acid showed strong free radical scavenging and antimutagenic effects in both *in vitro* antioxidant and antimutagenic assays.

**Keywords:** Ames assay, Antimutagenic, Antioxidant, DPPH, Gallic acid.

## 1. Introduction
Increasing environmental pollution and daily stresses in life are causing gene mutations and oxidative stress in Human beings. Oxidative stress is normally caused by excessive generation of free radicals or imbalance in antioxidant defense system of man. Free radicals are highly reactive and unstable compounds and are produced in the body during normal metabolic activities, detoxification processes in the immune system defense or are introduced into the body from external environment. They are unstable because of extra energy and lessen their energy by reacting with other molecules of cells and interfere with the normal functioning of cells. Many studies indicate that plants containing polyphenols such as gallic acid have protective functions against diseases. Gallic acid is found in red wines [1], Green Tea [2], *Terminalia chebula*, *Terminalia bellerica*, *Phyllanthus emblica* [3], *Chukrasia tabularis* A Juss [4] etc. It is found in free and bound form [5]. The increasing interest in gallic acid is due to its various pharmacological activities including anticancer [6], anti-inflammatory activities [7], antibacterial, antifungal and antiviral [8], antidiabetic [9], antimalarial [10], antiallergic activities [11]. Keeping all this in mind, the present study was planned to investigate antimutagenic and antioxidant potential of gallic acid.

## 2. Materials and Methods
### 2.1 Materials
All the chemicals used in the present study were of analytical grade and obtained from Merck, Mumbai, India. Gallic acid was purchased from Sigma Aldrich Company. The bacterial strains of *Salmonella typhimurium* TA98 and TA100 were procured from the Institute of Microbial Technology (CSIR), Chandigarh, India.

### 2.2 Antimutagenicity assay (Ames test):
Antimutagenicity of gallic acid was checked using Ames assay as proposed by Maron and Ames [12]. In this study, histidine requiring strains of *Salmonella typhimurium* i.e. TA98 and TA100 were used and the experiments were carried out with (+S9 mix) and without metabolic activation (-S9 mix) system. Mutagen 4-nitro-o-phenylene diamine (NPD) was used for TA98 and sodium azide was used for TA100 in experiments without S9, while 2-aminofluorene (2-AF) was used in experiments with (+S9) in both the tester strains. Fresh minimal agar medium, top agar and bacterial culture (density of 1-2x 109 CFU/ml) was used in all the experiments. Dimethyl sulfoxide (DMSO) was used as solvent for the preparation of different concentrations of gallic acid (100-2500 µg/0.1ml). Mutagens were used in non toxic

concentrations i.e. NPD (20 µg/0.1 ml), 2-AF (2.5 µg/0.1 ml) and sodium azide (20 µg/0.1 ml). The spontaneous reversion frequency of TA98 and TA100 was also found for each experiment. Toxicity of gallic acid was checked against all the used mutagens. The experiments were conducted in co-incubation and pre-incubation modes. All the experiments were conducted in triplicates and percent inhibition of mutagenic activity was calculated as follows:

The inhibitory activity of gallic acid was expressed as:

Inhibitory activity (%) = [(a-b) / (a-c)]* 100

Where 'a' is the number of histidine revertants induced by mutagen alone (positive control), 'b' is the number of histidine revertants induced by mutagen in the presence of gallic acid, and 'c' is the number of histidine revertants induced in the presence of gallic acid alone and solvent (negative control).

### 2.3 Antioxidant tests
The antioxidant potential of gallic acid was checked by using different concentrations (50-1000 µg/ml) of gallic acid. Various standard *in vitro* experiments were used to evaluate the antioxidant potential of gallic acid.

#### 2.3.1 DPPH free radical scavenging assay
This assay is widely used to asses the free radical scavenging activity of compounds. The method of Blois [13] was used in this assay and DPPH radical scavenging activity was determined by using the following formula.

% DPPH radical scavenging = (1- Absorbance of sample / Absorbance of control) ×100

The samples were measured against blank (methanol).

#### 2.3.2 Lipid peroxidation assay
The inhibitory effect of gallic acid on lipid peroxidation induced by ascorbate-$Fe^{2+}$ in rat liver homogenate was determined as per method given by Halliwell and Guttridge [14].

#### 2.3.3 Deoxyribose degradation assay
The hydroxyl radicals scavenging effect of the gallic acid was checked according to Halliwell *et al*. [15] and Arouma *et al*. [16]. Hydroxyl radicals cause oxidative stress and damage the cells. In this assay, hydroxyl radicals were generated through Fenton reaction system. The effect of gallic acid was checked in site-specific and non-site specific modes.

#### 2.3.4 Reducing power assay
The reducing ability of gallic acid was determined according to method of Oyaizu [17].

### 3. Statistical Analysis
Results of gallic acid were statistically confirmed through mean, standard deviation (SD), linear regression, one-way and two-way analysis of variance (ANOVA). The differences ($p \leq 0.001$, $p \leq 0.01$) among means were compared by honestly significant difference (HSD) using Tukey's test.

### 4. Results and Discussions
#### 4.1 Antimutagenic potential of gallic acid in Ames assay
The results obtained in TA98 strain of *S. typhimurium* are shown in Table 1. As evident from the results gallic acid exhibited 74.56% and 76.45% inhibitory activity at the maximum dose of 2500 µg/0.1ml/plate against NPD and 95.27% and 95.81% inhibitory activity against 2-AF in TA98 strain in co-incubation and pre-incubation modes respectively. In TA100 strain of *S. typhimurium* against sodium azide it showed 95.89% and 96.22% inhibition and against 2-AF it exhibited 99.76% and 99.56% inhibitory activity at the maximum dose tested in both co-incubation and pre-incubation modes respectively (Table 2). Results were found to be statistically significant in both one-way and two-way ANOVA (Table 1-2). Regression analysis of gallic acid on the percent inhibition against mutagen in TA98 and TA100 tester strain of *S. typhimurium* is given in Fig. 1-2. Antimutagens and antioxidant attenuate the risk of many diseases caused by oxidative stress and regular intake of these compounds can reduce the genotoxic effects of mutagenic and carcinogenic factors. Antimutagenic and antioxidant compounds can be used as chemopreventive agents [18].

#### 4.2 Antioxidant potential of gallic acid in various *in vitro* antioxidant assays
Gallic acid showed highest DPPH radical scavenging activity of 91.31% at 1000 µg/ml concentration (Table 3). In lipid peroxidation assay, gallic acid exhibited 87.63% inhibition at highest concentration tested. In deoxyribose degradation assay, the inhibitory effect of gallic acid on hydroxyl radicals generated *in vitro* was estimated. In deoxyribose degradation assay, ·OH radicals cause degradation of deoxyribose to malondialdehyde which on reaction with TBA produce pink chromogen. The results revealed good hydroxyl radical scavenging ability of gallic acid in site-specific as well as non-site specific deoxyribose degradation assay. Gallic acid showed 54.80% inhibition at 1000 µg/ml concentration in site-specific deoxyribose assay, while 80.89% inhibition in non-site specific deoxyribose degradation assay. It was observed that gallic acid showed pronounced effect in non-site specific deoxyribose degradation assay as compared to site-specific assay indicating that extracts act as better ·OH radical scavenger than chelating agents. Gallic acid exhibited highest activity in reducing power assay. One-way ANOVA (Table 3) and regression analysis (Fig. 3) for gallic acid represented statistically significant differences among mean percent inhibition values (at $p \leq 0.001$ and $p \leq 0.01$) in all the assays. Many studies indicate that plant rich in gallic acid are known to have antioxidant properties. The present study also supports the free radical scavenging potential of gallic acid.

**Table 1:** Antimutagenic potential of gallic acid in TA98 tester strain of *S. typhimurium* in Ames Assay.

| Treatment | Dose (µg/100 µl/plate) | TA98 | | | |
|---|---|---|---|---|---|
| | | Without S9 (-S9) | | With S9 (+S9) | |
| | | Revertants/plate | Percent inhibition | Revertants/plate | Percent inhibition |
| **Spontaneous** | | 29.00±1.00 | | 26.67±1.15 | |
| **Positive control** | | | | | |
| **NPD** | **20** | 929.33±38.99 | | | |
| **2-AF** | **20** | | | 2336.33±121.94 | |
| **Negative control** | **100** | 30.00±2.00 | | 27.33±1.15 | |
| | **400** | 25.00±2.65 | | 26.67±1.15 | |
| | **800** | 26.67±3.06 | | 25.67±3.79 | |
| | **1000** | 27.67±4.51 | | 28.00±2.00 | |
| | **1500** | 24.67±1.15 | | 24.33±1.15 | |
| | **2000** | 23.67±1.15 | | 23.00±2.00 | |
| | **2500** | 26.33±3.06 | | 23.33±3.06 | |
| **Co-incubation** | **100** | 534.33±51.54 | 43.92±5.69 | 341.00±13.89 | 86.42±0.63 |
| | **400** | 499.00±10.82 | 47.58±1.12 | 275.33±10.21 | 89.23±0.47 |
| | **800** | 442.00±16.52 | 53.99±1.90 | 252.33±6.81 | 90.19±0.42 |
| | **1000** | 403.67±7.37 | 58.30±1.06 | 226.33±10.02 | 91.41±0.38 |
| | **1500** | 385.33±11.02 | 60.13±1.25 | 208.00±3.46 | 92.06±0.18 |
| | **2000** | 371.00±18.52 | 61.65±1.97 | 174.33±39.72 | 93.46±1.69 |
| | **2500** | 256.00±12.12 | 74.56±1.09 | 132.67±12.66 | 95.27±0.48 |
| **Pre-incubation** | **100** | 509.33±59.91 | 46.69±6.57 | 292.00±9.54 | 88.54±0.44 |
| | **400** | 460.33±31.21 | 51.86±3.39 | 240.33±15.14 | 90.75±0.64 |
| | **800** | 428.67±9.02 | 55.47±1.18 | 235.33±13.20 | 90.93±0.72 |
| | **1000** | 401.67±9.07 | 58.52±0.71 | 225.00±13.08 | 91.47±0.63 |
| | **1500** | 360.00±9.64 | 62.93±1.02 | 189.67±21.73 | 92.85±0.96 |
| | **2000** | 328.00±34.00 | 66.39±3.68 | 143.33±22.19 | 94.80±0.90 |
| | **2500** | 239.00±18.73 | 76.45±2.29 | 120.33±13.87 | 95.81±0.47 |
| Statistical Analysis | | | | | |
| **One-way Anova** | | | | | |
| Positive control and co-incubation | | F(7,16)=185.74***; HSD=72.35 | | F(7,16)=785.61***; HSD=130.66 | |
| Positive control and pre-incubation | | F(7,16)=132.50***; HSD=88.61 | | F(7,16)=819.55***; HSD=129.23 | |
| **Two-way Anova** | | | | | |
| Co-incubation and pre-incubation | | | | | |
| Treatment | | F(1,28)=8.07** | | F(1,28)=20.09*** | |
| Concentration | | F(6,28)=68.31*** | | F(6,28)=84.55*** | |
| Treatment x Concentration | | F(6,28)=0.43 | | F(6,28)=1.33 | |
| | | HSD=80.133 | | HSD=50.684 | |

Data shown are Mean±SD of experiments with triplicate plates/concentration/experiment. Significant at ***p≤0.001, **p≤ 0.01.

**Table 2:** Antimutagenic potential of gallic acid in TA100 tester strain of *S. typhimurium* in Ames Assay.

| Treatment | Dose (µg/100µl/plate) | TA98 | | | |
|---|---|---|---|---|---|
| | | Without S9 (-S9) | | With S9 (+S9) | |
| | | Revertants/plate | Percent inhibition | Revertants/plate | Percent inhibition |
| **Spontaneous** | | 251.33±12.22 | | 240.67±15.37 | |
| **Positive control** | | | | | |
| **Sodium azide** | **20** | 1949.67±68.06 | | | |
| **2-AF** | **20** | | | 2578.67±48.91 | |
| **Negative control** | **100** | 216.67±14.84 | | 226.67±4.04 | |
| | **400** | 227.67±19.66 | | 230.67±17.79 | |
| | **800** | 244.00±10.82 | | 253.67±9.29 | |
| | **1000** | 248.67±13.05 | | 244.67±11.59 | |
| | **1500** | 241.00±12.12 | | 229.00±5.57 | |
| | **2000** | 248.67±10.50 | | 234.33±3.51 | |
| | **2500** | 238.00±20.88 | | 228.00±8.89 | |
| **Co-incubation** | **100** | 628.33±2.31 | 76.25±0.53 | 417.67±14.74 | 91.88±0.78 |
| | **400** | 554.33±6.11 | 81.04±1.19 | 356.33±11.50 | 94.65±0.93 |
| | **800** | 480.33±12.58 | 86.15±0.73 | 359.67±3.79 | 95.44±0.47 |
| | **1000** | 421.67±7.02 | 89.83±0.29 | 321.67±7.02 | 96.70±0.18 |

| | | | | | |
|---|---|---|---|---|---|
| | **1500** | 392.33±5.51 | 91.15±0.83 | 263.67±11.68 | 98.52±0.51 |
| | **2000** | 355.67±7.77 | 93.71±1.03 | 249.33±16.44 | 99.36±0.59 |
| | **2500** | 308.67±13.32 | 95.89±1.95 | 233.67±18.34 | 99.76±0.84 |
| **Pre-incubation** | **100** | 508.67±73.08 | 83.14±3.78 | 459.00±8.19 | 90.42±0.77 |
| | **400** | 435.67±15.04 | 87.92±0.26 | 420.00±14.80 | 92.22±0.31 |
| | **800** | 398.33±3.06 | 90.95±0.62 | 358.00±9.64 | 94.41±0.97 |
| | **1000** | 384.00±7.94 | 92.05±0.43 | 301.33±10.26 | 96.85±0.36 |
| | **1500** | 355.33±14.57 | 93.32±1.45 | 255.00±9.17 | 98.70±0.36 |
| | **2000** | 343.33±15.57 | 94.44±1.28 | 239.67±13.01 | 99.03±0.73 |
| | **2500** | 302.67±24.44 | 96.22±0.82 | 236.67±13.58 | 99.56±0.78 |
| Statistical Analysis | | | | | |
| **One-way Anova** | | | | | |
| Positive control and co-incubation | | F(7,16)=1363.08***; HSD=71.78 | | F(7,16)=4360.47***; HSD=59.58 | |
| Positive control and pre-incubation | | F(7,16)=652.98***; HSD=106.43 | | F(7,16)=4650.71***; HSD=57.58 | |
| **Two-way Anova** | | | | | |
| Co-incubation and pre-incubation | | | | | |
| Treatment | | F(1,28)=71.09*** | | F(1,28)=6.61** | |
| Concentration | | F(6,28)=94.21*** | | F(6,28)=247.92*** | |
| Treatment x Concentration | | F(6,28)=6.65*** | | F(6,28)=9.60*** | |
| | | HSD=67.905 | | HSD=36.459 | |

Data shown are Mean±SD of experiments with triplicate plates/concentration/experiment. Significant at ***p≤0.001, **p≤ 0.01.

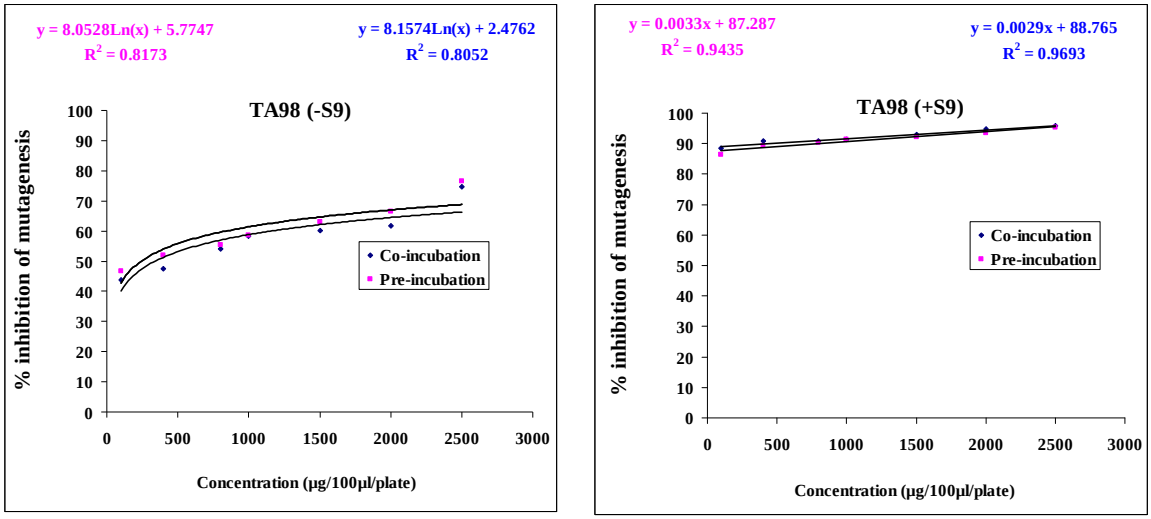



**Fig 1:** Regression analysis of gallic acid on the percent inhibition of mutagenicity of NPD and 2-AF in TA98 tester strain of *S. typhimurium* in Ames Assay.

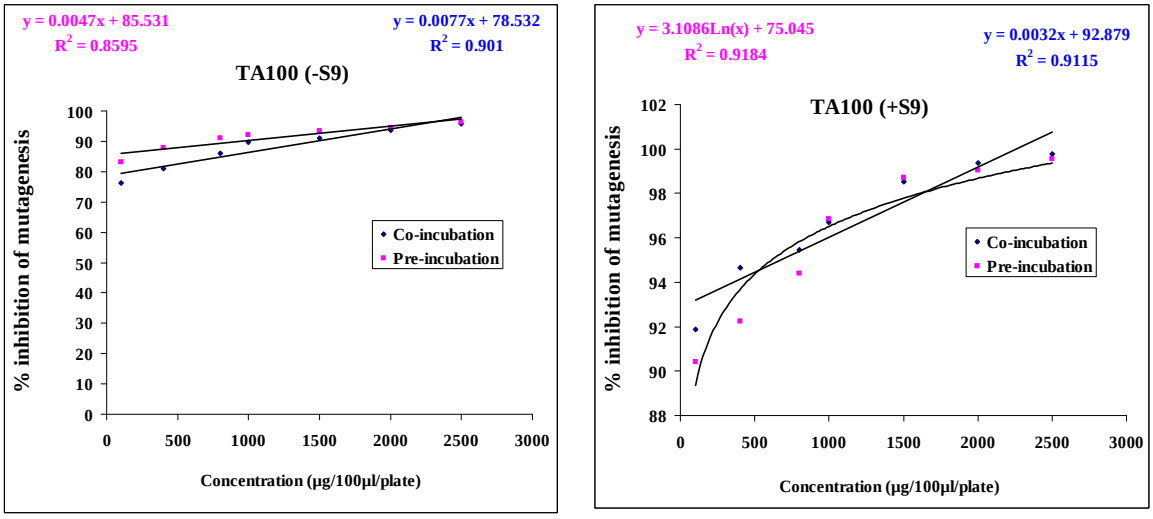
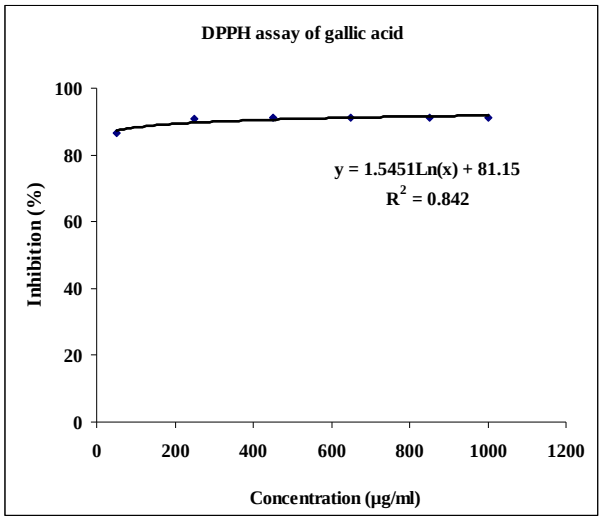


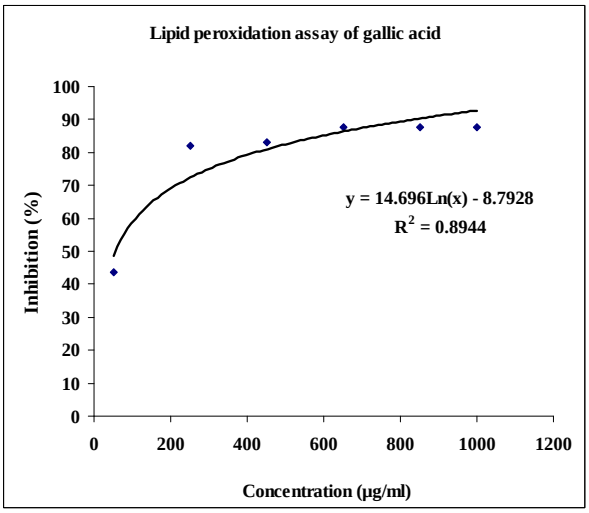

**Fig 2:** Regression analysis of gallic acid on the percent inhibition of mutagenicity of sodium azide and 2-AF in TA100 tester strain of *S. typhimurium* in Ames Assay.

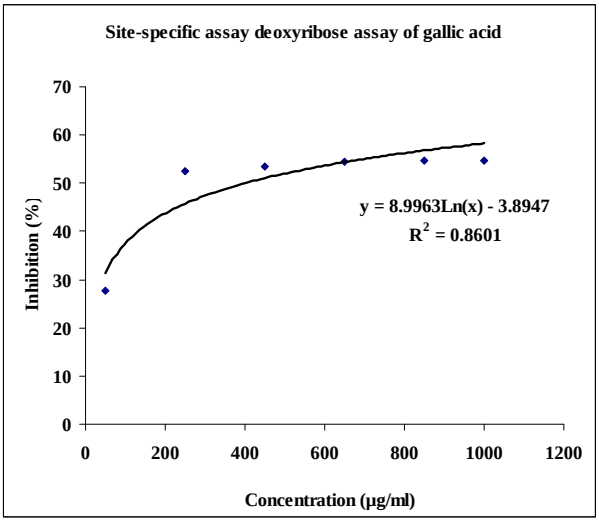
**Table 3:** Antioxidant potential of gallic acid in various *in vitro* antioxidant assays

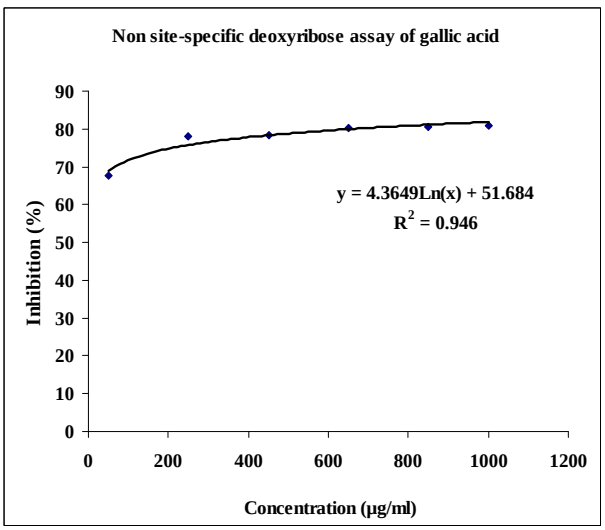
| S. No. | Concentration (µg/ml) | DPPH Assay | Lipid Peroxidation Assay | Site-Specific Deoxyribose Assay | Non-Site Specific Deoxyribose Assay | Reducing Power Assay |
|---|---|---|---|---|---|---|
| | | Inhibition (%) Mean ± SD | | | | |
| 1 | **50** | 86.52±0.53 | 43.67±0.16 | 27.63±0.06 | 67.78±0.09 | 45.78±0.20 |
| 2 | **250** | 90.96±0.35 | 82.05±0.27 | 52.53±0.59 | 77.89±0.09 | 99.34±0.09 |
| 3 | **450** | 91.05±0.00 | 82.96±0.22 | 53.55±0.97 | 78.23±0.09 | 99.90±0.05 |
| 4 | **650** | 91.05±0.18 | 87.56±0.22 | 54.35±0.17 | 80.38±0.31 | 99.90±0.00 |
| 5 | **850** | 91.13±0.18 | 87.63±0.15 | 54.69±0.06 | 80.63±0.15 | 99.90±0.00 |
| 6 | **1000** | 91.31±0.18 | 87.63±0.08 | 54.80±0.01 | 80.89±0.23 | 99.90±0.00 |
| 7 | **$IC_{50}$ Value** | 7.79 | 54.598 | 399.41 | 23.76 | 59.218 |
| 8 | **F-ratio $_{(5,12)}$** | **125.32$^{***}$** | **23987.21$^{***}$** | **1576.96$^{***}$** | **2350.52$^{***}$** | **164058.07$^{***}$** |
| 9 | **HSD** | **0.79** | **0.53** | **1.29** | **0.49** | **0.26** |
| 10 | **Regression Equation** | **y = 1.5451Ln(x) + 81.15** | **y = 14.696Ln(x) - 8.7928** | **y = 8.9963Ln(x) - 3.8947** | **y =4.3649Ln(x)+51.684** | **y = 17.818Ln(x) - 15.149** |
| 11 | **R-value** | **R=0.9176$^{**}$** | **R =0.9457$^{***}$** | **R =0.9274$^{***}$** | **R =0.9726$^{***}$** | **R =0.8991$^{**}$** |

Data shown are Mean±SD of experiments with triplicate plates/concentration/experiment. Significant at ***p≤0.001, **p≤ 0.01



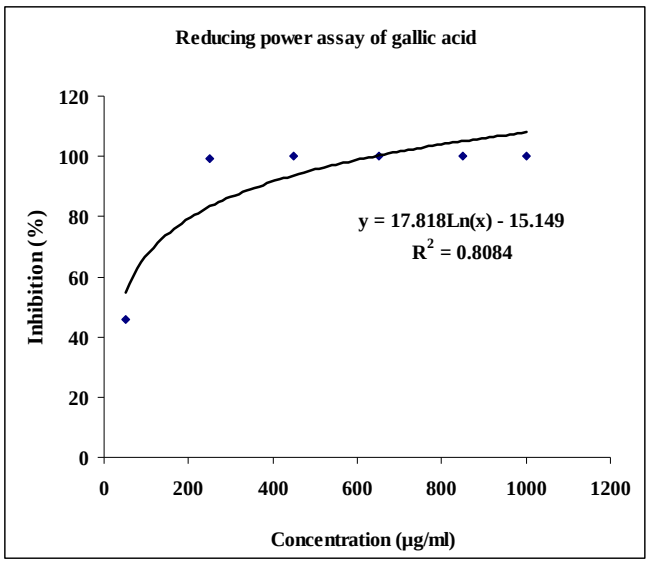



**Fig 3:** Regression analysis of gallic acid in various *in vitro* antioxidant assays.

## 5. Conclusion

Gallic acid showed potent effects in both antimutagenic and antioxidant assays. It showed strong inhibitory effects against mutagen inducing frameshift mutations on *S. typhimurium* TA98 and base pair substitutions on *S. typhimurium* TA100. On the other hand, antioxidant assays used in this study established the free radical scavenging abilities of gallic acid. However, *in vivo* studies on eukaryotic models are required to confirm these protective effects against mutagens and free radical mediated reactions.

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