

## Amelioration of oxidative damage by Methyl gallate in different *in vitro* models

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### Abstract

The present study aims to evaluate the antioxidant potential of methyl gallate - an ester of gallic acid in different *in vitro* assays. The antioxidant capacity was assessed using Molybdate ion reduction, DPPH, ABTS radical cation scavenging, deoxyribose degradation (site specific and non-site specific), reducing power, lipid peroxidation, superoxide anion scavenging, chelating power and DNA nicking assays and the results were compared with known antioxidant i.e. gallic acid. From the results obtained, it was found that methyl gallate exhibited potent antioxidant activities in different *in vitro* models and the activity was related to its chemical structure. The reduction ability of methyl gallate was found to be 99.58mg AAE /100mg dry weight of pure compound. The IC<sub>50</sub> value of methyl gallate in DPPH, ABTS<sup>+</sup> scavenging, deoxyribose degradation (site specific and non-site specific), reducing power, lipid peroxidation, superoxide anion scavenging, chelating power was found to be 21.679, 8.689, 19.884 and 20.086, 91.169, 15.825, 32.699 and 4990.2 µg/ml respectively. The methyl gallate also has tendency to conserve the supercoiled DNA (pBR 322) from the OH radicals mediated damage in DNA nicking assay. However, it has been found that hydrogen donation, free radical scavenging potential of methyl gallate was lower than standard antioxidant compound i.e. gallic acid and the lower effect was related to substitution of -OH group by -CH<sub>3</sub> group.

**Keywords:** Methyl gallate; gallic acid; antioxidant; hydrogen donation; free radical scavenging assay

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### Introduction

The overproduction of reactive oxygen species causes degradation of biomolecules including DNA, lipids and proteins and is furthermore linked to most prevalent degenerative diseases including aging, cancer and diabetes. Several environmental and life style factors act

as contributory elements for the production of free radicals. Reduction of reactive species is one of the main efforts that are involved in providing protection as these processes maintain the integrity of biomolecules (Hsieh et al., 2004). The natural products including flavonoids, coumarins and polyphenols are explored for their antioxidative properties such as hydrogen donating, radical scavenging and metal chelating activities. On the basis of these activities, a number of compounds has been characterized and developed as antioxidative agents (Lee et al., 2002; Finkel and Holbrook, 2004). Methyl gallate (MEG) - an ester of gallic acid (Figure 1), is an extremely potent compound with remarkable biological activities including antimicrobial, anti-inflammatory activities, antiplatelet activity, tendency to act as inhibitor of cell adhesion, cancer metastasis, promotion of skin papillomas and carcinomas (Schmidt et al., 2003; Chaubal et al., 2005; Kim et al., 2006). The compound was also reported to act as inhibitor of collagenase and protein tyrosine kinase, protector of cultured Madin-Darby canine kidney (MDCK) cells against hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>)-mediated oxidative stress. The compound is used as an active ingredient of many pharmaceutical preparations that are used for the treatment of enteritis, dysentery and itching. The biological properties of methyl gallate are related to their tendency to enhance the bioavailability of active ingredients which are further related to the peculiar structure of methyl gallate. The occurrence of methyl gallate has been reported in different plants including grape seeds, *Acacia nilotica*, *Acer cissifolium*, *Acer truncatum*, *Cedrela sinensis*, *Haematoxylon campechianum*, *Geranium niveum*, *Paeonia lactiflora* Pall., *Phyllanthus myrtifolium*, *Oenothera biennis*, *Rubus coreanum*, *Rhus glabra*, *Sedum aizoon* L. *Toona sinensis* etc (Hsieh et al., 2004; Lim et al., 2004; Schmidt et al., 2003; Lee et al., 2005; Ma et al., 2005).

It is a well known fact that the radical scavenging ability of any compound is a main focus to explain its protective function against oxidative stress. Keeping this in mind, the present study aims to evaluate the amelioration capacity of methyl gallate against oxidative damage using different *in vitro* assays. The electron and hydrogen donating ability of isolated compound is determined using molybdate ion reduction assay, DPPH, ABTS<sup>+</sup>, reducing power assay whereas hydroxyl radical scavenging ability is determined using deoxyribose degradation and DNA nicking assay. The superoxide anion radical scavenging ability is determined in PMS-NADH-NBT system and ferrozine is used as an indicator to determine metal ion chelation ability.

## Materials and methods

### Chemicals

Methyl gallate (isolated from *Chukrasia tabularis* leaves), DPPH (2,2'-diphenyl-1-picryl hydrazyl), TBA (2-thiobarbituric Acid), ABTS ((2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)), Ethidium Bromide were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Supercoiled plasmid pBR 322 DNA and agarose were obtained from Genei, Bangalore. Ammonium molybdate, sulphuric acid were obtained from Qualigens Ltd. Phenazine methosulphate, Sodium phosphate, Ferrozine, Nicotinamide adenine dinucleotide reduced (NADH), 2- deoxyribose and Potassium persulfate were obtained from Himedia Chemicals Ltd. Mumbai. Bromophenol blue, L-ascorbic acid, Tris (hydroxymethyl) aminomethane, Folin Ciocalteu Reagent, BHT (butylated hydroxy toluene), Trichloroacetic

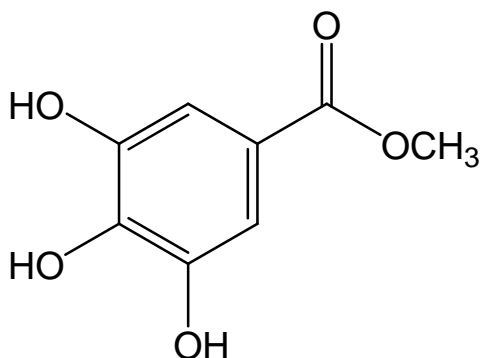


Figure 1: Structure of Methyl gallate

acid (TCA), Gallic acid, Potassium Chloride (KCl), Ferric Chloride (FeCl<sub>3</sub>), Hydrochloric acid (HCl), Sodium hydroxide (NaOH), Rutin, Nitroblue tetrazolium chloride, Dipotassium hydrogen phosphate (K<sub>2</sub>HPO<sub>4</sub>), Potassium dihydrogen phosphate (KH<sub>2</sub>PO<sub>4</sub>) and Ethylenediaminetetraacetic acid (EDTA) were of analytical grade.

### ***Determination of Antioxidant Activities***

The determination of antioxidant potential of methyl gallate in different *in vitro* models is as follows:

#### ***Molybdate ion Reduction assay***

The tendency of methyl gallate to reduce molybdate ion was determined following the method given by Prieto et al. (1999). The reduction ability of methyl gallate was determined by incubating the reaction mixture comprising of 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate with 0.3 ml of solution at 95 °C for 90 minutes. The mixture was cooled to room temperature and the absorbance of coloured solution was measured at 695 nm against a blank. The results were expressed as mg Ascorbic Acid Equivalents (AAE)/ 100 mg dry weight of extract or fractions.

#### ***DPPH radical scavenging activity***

The hydrogen donating ability of methyl gallate was assessed using DPPH radical scavenging method described by Blois with slight modifications (Blois, 1958). In this assay, 0.2 ml of isolated compound solution (20-200 µg/ml) was added to 3ml of 0.1 mM methanolic DPPH solution in a cuvette and absorbance was read at 517 nm. The radical scavenging activity was expressed as the inhibition percentage and monitored as per the equation: % DPPH radical scavenging =  $(1 - A_S/A_C) \times 100$ ; A<sub>C</sub> = Absorbance of Control, A<sub>S</sub> = Absorbance of Sample solution. The DPPH solution without sample solution was used as control. IC<sub>50</sub> value is the concentration of sample (µg/ml) required to scavenge 50 % DPPH free radical and was calculated from inhibition curve. Gallic acid was used as positive control.

*ABTS Radical cation scavenging assay*

The method given by Re et al. (1997) was followed to determine the ABTS<sup>+</sup> scavenging potential. In this method, 1.9 ml of ABTS<sup>+</sup> radical cation solution (generated by mixing two reagents i.e. 7mM of ABTS and 140mM of Potassium Persulfate in a proportion to make the final concentration of Potassium Persulfate 2.45 mM in mixture; the reaction mixture was kept at 30°C for 12-16 hours at dark place and then diluted with ethanol or PBS (pH=7.4) to obtain the absorbance of  $0.700 \pm 0.020$  at 734nm.) was mixed with 0.1 ml of methyl gallate solution (20-200 µg/ml) and the absorbance was measured spectrophotometrically for 0 to 6 minutes at 734nm. The radical scavenging activity was expressed as the inhibition percentage as per the equation: % ABTS<sup>+</sup> radical scavenging =  $(1 - A_S/A_C) \times 100$ ;  $A_C$  = Absorbance of Control,  $A_S$  = Absorbance of Sample solution. Gallic acid was used as a positive control.

*Reducing Power Assay*

The reducing power of methyl gallate was determined by the method of Oyaizu, 1986. 1 ml of methyl gallate solution (20-200 µg/ml) was mixed with 2.5 ml of phosphate buffer (200mM, pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 minutes. A volume of 2.5 ml of 10% TCA was then added to the mixture and centrifuged at 3000 rpm for 10 minutes. 2.5 ml of supernatant was mixed with 2.5 ml of distilled water and 0.5 ml of FeCl<sub>3</sub> (0.1%) and the absorbance was measured spectrophotometrically at 700 nm. Increase in absorbance of the reaction mixture was interpreted as increase in reducing activity of the methyl gallate and the results were compared with gallic acid that was used as a positive control. The percentage reduction of the sample as compared to standard i.e. Gallic acid was calculated by using the formula  $[1 - (1 - A_S/A_C)] \times 100$ . Here,  $A_C$  = Absorbance of standard at maximum concentration tested and  $A_S$  = Absorbance of sample.

*Deoxyribose degradation assay*

Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and methyl gallate for hydroxyl radicals generated by the Fe<sup>3+</sup>-ascorbate-EDTA-H<sub>2</sub>O<sub>2</sub> system according to the method of Halliwell et al. (1987) and Arouma et al. (1987) with slight modifications. Depending upon the use of EDTA, this method comprised of non-site and site specific scavenging of ·OH radicals. For non-site specific hydroxyl radical scavenging assay, the Haber-Weiss reaction mixture (1 ml) contained 2-Deoxyribose (10 mM), Fe (III) chloride (10 mM), EDTA (1mM), and H<sub>2</sub>O<sub>2</sub> (10 mM) without or with the methyl gallate solution (10-100 µg/ml) in 50 mM potassium phosphate buffer, pH 7.4. In site-specific hydroxyl radical scavenging assay, EDTA was replaced with same amount of buffer. The reaction was triggered by adding ascorbic acid (1mM) which served as a reducing agent by reducing Fe<sup>3+</sup> to Fe<sup>2+</sup> ions and subsequent incubation of the mixture for 1 h at 37°C. Solutions of Fe (III) chloride, ascorbic acid and H<sub>2</sub>O<sub>2</sub> were prepared in distilled water just prior to use. To 1 ml solution of above mixture, TBA in 25 mM NaOH (1 ml, 0.5%) and TCA (1 ml, 10% w/v aqueous solution) were added. The mixture was heated for 90 minutes on water bath at 80°C and the amount of pink chromogen produced was spectrophotometrically measured at 532 nm. The hydroxyl radical

scavenging ability of methyl gallate was compared with gallic acid used as an internal standard. The inhibitory effect on the hydroxyl radicals was calculated as: % Hydroxyl radical scavenging capacity =  $(1-A_S/A_C) \times 100$ ;  $A_C$  = Absorbance of Control,  $A_S$  = Absorbance of Sample solution.

#### *DNA nicking assay*

The ability of methyl gallate to protect super coiled pBR 322 from the harmful effects of hydroxyl radicals generated by Fenton's reagent was assessed by the DNA nicking assay described by Lee and co-workers with slight modifications (Lee et al., 2002). The reaction mixture contained 0.3 $\mu$ l of plasmid DNA, 10 $\mu$ l Fenton's reagent (30 mM H<sub>2</sub>O<sub>2</sub>, 50  $\mu$ M ascorbic acid, and 80  $\mu$ M FeCl<sub>3</sub>) followed by the addition of methyl gallate (100-1000 $\mu$ g/ml) and the final volume of the mixture was brought up to 20  $\mu$ l using distilled water. The mixture was then incubated for 30 min at 37 °C and the DNA was analyzed on a 1% agarose gel (prepared by dissolving 0.5g of agarose in 50 ml of 1X TBE Buffer) followed by ethidium bromide staining. Rutin was used as a positive control.

#### *Lipid Peroxidation assay*

Normal albino rats of the Wistar strain (250g) were used for the preparation of liver homogenate. The perfused liver was isolated, and 10% (w/v) homogenate was prepared with homogenizer at 0-4 °C with 0.15 M KCl. The homogenate was centrifuged at 800g for 15 min and clear cell free supernatant was used for the study of *in vitro* lipid peroxidation.

The method given by Halliwell and Gutteridge (1989) with slight modifications was followed, to determine the peroxy radical scavenging ability by estimating the amount of malondialdehyde (MDA) using TBA as reagent. The protective effect of methyl gallate was determined by mixing its different concentrations (1-100 $\mu$ g/ml) with 0.15 M KCl and rat liver homogenate. Peroxidation was initiated by adding 100  $\mu$ l of 10mM ferric chloride. After incubation at 37 °C for 30 min., lipid peroxidation was monitored by the formation of thiobarbituric acid reactive substances (TBARS). TBARS were estimated by adding 2 ml of ice-cold HCl (0.25 N) containing 15% trichloroacetic acid (TCA), 0.5% thiobarbituric acid (TBA) and 0.5% butylated hydroxytoluene (BHT) to the reaction mixture, followed by heating at 100°C for 60 min. The samples were then cooled and centrifuged, and absorbance of the supernatants was measured at 532 nm. The protective effect of methyl gallate against lipid peroxidation was calculated as follows and the results were compared with gallic acid used as an internal standard:

$$\% \text{ Inhibition} = (1-A_S/A_C) \times 100$$

$A_C$  = Absorbance of Control,  $A_S$  = Absorbance of Sample solution. For calculating  $A_C$ , an identical experiment was performed without methyl gallate solution.

#### *Chelating ability of Ferrous ions*

The potential of methyl gallate to chelate Fe<sup>2+</sup> was determined as per the method given by Dinis et al. (1994) with slight modification. The method comprised of mixing of

different concentration of methyl gallate (1ml, 20-200 µg/ml) with 3.7 ml of methanol and 2mM ferrous chloride (0.1ml). The reaction was initiated by 5 mM of ferrozine. Here, Ferrozine served as an indicator which complexed with freely available Fe<sup>2+</sup> ions in solution to form purple coloured complex. The mixture was kept at room temperature for 10 minutes. The absorbance of the mixture was determined at 562nm against a blank. EDTA was used as positive control. The inhibition percentage was calculated using the formula.

$$\text{Inhibition (\%)} = (1 - A_S/A_C) \times 100$$

Here, A<sub>S</sub> = absorbance of sample and A<sub>C</sub> = absorbance of control. In control, the solvent used for making concentration replaced the sample.

#### *Superoxide anion scavenging assay*

In order to assess the superoxide anion scavenging activities of methyl gallate, the method given by Nishikimi et al. (1972) was followed with modifications. The superoxide anions were generated non-enzymatically in a phenazine methosulphate-NADH system and assayed by development of blue coloured formazon dye upon reduction of nitro blue tetrazolium. Briefly, 1ml of methyl gallate solution of different concentrations (20-200 µg/ml) was mixed with 156 µM NADH, 60 µM NBT and 468 µM phenazine methosulphate (PMS) in phosphate buffer (pH = 8.3). The reaction was initiated with the addition of PMS and the reaction mixture was incubated at 25°C for 10 minutes. The absorbance of coloured complex was measured at 560 nm. The decrease in absorbance indicates the scavenging of superoxide anions. The inhibition percentage of methyl gallate was calculated as  $(1 - A_S/A_C) \times 100$  and the results were compared with gallic acid that was used as an internal standard.

#### *Statistical Analysis*

Each experiment was performed at least three times and results were presented as the mean ± SEM. Regression analysis were performed along with multiple comparisons by one-way analysis of variance (ANOVA). Data was considered statistically significant at  $p \leq 0.05$ .

## **Results**

### ***In vitro* antioxidant studies**

Methyl gallate, a derivative of gallic acid, isolated from the ethyl acetate fraction of *Chukrasia tabularis* leaves, was analysed for its ability to scavenge different radicals generated in different *in vitro* systems. It was found that methyl gallate has got strong ability to reduce Mo(VI) to Mo(V) by electron donation at acidic pH. The reduction ability of methyl gallate was found to be 99.58 mg AAE /100 mg dry weight of pure compound and was calculated from the regression equation obtained for ascorbic acid. The regression equation was  $y = 0.004x + 0.067$  ( $R^2 = 0.990$ ); Here,  $y$  = absorbance obtained at 695 nm and  $x$  = concentration of ascorbic acid used.

The methyl gallate was quite efficient in converting radical form to non-radical form through the process of hydrogen donation. It exhibited an inhibitory percentage of 85.88% at

80 µg/ml in DPPH radical scavenging assay whereas gallic acid showed an inhibition of 86.88% at the same concentration (Figure 2A). Figure 2B showed that methyl gallate exhibited a remarkable activity of 93.31% at 60 µg/ml in scavenging ABTS<sup>+</sup> generated by mixing two reagents (ABTS and Potassium persulfate). However, as indicated from the IC<sub>50</sub> values of gallic acid and methyl gallate, it was concluded that the radical scavenging ability of methyl gallate was less than gallic acid. The IC<sub>50</sub> value of gallic acid in DPPH assay and ABTS<sup>+</sup> scavenging assay was found to be 8.499 µg/ml and 2.569µg/ml respectively as compared to 21.679 µg/ml and 8.689 µg/ml respectively in case of methyl gallate. Figure 2D show the hydroxyl radical scavenging ability of methyl gallate in non-site specific manner and Figure 2C demonstrate the scavenging ability in site-specific assay. It has been found that like the standard compound (gallic acid) methyl gallate showed the ·OH scavenging ability in a dose dependent manner. The results revealed that methyl gallate exhibited a remarkable scavenging potential of 76.25% at 90 µg/ml in non-site specific assay whereas in site specific assay, it showed 83.34% inhibition at the same concentration i.e. 90 µg/ml. The results so obtained indicated that methyl gallate has stronger affinity to bind to deoxyribose molecule as compared to Fe<sup>3+</sup> ion and thus protect deoxyribose molecule from ·OH radical damage generated at particular site. However, gallic acid exhibited hydroxyl radical scaveng-

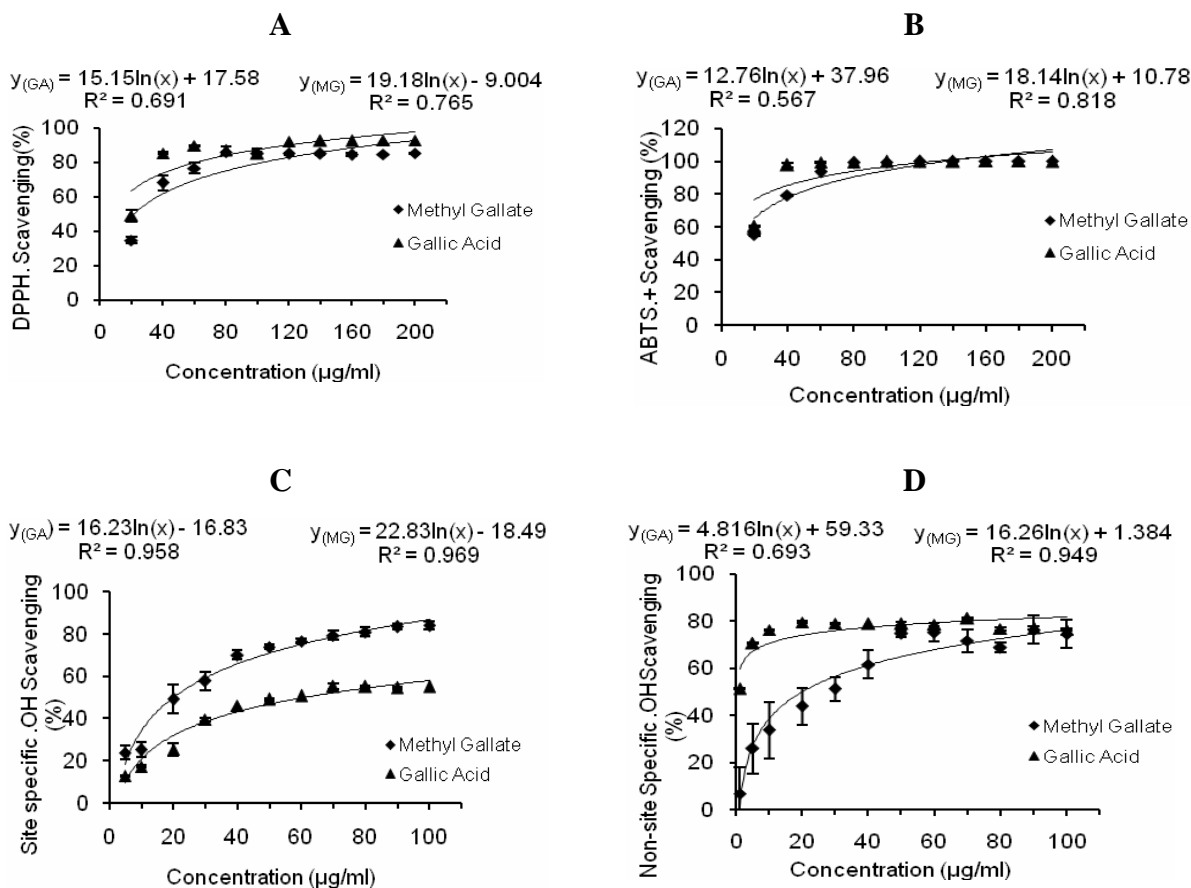


Figure 2 depicts the antioxidative activities of methyl gallate in different *in vitro* assays. (A) DPPH assay (B) ABTS radical cation scavenging (C) Site specific hydroxyl radical scavenging (D) non-site specific hydroxyl radical scavenging assay.

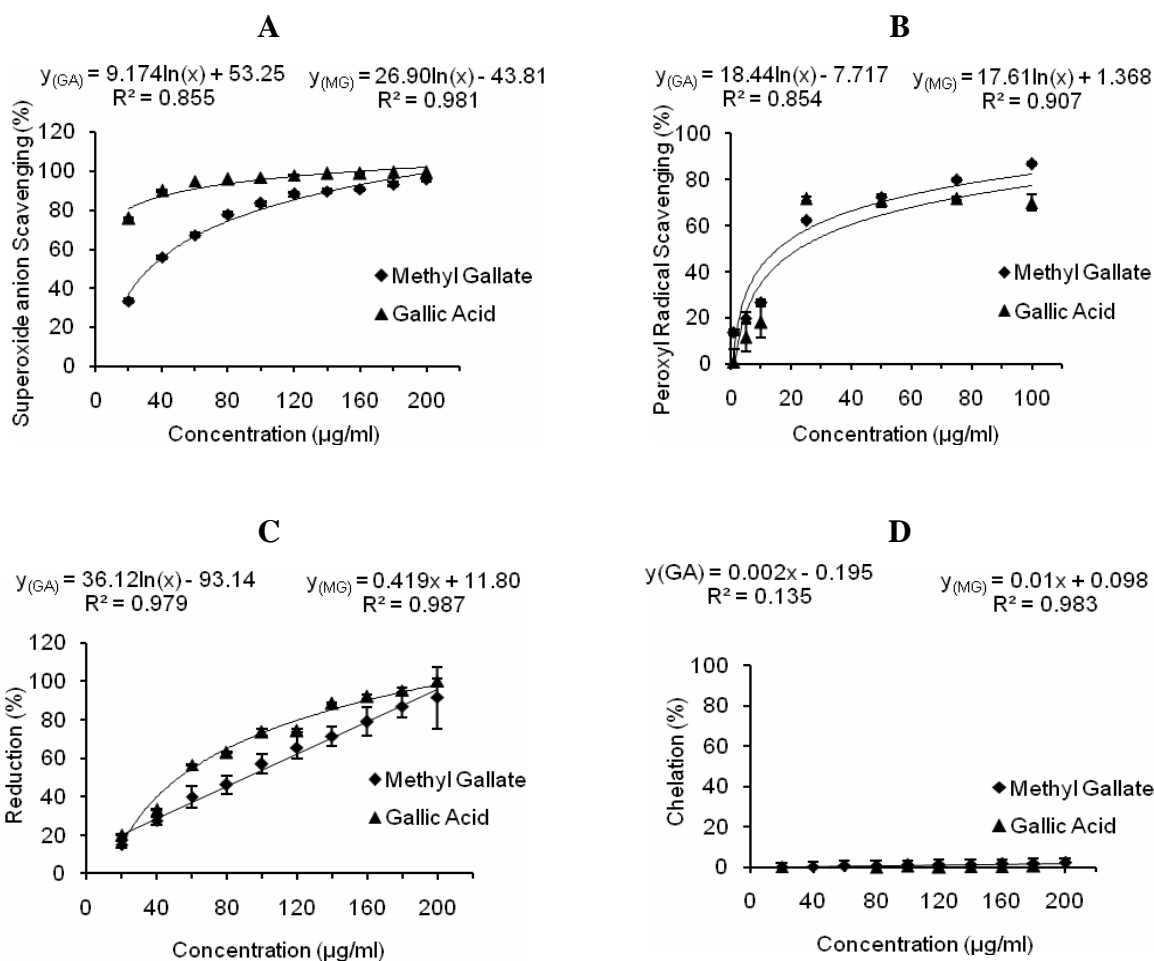


Figure 3 depicts the antioxidative activities of methyl gallate in different *in vitro* assays. (A) Superoxide anion scavenging (B) Lipid peroxidation (C) reducing power (D) chelating power assay

ing potential of 54.46% at 90  $\mu\text{g/ml}$  in site specific assay whereas the inhibitory effect of 81.12% for  $\cdot\text{OH}$  radicals was observed in non-site specific assay at the 70  $\mu\text{g/ml}$  concentration.

Figure 3A shows the tendency of methyl gallate to scavenge superoxide anion radicals generated in PMS-NADH-NBT system. It was found that methyl gallate acted as potent scavenger of  $\text{O}_2^{\cdot-}$  as it exhibited an inhibitory effect of 95.68% at 200  $\mu\text{g/ml}$ . The methyl gallate exhibited less activity than gallic acid. The  $\text{IC}_{50}$  value of gallic acid and methyl gallate for scavenging  $\text{O}_2^{\cdot-}$  was found to be 5.837  $\mu\text{g/ml}$  and 32.699  $\mu\text{g/ml}$  respectively. The ability of methyl gallate to scavenge *in vitro* generated peroxyl radicals in the presence of  $\text{Fe}^{3+}$  using liver homogenate as substrate and thus, inhibit lipid peroxidation is shown in Figure 3B. The correlation among different concentrations used and the results obtained was also calculated using MS Excel software. It was found that methyl gallate exhibited 86.71% peroxyl radical scavenging ability at 100  $\mu\text{g/ml}$  whereas gallic acid exhibited an inhibitory effect of 70.01% at the same concentration. The inhibitory effect of



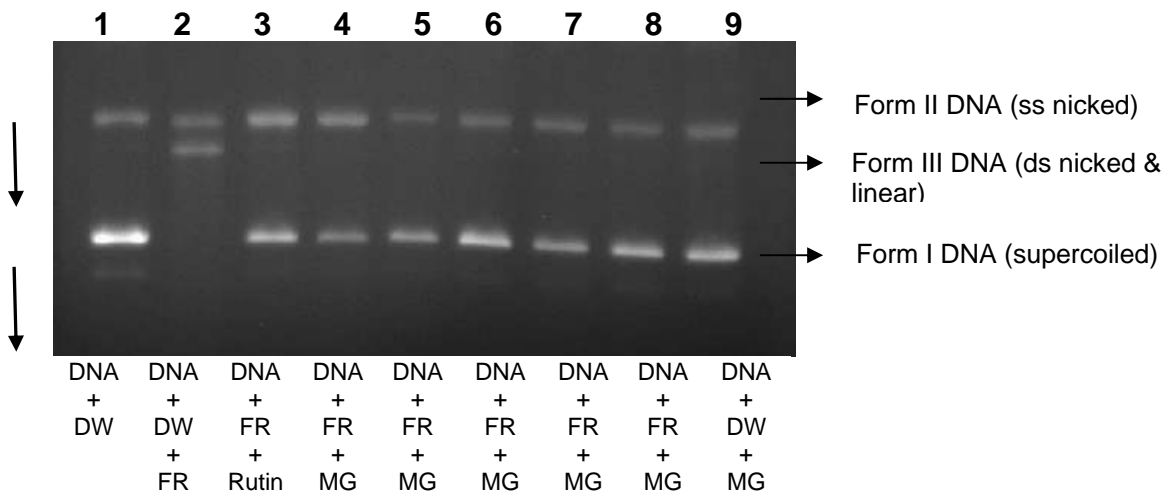


Figure 4. DNA Nicking Assay for methyl gallate (MG) at different concentrations (1000-100 µg/ml). Lane 1 = Negative control (Distilled water + DNA); Lane 2 = Control (DNA + Fenton’s reagent); Lane 3 = Rutin (1000 µg/ml) + FR as positive control; Lane 4 = 1000 µg/ml of MG; Lane 5 = 800 µg/ml of MG; Lane 6 = 600 µg/ml of MG; Lane 7 = 400 µg/ml of MG; Lane 8 = 200 µg/ml of MG; Lane 9 = 100 µg/ml of MG.

gallic acid and methyl gallate was found to occur in a dose dependent manner. The peroxy radical scavenging ability of methyl gallate was compared to standard compound (gallic acid) and it was found that methyl gallate (IC<sub>50</sub> value = 15.825 µg/ml) was more effective in scavenging peroxy radicals as compared to gallic acid (IC<sub>50</sub> value = 22.874 µg/ml). Figure 3C shows the tendency of methyl gallate to donate electrons in comparison to gallic acid in reducing power assay. It was found that methyl gallate exhibited reduction percentage of 91.57% in terms of gallic acid at 200 µg/ml concentration. It was concluded from Figure 3D that methyl gallate was inefficient in sequestering Fe<sup>2+</sup> ions just like gallic acid but has slightly higher chelation potential than gallic acid. It was found that methyl gallate exhibited metal chelating ability of 1.85% at 180 µg/ml whereas gallic acid has chelation ability of 0.654% at the same concentration. Figure 4, Figure 5 and Table 1 depict the potency of methyl gallate at different concentrations (1000-100 µg/ml) to conserve the supercoiled DNA (pBR 322) from the OH

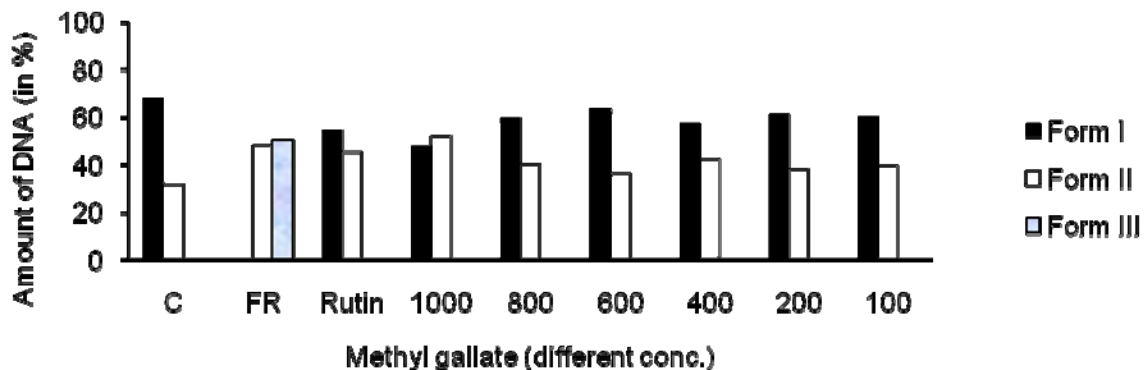


Figure 5 depicting the amount of plasmid DNA (in %) after treatment with different concentrations (1000-100 µg/ml) of methyl gallate

Table 1. Densitometric analysis of different forms of DNA after treatment with different concentrations (1000-100 µg/ml) of methyl gallate isolated from the leaves *C. tabularis*

	C	FR	Rutin	1000	800	600	400	200	100
<b>Form DNA I</b>	3636.36 (68.09)	0	2452.48 (54.32)	1749.73 (47.94)	2052.32 (59.42)	2596.51 (63.33)	1950.81 (57.16)	2230.03 (61.52)	2210.83 (60.11)
<b>Form DNA II</b>	1703.72 (31.91)	1578.74 (48.49)	2062.27 (45.68)	1900.46 (52.06)	1401.37 (40.58)	1503.30 (36.67)	1462.33 (42.84)	1394.84 (38.48)	1467.10 (39.89)
<b>Form DNA III</b>	0	1676.86 (50.51)	0	0	0	0	0	0	0
<b>Total DNA</b>	5340.08	3255.60	4514.75	3650.19	3453.69	4099.81	3413.14	3624.87	3677.93

radicals mediated damage in DNA nicking assay. These radicals converted Form I DNA to Form II DNA though single strand breakage. It is evident from Table 1 that 68.01% of Form I DNA was present in negative control whereas Form I DNA was absent in positive control (Fenton's reagent treated DNA) and the supercoiled DNA was completely degraded to Form II (ss nicked) and Form III (ds nicked and linear DNA) having 48.49% and 50.51% amount of degraded DNA respectively. The addition of 1000 µg/ml rutin solution along with Fenton's Reagent preserved 54.32% of Form I DNA (supercoiled) as compared to negative control which was having 68.01% of Form I DNA. Furthermore, the different concentration of methyl gallate preserved 47.94%-63.33% of supercoiled DNA and thus prevented its conversion to nicked DNA by scavenging hydroxyl radicals. The lower concentrations were found to be more effective in preserving supercoiled form of DNA.

## Discussion

From the present investigation, it was found that Methyl gallate, isolated from the ethyl acetate fraction of *Chukrasia tabularis*, has pronounced antioxidant efficacy but the effect was lower than that of gallic acid (used as standard antioxidant compound). The substitution of -OH group of gallic acid with electron releasing (-CH<sub>3</sub>) group might be the reason for less activity of methyl gallate as number and arrangements of -OH groups in phenols and flavonoids altered their antioxidant potential. It was found that the degree of hydroxylation, substitution by electron withdrawing groups and electron releasing groups alter the activity of phenolic acids. In hydroxycinnamic acids, it was found that caffeic acid with two hydroxyl groups attached to benzene ring has higher antioxidant activity than ferulic acid that is characterized by the presence of one hydroxyl groups and one methoxy groups in the ring structure. The ferulic acid in turn is found to be more active than *p*-coumaric acid having single -OH group (Pekkarinen et al., 1999; Shahidi and Chandrasekara, 2010). In 1995, Castelluccio and co-workers reported the effect of methoxylation and hydroxylation on the activity of hydroxycinnamic acids (caffeic acid, ferulic acid and *p*-coumaric acid). It was found that in metmyoglobin mediated peroxidation of lipids, caffeic acid was most active with IC<sub>50</sub> value of 0.33 µM less than ferulic acid (0.9 µM) and *p*-coumaric acid (4 µM) (Castelluccio et al., 1995). In a similar manner, it was found that rosmarinic acid with four hydroxyl groups has higher antioxidant potential in different *in vitro* models as compared to caffeic acid with two hydroxyl groups (Chen and Ho, 1997).

Among hydroxybenzoic acids, it was found that hydroxylation has resulted in enhanced hydrogen and electron donating activity. 3,4,5-trihydroxybenzoic acid (gallic acid) was found to be more active than 2,3-dihydroxybenzoic acid that in turn was found to be more active than 3-hydroxybenzoic acid. However, methoxylation i.e. substitution of hydroxyl group with electron releasing group lower the activity of resultant derivative (Pekkarinen et al., 1999; Chen and Ho, 1997).

In the present study, it was found that although methyl gallate showed pronounced electron and hydrogen donating potential in DPPH, ABTS radical cation scavenging assay, reducing power assay but the effect was lower as compared to gallic acid. It was found that methyl gallate with an IC<sub>50</sub> value of 21.679 µg/ml and 8.689 µg/ml respectively in DPPH assay and ABTS<sup>+</sup> scavenging assay has lower activity than gallic acid that exhibited the 50% inhibitory values of 8.499 µg/ml and 2.569µg/ml respectively. In a similar manner, the higher inhibitory effect of gallic acid was observed in superoxide anion radical scavenging and hydroxyl radical scavenging assay than its methyl derivative. However, in site-specific deoxyribose degradation assay and lipid peroxidation assay, methyl gallate has higher activity with IC<sub>50</sub> value of 20.086 µg/ml and 15.825 µg/ml respectively than gallic acid (IC<sub>50</sub> value = 61.417 µg/ml and 22.874 µg/ml respectively). The higher activity of methyl gallate in these assays might be related to its ferrous ion chelation ability whereas gallic acid lacks such activity. The presence of electron releasing group in methyl gallate might contribute to its metal ion chelation potential. The integrity of supercoiled DNA by methyl gallate in plasmid nicking assay was also due to their hydroxyl radical scavenging potential.

It has been concluded from the present study, methyl gallate, isolated from ethyl acetate fraction of *C. tabularis* using column chromatography, has been found to exhibit potent antioxidant activity in different *in vitro* models and the activity was related to its chemical structure.

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