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Synergetic Effect of Green Tea Catechins in Presence of Metal Ions

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Abstract: **Preventive and therapeutic activity of green tea is well known, the reports pertaining to the synergetic effect of its constituent polyphenolic catechins are of current interest to many, such as chemists, biologists, scientists from pharma industry, naturopathy, etc. Present report describes the results from an experimental investigation about the effect of two metal ions - Al(III) and Fe(II) on binding affinity of two catechin compounds, (–)-epigallocatechin (EGC) and (–)-epigallocatechin gallate (EGCG) with bovine serum albumin (BSA) and calf thymus 2- deoxyribonucleic acid(CT-DNA) using UV-visible spectroscopic method. Calculated Binding constant values indicate that the interaction of EGCG and EGC with BSA increases significantly for each metal-catechin conjugates.**

Keywords: **Green tea catechins, EGCG, EGC, BSA, Binding constant, UV-titration.**

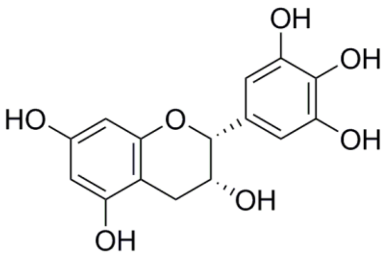
1. INTRODUCTION

Polyphenolic catechins that constitute up to 30% of dry green tea leaves have (–)-epicatechin (EC), (–)-epicatechin gallate (ECG), (–)-epigallocatechin (EGC) and (–)-epigallocatechin gallate (EGCG) as main active components [1,2] and are accountable for the varied pharmaceutical properties of *Camellia sinensis*[3,4]. Green tea and its constituent catechins in particular, due to their long known strong antioxidant activity, are subject of active interest for interdisciplinary research – especially to biologists and pharmaceutical chemists [5]. These catechins are known to exhibit preventive and therapeutic effect against a variety of lethal diseases, such as cancer [6], cell proliferation [7], tumor growth [8], tumor invasion & angiogenesis [9,10], atherosclerosis [11], cardiovascular diseases [12] and also possess antimicrobial activity against a large range of pathogenic microorganisms [13-15]. Essential metals are micro components in organism bodies that are indispensable for healthy functioning of organs. Although, some of them may be considered as macro metal due to their requirement in higher amount for survival of the organisms, most of these are present in very low quantity yet are able to regulate functioning of various organs. Properties of ligand molecules may be altered, either enhanced or diminished, because of the conjugation of ligand with metal. Green tea catechins, due to their polyphenolic nature may also act in synergy with metal ions in the body of organism. Noritaka Kugaya *et al.* investigated the preventive activity of EGCG against hepatotoxin-induced cell injury and reported that EGCG alone was not much effective as hepatoprotecting agent, however, its hepatoprotecting activity was found to be enhanced considerably in presence of zinc due to its complexation with the latter [16].Free radical scavenging ability and growth inhibitory capacity of EGCG on PC-3 cells were also found to be enhanced by the presence of free Zn^{+2} ions while zinc-EGCG complex had no effect [17].UV-visible and liquid state ^{1}H NMR spectroscopic techniques have been utilized to identify the conjugation of EGCG with Zn(II) ion[18].Measurement of the ^{1}H nuclear spin relaxation time T_1 of EGCG and EGC has revealed that as compared togallocatechin ring, the gallate ring of EGCG has stronger capacity to coordinate with Mn^{+2}ion [19].

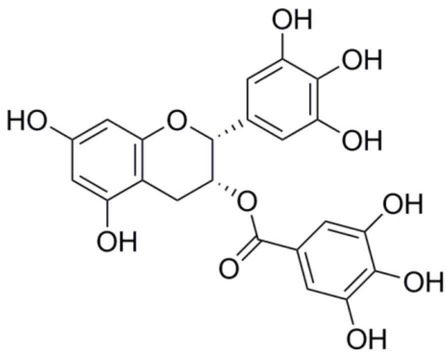
In the present investigation an attempt has been made to study the effect of two metal ions - Al(III) and Fe(II) to form EGCG/EGC conjugates and to identify the effect on the interaction with bovine serum albumin (BSA) and calf thymus 2-deoxyribonucleic acid(CT-DNA) using UV-visible spectroscopic method. Binding constants for each metal-catechin conjugates with BSA and DNA have been calculated from double reciprocal plots and compared.

2. MATERIALS AND METHODS

Calf thymus DNA (CT-DNA), (-)-epigallocatechin gallate (EGCG) and (-)-epigallocatechin (EGC) (Fig.1) were purchased from TCI Chemicals (India) Pvt. Ltd., bovine serum albumin (BSA) was bought from Sigma-Aldrich and all other reagents viz. anhydrousdisodium hydrogen phosphate (Na_2HSO_4), sodium dihydrogen orthophosphate ($NaH_2PO_4.2H_2O$), acetic acid (CH_3COOH), sodium acetate (CH_3COONa), aluminium sulphate ($Al_2(SO_4)_3$), ferrous sulphate ($FeSO_4$) and EDTA of AR grade were used without further purification. The pH values of the solutions prepared were determined on pH meter in reference to the standard. UV-Visible spectra measurements were carried out using a UV-2700 spectrophotometer (Shimadzu, Japan) at room temperature.



(a) *EGC*



(b) *EGCG*

Fig 1: Green tea Catechins- a) EGC and b) EGCG

2.1 Preparation of stock solutions

Phosphate buffer of 7.2 pH was prepared by the accurate combination of 10 mM disodium hydrogen phosphate (Na_2HPO_4) solution with 10 mM sodium dihydrogen orthophosphate ($NaH_2PO_4.2H_2O$) solution. 100 mL 1mM EDTA phosphate buffer solution was prepared by dissolving 0.0292 g EDTA (ethylenediaminetetraacetic acid) in the phosphate buffer solution. Acetate buffer of 5pH was prepared by mixing 20 mM acetic acid (CH_3COOH) solution in 20 mM sodium acetate (CH_3COONa) solution. BSA and DNA solutions were prepared in phosphate buffer of 7.2 pH, while acetate buffer of 5pH was used to prepare 0.58 mM solutions of EGCG and EGC and 0.17 mM solution of selected metal salts i.e., $Al_2(SO_4)_3$ and $FeSO_4$.

2.2 UV spectroscopic measurements of EGCG /EGC metal Complex

To investigate the effect of metal ions on the interaction of catechins (EGCG/EGC) with BSA, two sets of experiments were undertaken – i) UV spectroscopic measurements of BSA-EGCG/EGC mixture, initially made with 10 μL solution of each, followed by nineteen successive additions of 5μL of EGCG or EGC solution (Fig 2), ii) UV spectroscopic measurements of mixture of solutions of BSA and metal-catechin conjugate with increasing ratio of catechin in latter. For the second set of experiments, total 19 sets of metal-catechin complex were arranged by successive addition of5μL of EGCG or EGC solution to the initial mixture of 10 μL Catechin and 10 μL metal ionic solutions. During this, the range of metal:catechin molar ratio increased from the initial 1:3.41 to 1:68.24. To all sets prepared for the four metal-catechin complexes, i.e. Al(III)-EGCG, Al(III)-EGC, Fe(II)-EGCG and Fe(II)-EGC, 10μl aliquot of BSA was added and resulting mixtures were incubated at room temperature for 1h followed by the recording of UV-visible spectrum in each case. In case of the investigation of the effect of the same metals on binding of EGCG with DNA, similar process was repeated for the interaction of Al(III)-EGCG and Fe(II)-EGCG with DNA, for which 10 sets of metal-catechin conjugates were arranged by successive addition of 10μL of EGCG solution to10 μL metal ionic solutions.

3. RESULT AND DISCUSSION

3.1 Interaction of EGCG/EGC and metal-EGCG/EGC conjugates with BSA

UV-visible spectra of BSA-Catechin complex

The absorbance plots of BSA at different concentrations of EGC/EGCG in 20 mM phosphate buffer at pH 7.2have been presented in Figure 2. The absorbance maxima corresponding to the BSA at 279 nm is found to be increasing with increasing concentration of EGC or EGCG. This increment in the absorbance of BSA may be attributed to the greater possibility of interaction between BSA and EGC/EGCG at the higher concentration of latter.

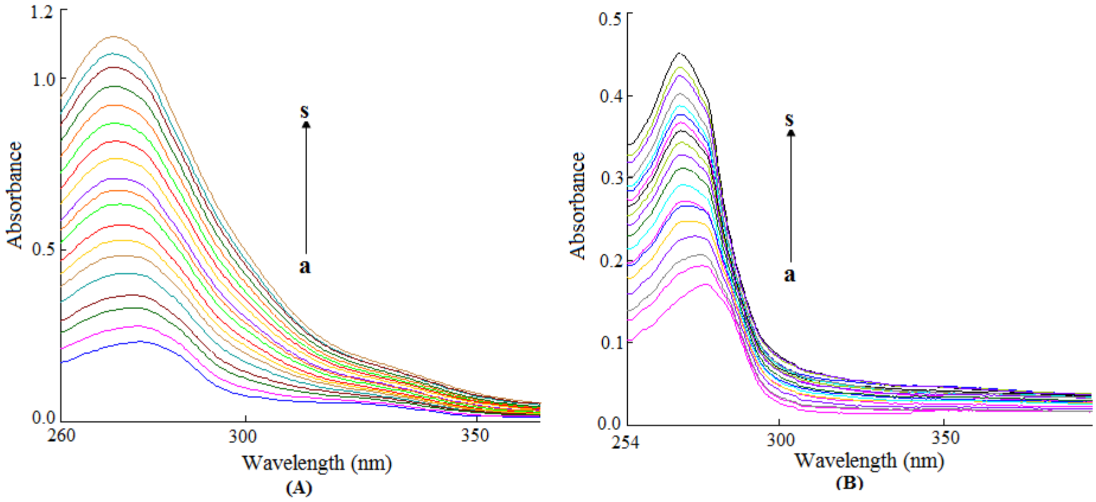


Fig 2: Absorbance plot of BSA-catechin complex

(A)BSA (10 µL) with increasing amount of EGCG from *a* (10µL) to *s* (100 µL) at 5 µL interval

(B)BSA (10 µL) with increasing amount of EGC from *a* (10µL) to *s* (100 µL) at 5 µL interval

UV-visible spectra of BSA with Catechin-metal conjugates

To investigate the effect of metal on interaction of selected catechins with BSA, UV-visible spectra of metal-catechin conjugates have been measured for Al(III) and Fe(II)] ions. Observed UV-visible absorption spectra of Al(III)-EGCG, Fe(II)-EGCG, Al(III)-EGC and Fe(II)-EGC with BSA have been presented in Fig. 3.

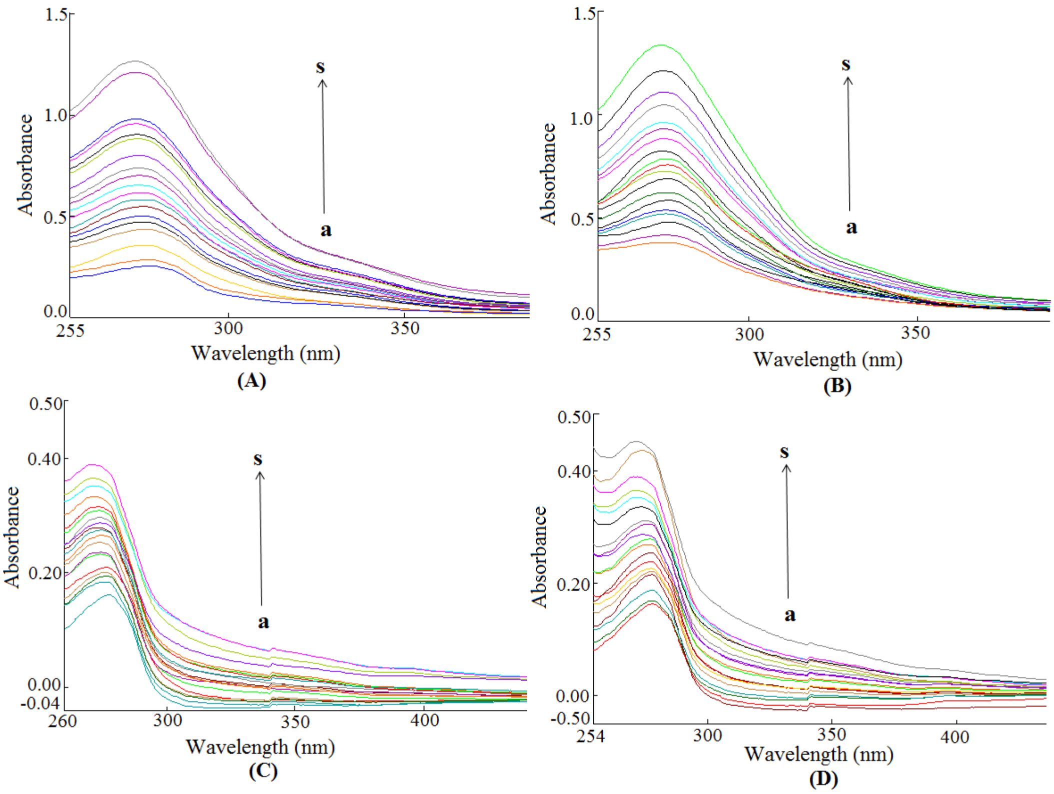


Fig 3: Absorbance plot of BSA-metal-catechin conjugate[BSA (10 µL)

EGCG - *a*(10µL) to *s* (100 µL) at 5 µL interval-(A) with 10µL of Al(III) and (B) 10µL of Fe(II) ions

EGC - *a* (10µL) to *s* (100 µL) at 5 µL interval-(C) with 10µL of Al(III) and (D) 10µL of Fe(II) ions

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The absorbance plots of metal-EGCG/EGC conjugates with BSA (Fig. 3) vary from those of EGCG/EGC with BSA (Fig. 2) indicating that the presence of selected metal ions has sufficient effect on the binding of catechins with BSA so as to alter the absorbance of BSA. Irregular increment in the λ_{max}value of BSA with the increase in the concentration of metal-EGCG/EGC conjugates were observed in all cases, which may be either due to the incomplete conjugation of EGCG/EGC with metal ions or due to incomplete complexation of metal-EGCG/EGC conjugates with BSA. Binding constant denotes the affinity of interaction for any ligand with its target. Therefore, association constant for BSA with EGCG and EGC has been derived using Benesi-Hildebrand plot based on absorbance. Double reciprocal plots between 1/ΔA and 1/[EGCG] and 1/ΔA and 1/[EGC] have been made and binding constants have been calculated from the ratio of the intercept to the slope of the corresponding plots (Fig. 4). Greater binding constant value of EGCG indicates its greater capacity to interact with BSA as compared to EGC (Table 1). The higher binding affinity of EGCG with BSA may be due to the presence of galloyl moiety having larger number of hydroxyl groups that consequently increase possibility of hydrogen bonds between EGCG and BSA.

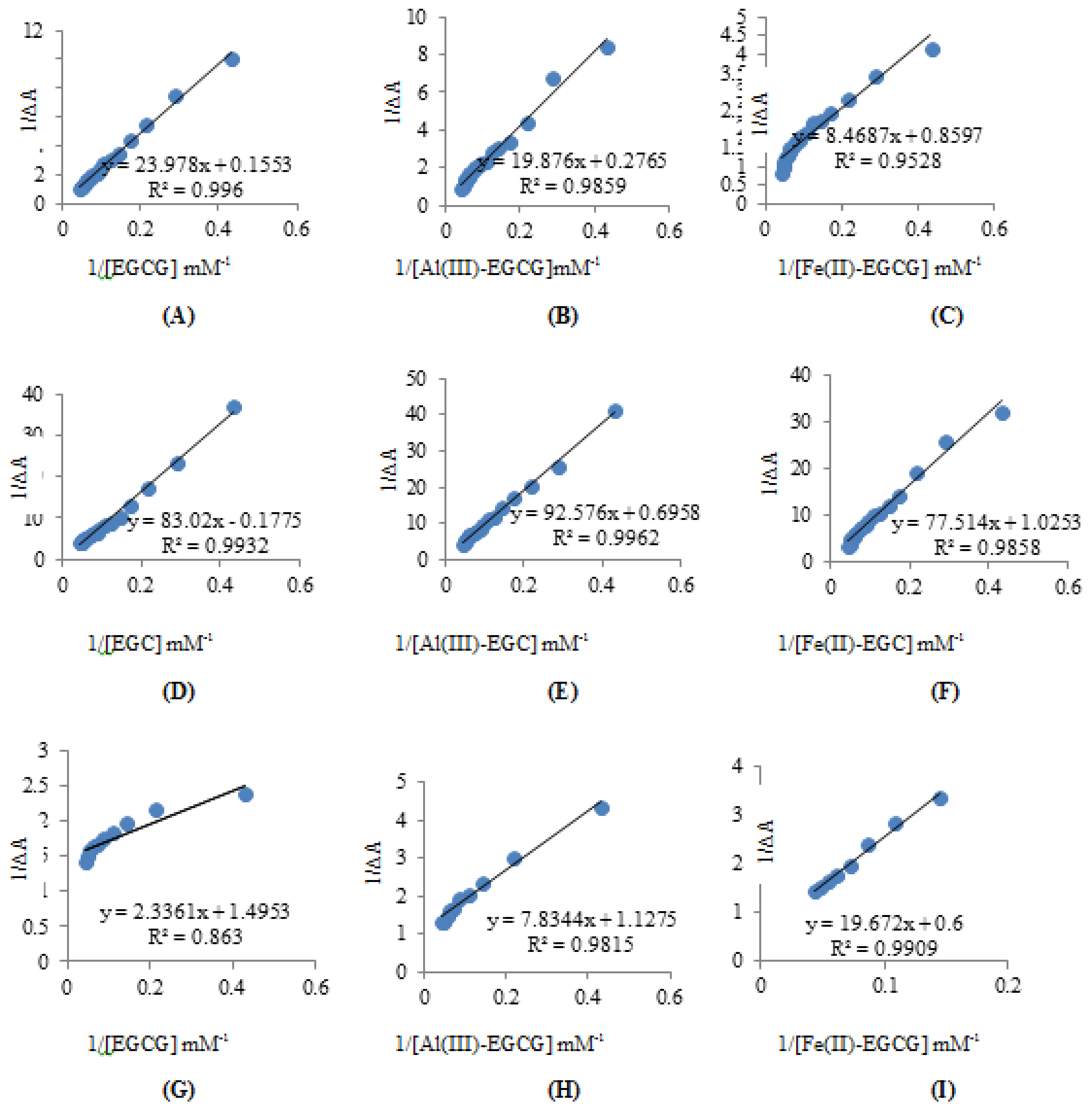

Fig 4: Double Reciprocal Plots between variation in absorbance of BSA (ΔA)and concentration of - (A) EGCG, (B) Al(III)-EGCG, (C) Fe(II)-EGCG, (D) EGC, (E) Al(III)-EGC, (F) Fe(II)-EGC and absorbance of DNA(ΔA) and concentration of (G) EGCG, (H) Al(III)-EGCG and (I) Fe(II)-EGCG

TABLE 1: Binding constant and R^2 values of EGCG, EGC and their metal conjugates for BSA

EGCG & EGCG-metal conjugates	Binding constant (M^{-1})	R^2	EGC & EGC-metal conjugates	Binding constant (M^{-1})	R^2
EGCG	6.476 x 10^3	0.996	EGC	2.138 x 10^3	0.9932
Al(III)-EGCG	1.389 x 10^4	0.9859	Al(III)-EGC	7.507 x 10^3	0.9962
Fe(II)-EGCG	1.014 x10^3	0.9528	Fe(II)-EGC	1.322 x 10^4	0.9858

It is evident from the analysis of data for interaction with BSA, all metal-catechin [Al(III)-EGCG, Al(III)-EGC and Fe(II)-EGC] have the higher binding constant values (Table 1) except the case of Fe(II)-EGCG, for which the binding constant value is same as EGCG. Higher values of binding constants indicate that presence of metal ions increases the binding affinities of catechins with BSA. The enhancements in binding constant values may be attributed to the fact that due to the presence of metal ions, the charge distribution provides more sites for the generation of attractive forces between catechins and BSA. In the case of EGCG, higher value of binding constant was obtained for Al(III)-EGCG (1.389 x 10^4M^{-1}) complex than the Fe(II)-EGCG (1.014 x10^3M^{-1}). However, in case of EGC, binding constant value for Fe(II)-EGC (1.322 x 10^4M^{-1}) is higher than Al(III)-EGC (7.507 x 10^3M^{-1}), the reason for which is not obvious

3.2Ineractionof EGCG and metal-EGCG conjugates with DNA

Nucleic acid is the genetic unit of organism and in many cases attacked by pathogens or their metabolites, which alters its geometry and chemical composition and causes various diseases. Therefore, investigation of drug–nucleic acid interaction is one of the important tools for drug designing. In the present case, the binding constant values calculated for the DNA and EGCG are presented in Table 2 and absorbance plots in Fig 5.

TABLE 2: Binding constant and R^2 values of EGCG and its metal conjugates for DNA

EGCG & its metal conjugates	Binding constant(M^{-1})	R^2
EGCG	6.3 x 10^5	0.863
Al(III)-EGCG	1.4 x 10^5	0.9815
Fe(II)-EGCG	3.0 x 10^4	0.9909

The results obtained reveal that EGCG binds more strongly with DNA than BSA. A regular increasing interval in absorbance of DNA at 260 nm was observed with increasing the concentration of EGCG in 20 mM phosphate buffer at pH 7.2 (Fig. 5).

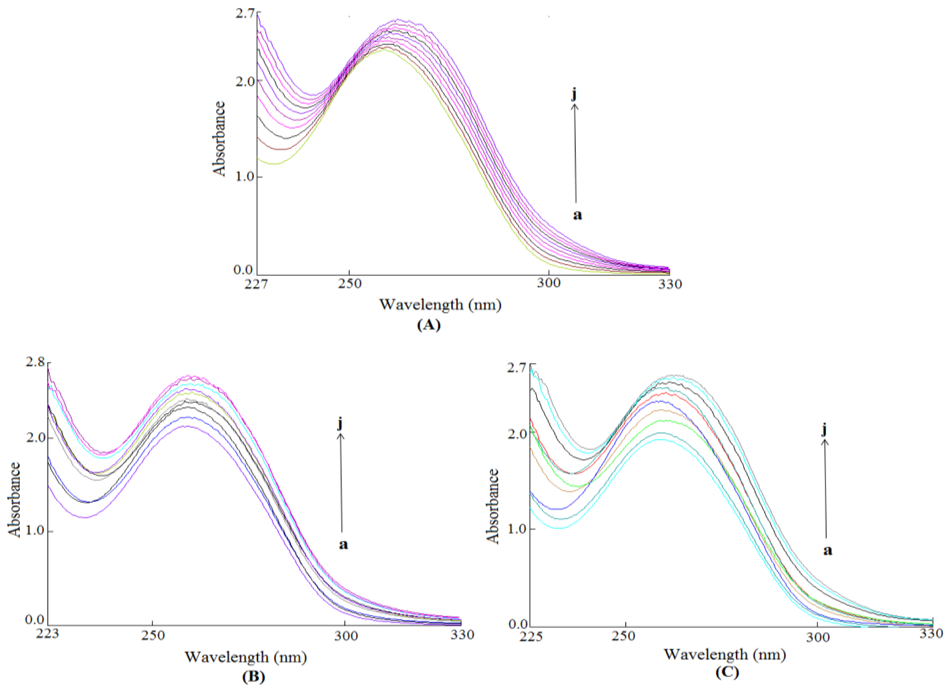

Fig 5: Absorbance plot of DNA-EGCG/metal-EGCG complex

(A)DNA (10 µL) with increasing amount of EGCG from *a* (10µL) to *j* (100 µL) at 10µL interval

(B) in addition with 10µL of Al(III) and (C) in addition with 10µL of Fe(II) ions

The difference observed between spectra of EGCG alone and spectra of Al(III)-EGCG and Fe(II)-EGCG conjugates indicates that the influences of metal ions on the interaction of EGCG with DNA (Fig.5). However, both metal ions diminish the affinity of EGCG with DNA (Table 2). This reduction was obtained due to the large size of metal ions which creates steric hindrance during binding of EGCG with DNA.

4. CONCLUSION

The present investigation centered on the effect of the binding of catechins with biomolecules like, DNA and BSA in presence or absence of metal ions indicates that the influences of metal ions on the interaction of green tea catechins are quite significant. During the processing or preparation of the green tea, the catechins come in the contact of metal ions to formmetal-catechin conjugates or alternatively, the metal ions present in organism form catechin metal conjugates having sufficient affinity to alter the physiological properties of catechins. In the present investigation the UV spectroscopic technique has been shown to be a handy tool to identify such interaction.

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