J Fluoresc (2012) 22:1113–1125
DOI 10.1007/s10895-012-1050-4

ORIGINAL PAPER

# Investigations on Photoinduced Interaction of 9-Aminoacridine with Certain Catechols and Rutin

**C. Manivannan · K. Meenakshi Sundaram · R. Renganathan · M. Sundararaman**

Received: 25 October 2011 / Accepted: 19 March 2012 / Published online: 3 April 2012


**Abstract** The fluorescence quenching of 9-aminoacridine by certain biologically important catechols and rutin was investigated using absorption, steady state and time resolved fluorescence measurements. The in vitro-antioxidant activities of the above compounds were studied using deoxyribose degradation assay and nitric oxide scavenging assay. The experimental results showed that the fluorescence of 9-aminoacridine was quenched by quencher molecules via forming ground state complex. The bimolecular quenching rate constant $k_q$, binding constant (K) and number of binding sites (n) were calculated at different temperatures from relevant fluorescence data. Static quenching mechanism was supported by lifetime measurement. The free energy change ($\Delta G_{et}$) for electron transfer process was calculated by Rehm-Weller equation. The binding distance of 4-nitrocatechol with 9-aminoacridine was obtained according to Forster's non-radiative energy transfer theory. Nature of binding forces and their interactions was probed based on thermodynamic parameters.

**Keywords** 9-aminoacridine · Antioxidant activity · Quenching mechanism

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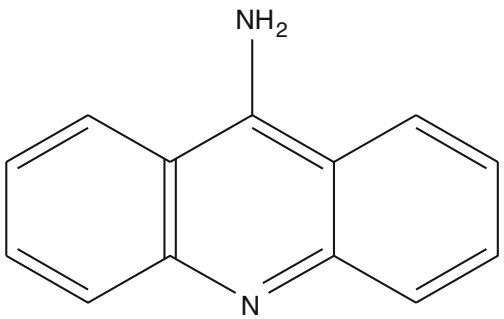
## Introduction

9-Aminoacridine (9-AA) is a polynuclear hetreocycle base and interacts strongly with biomolecules such as DNA and proteins [1–4].9-AA is an antibacterial, and antitumor drug that has been proposed as a specific fluorescent probe capable of binding the active center of guanidinobenzotase [5].



9 - Aminoacridine

Antioxidants play a vital role in biology, polymer chemistry and food industry. By intercepting oxidizing species, predominantly reactive radicals, they prevent cellular damage and polymer or food degradation. Catechols and catechol amines are antioxidants and play a role in scavenging reactive oxygen species (ROS), typically superoxide [6]. Rutin is naturally occurring flavonoid used widely in biochemical and pharmacological activities [7]. It scavenges free radicals [8] and depresses cellular immunity [9]. Knowledge of the bimolecular reaction kinetics by which antioxidants intercept reactive oxidizing species, as studied herein, is of utmost importance for modeling their actual activity and understanding the mechanism by which they act [10]. Fluorescence quenching can be resolved with high sensitivity towards mechanism and action of antioxidants

than other conventional methods like absorption measurements involving DPPH [11] or ABTS radicals [12]. Recently we have reported the fluorescence quenching of 9-AA showing pronounced tendency to undergo charge transfer process with certain antioxidants such as estrogens and flavonoids [13]. Similarly the effect on substitution of electron donor and acceptor groups on fluorescence was examined by Nandhikonda et al. [14].

In the present work, the fluorescence quenching of 9-AA by various catechols (such as catechol, 3-methylcatechol, 4-tert-butyl catechol, 4-nitrocatechol, Rutin, DPMN, and L-DOPA) having different substituents on the skeleton has been measured in two different solvents of varying polarities. The oxidation potential of catechol moieties was also measured using cyclic voltammetry techniques and Rehm-Weller equation was applied to measure free energetics for electron transfer process. For supporting this radical scavenging mechanism, in vitro-antioxidant activity was also investigated by deoxyribose degradation assay and nitric acid scavenging assay. The quenching mechanism was confirmed from lifetime measurements.

## Materials and Methods

### Materials

9-Aminoacridine hydrochloride monohydrate (9-AA) was purchased from Sigma. All catechols such as catechol, 3-methylcatechol, 4-nitrocatechol, 4-tert-butyl catechol, rutin, dopamine hydrochloride (DPMN) and 3, 4-dihydroxy-L-phenylalanine (L-DOPA) were obtained from Sigma Aldrich. 2-deoxyribose, Ascorbic acid, ferrous sulphate heptahydrate, thiobarbutric acid, sulphonamide were purchased from Himedia. Trichloroacetic acid, Sodiumnitroprusside, orthophosphoricacid,naphthylethylenediamine dihydrochloride were purchased from Merck. Spectroscopic grade solvent, $CH_3CN$ and double distilled water were used for preparing the solutions. All measurements were performed at room temperature.

### Steady State Measurements

The steady state fluorescence quenching measurements were carried out in JASCO FP-6500 Spectrofluorimeter. The excitation wavelength of 9-AA was 400 nm and the emission maximum was monitored at 434 nm. The excitation and emission slit widths (3 nm) and scan rate (200 nm/min) were maintained constant for all experiments. Samples were prepared by dissolving 9-AA in water and administering the appropriate amounts of catechols. The samples were carefully degassed using pure nitrogen gas for 15 min. Quartz cells (4×1×1) cm with high vacuum Teflon stopcocks were used for degassing. Absorption measurements were recorded using JASCO V-630 UV–vis spectrophotometer. Radical scavenging mechanism was investigated using SYSTRONICS 119 UV–vis spectrophotometer. ELCO LI-120 pH meter was used to maintain the pH of buffer solution.

### Time Resolved Fluorescence Measurements

Fluorescence lifetime measurements were carried out in a picosecond time correlated single photon counting (TCSPC) spectrometer. The excitation source was the tunable Ti-sapphire laser (TSUNAMI, Spectra Physics, USA). The diode laser pumped millennia V (Spectra Physics) CW Nd-$YVO_4$ laser was used to pump the sapphire rod in the Tsunami mode locked picosecond laser (Spectra Physics). The diode laser output was used to pump the Nd-$YVO_4$ rod in the Millennia. The time-resolved fluorescence emission was monitored at 434 nm. The emitted photons were detected by a MCP-PMT (Hamamtsu R3809U) after passing through the monochromator (*f*/3). The laser source was operated at 4 MHz and the signal from the photodiode was used as a stop signal. The signal from the MCP-PMT was used as start signal in order to avoid the dead time of the TAC. The difference between the start and stop signal is due to the time taken by the pulses traveling through the cables and electronic relaxation of the excited state. The data analysis was carried out by the software provided by IBH (DAS-6). The kinetic trace was analyzed by non-linear least square fitting of mono exponential function.

### Cyclic Voltammetric Measurements

In the present study, the reduction potential and oxidation potential of the compounds were measured in water with potassium chloride (0.1 M) as the supporting electrolyte. The experimental setup consisted of a platinum working electrode, a glassy carbon-counter electrode and a silver reference electrode. Irreversible peak potential of catechol compounds were measured at different scan rates (0.05 V/s). All samples were deaerated by bubbling with pure nitrogen gas for ca. 5 min at room temperature.

### Deoxyribose Degradation Assay

Deoxyribose degradation assay was determined according to the method described by Chung et al., [15]. The extent of deoxyribose degradation was measured by the thiobarbutric acid reaction. Reaction mixtures, in a total volume of 1 ml, contained 6 mM-deoxyribose, 0.01 mM- ascorbic

acid, 0.1 mM—phosphate buffer, pH 7.4, 0.01 mM—$H_2O_2$ and 0.1 mM-$FeSO_4$. 7 $H_2O$. The reaction mixture was incubated with test samples at 310 K for 60 min. After incubation time 3 ml trichloroacetic acid and 3 ml thiobarbutric acid was added and heated for exactly 10 min at 353 K. The mixture was cooled at room temperature and the absorbance at 532 nm was noted. Similarly the control solution was prepared without test samples. The change in absorbance at 532 nm was noted against the control solution.

The hydroxyl radical scavenging activity is reported as percentage inhibition of deoxyribose degradation and calculated as,

$$\%\,\text{Inhibition} = [\text{A(control)} - \text{A(standard)}/\text{A(control)}] \times 100 \quad (1)$$

where,

| | |
|---|---|
| A (control) | Absorbance of the reactant mixture without test samples and |
| A(standard) | Absorbance of the reactant mixture with test samples. |

### Nitric Oxide Radical Inhibition Assay

Nitric oxide radical inhibition assay was carried out according to the literature report [16, 17]. Nitric oxide production was initiated by the addition of 1 ml of sodiumnitroprusside (10 mM) in 1 ml phosphate buffer (0.1 mM, pH 7.4). The reaction mixture was incubated with test samples at various concentrations (50 μg/ml, 100 μg/ml and 150 μg/ml) at 398 K for 150 min. After the incubation, 1 ml of Griess reagent was added and kept at room temperature for 30 min. Pink colour was developed and the absorbance was read at 546 nm. Similarly, a control solution was also prepared in the same way without test compound. The nitric oxide radical scavenging activity was calculated as percentage inhibition of NO

$$\%\,\text{Inhibition of NO} = [\text{A(control)} - \text{A(standard)}/\text{A(control)}] \times 100 \quad (2)$$

where,

| | |
|---|---|
| A (control) | Absorbance of the reactant mixture without test samples and |
| A (standard) | Absorbance of the reactant mixture with test samples. |

## Results and Discussion

The fluorescent dye, 9-AA absorbs maximum at 400 nm and shows maximum emission at 434 nm as shown in the Fig. 1. The structure of quencher molecules used for our study is shown in Scheme 1.

### Ground State Interaction

Initially before the accomplishment of quenching experiments it is essential to measure the interaction between 9-AA and quencher molecules in the ground state. Measurements of UV–vis absorption spectra have been performed for all the compounds in aqueous solution. All catechol compounds absorbs below 290 nm. But, 4-nitrocatechol and rutin absorbs at 350 and 356 nm respectively.

9-AA shows maximum absorbance at 400 nm and DPMN absorbs at 280 nm. However, addition of DPMN to 9-AA decreases the absorption intensity of 9-AA followed by bathochromic shift (red shift) . The expansion of peaks from 375 nm to 435 nm is shown in the Fig. 2 for clear understanding. Therefore, it is inferred that there is a possibility of ground state complex formation exist between 9-AA and DPMN. It may be noted that similar type of

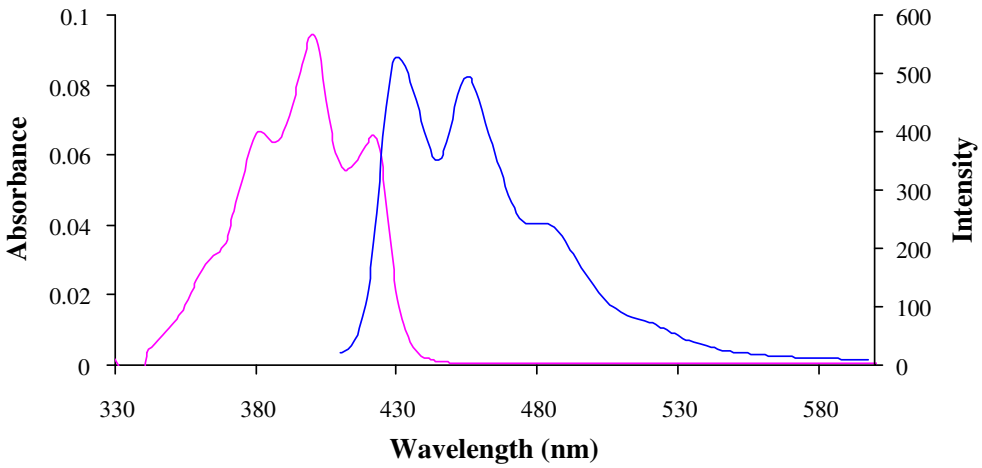
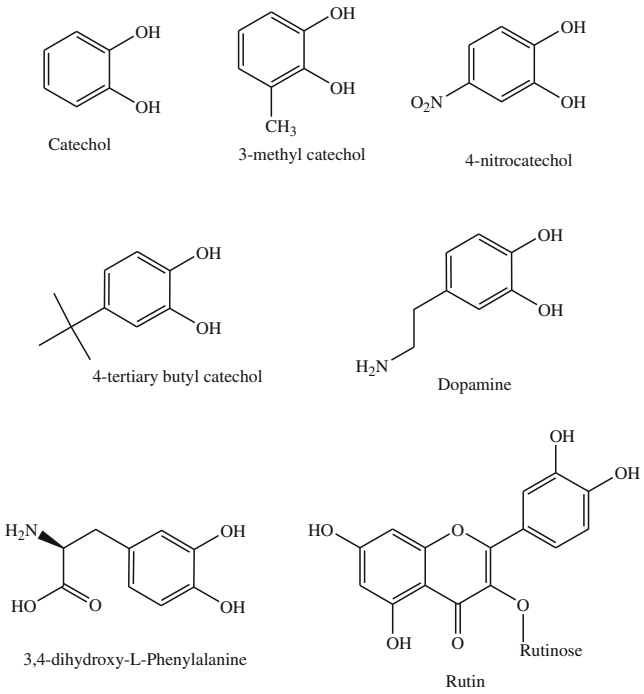



**Fig. 1** Absorption and Emission spectrum of 9-Aminoacridine




**Scheme 1** Structure of quencher molecules

behavior was observed for other quencher molecules chosen in our present study.

## Fluorescence Quenching of 9-AA by Catechols

The fluorescence intensity of 9-AA decreased by the addition of catechols as shown in the Fig. 3. The Stern-Volmer quenching plots obtained by the plot $I_0/I$ vs [Q] as shown in the Figs. 4 and 5 were linear and gave consistent results. The quenching rate constants ($k_q$) were determined by the Stern-Volmer equation Eq. (3)

$$I_0/I = 1+K_{SV}[Q]= 1+k_q\,\tau_0\,[Q] \qquad (3)$$

where, $I_0$ is the fluorescence intensity of the fluorophore in the absence of quencher, I is the fluorescence intensity of the fluorophore in the presence of quencher, $K_{SV}$ is the Stern-Volmer constant, $\tau_0$ is the fluorescence lifetime in the absence of quencher, [Q] is the concentration of the quencher and $k_q$ is the bimolecular quenching rate constant. From the slope $K_{SV}$ value can be assessed. The lifetime of 9-AA in water is 15.6 ns. Thus, the bimolecular quenching rate constant ($k_q$) is calculated based on Eq. (3). The bimolecular quenching rate constants and the corresponding electrochemical datas are compiled in Table 1. The quenching rate constant is dependant on the structure and electronic effects of substituents in quenchers. From the $k_q$ values given in the Table 1 the following trend is observed in water among the catechols

$$4-\text{nitrocatechol} > \text{DPMN} > \text{rutin} > \text{L}-\text{DOPA} > \text{t}-\text{butyl}-\text{catechol} > 3-\text{methyl catechol} > \text{catechol}.$$

Catechols having different substitutions i.e., electron donating and electron withdrawing were employed for the quenching of 9-AA. Among the catechols, 4-nitrocatechol shows dramatic increase in $k_q$ value which is not expected from the electron withdrawing nature of nitro group. Such a trend is supported by the absorption spectrum of 4-nitrocatechol which overlaps with emission spectrum of 9-AA which reveals energy transfer from the excited 9-AA to 4-nitrocatechol. Dopamine consist of a benzene ring with two adjacent hydroxyl groups (3 and 4th position) and a side chain of ethylamine. Presence of an electron donating group in the catechol moiety such as ethylamine promotes the antioxidant activity and leads to an enhanced release of $H^{\bullet}$. The lower $k_q$ value of rutin compared to DPMN is also due to the diminished π-conjugation between B and C rings. The steric repulsion arises between the 6′ or 2′ ring protons at the B ring and rutinose group (sugar substituents) in the C ring. L-DOPA exhibits relatively lower $k_q$ than rutin, because the

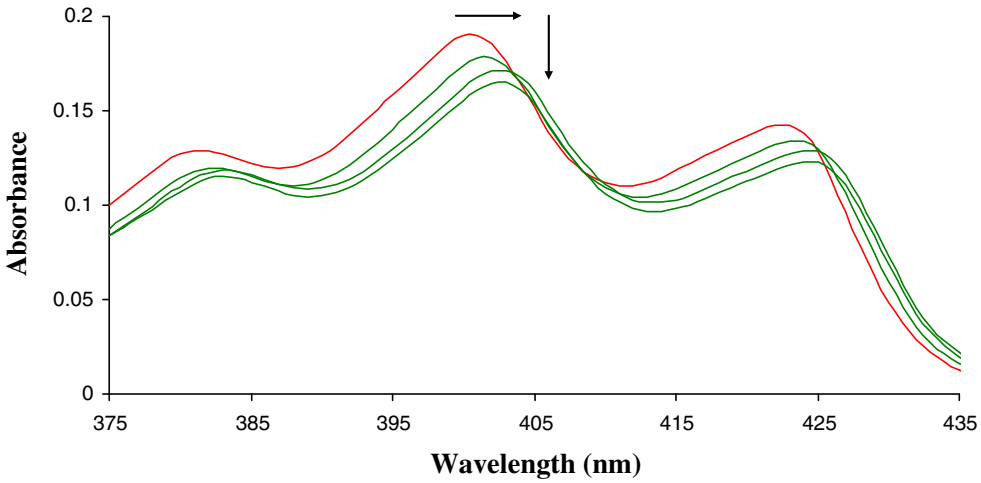



**Fig. 2** Absorption spectrum of 9-Aminoacridine ($4\times10^{-5}$ M; *Red line*) in the presence of Dopamine (0, 3, $5\times10^{-4}$M; *Green line*) in water.(The *arrows* indicates the decrease in absorbance followed by red shift)

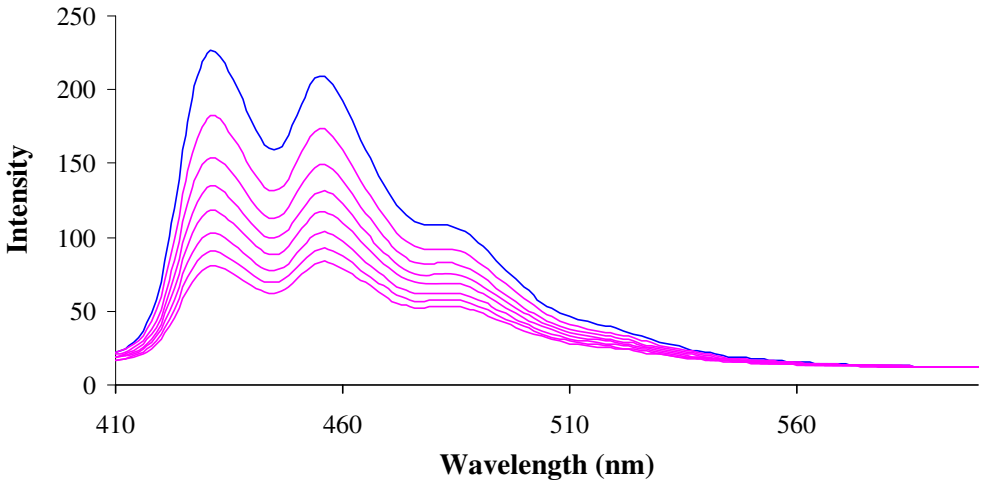



**Fig. 3** Fluorescence quenching of 9-Aminoacridine ($2\times10^{-6}$ M; *Blue line*) in the presence of 4-nitrocatechol (0, 1, 2,3,4, 5, 6, $7\times10^{-4}$ M; *Pink line*) in water

electron releasing amine becomes less effective owing to the presence of electron withdrawing nature of –COOH group. All substituted catechols shows high $k_q$ value than the parent catechol. The 4-t-butyl catechol shows higher $k_q$ value than 3-methyl catechol and parent catechol which reveals the fluorescence quenching of 9-AA increases quite systematically with increase in the number of alkyl groups due to the electron donating abilities in them.

Static and dynamic quenching is distinguished by their dependence on different temperature and excited state life time. The bimolecular quenching constants measured at different temperatures were shown in Table 1. It was observed that $k_q$ decreased with increasing temperature for all quenchers. Therefore, it can be concluded that the quenching is not initiated by dynamic but probably by a static process.

## Fluorescence Lifetime Measurement of 9-AA with Certain Catechols

Fluorescence quenching shall be dynamic resulting from collisional counters between fluorophore and quencher or static resulting from the formation of ground state complex between fluorophore and quencher [18]. Fluorescence lifetime measurements is a definite method for understanding the interaction between donor and acceptor systems. In general, the measurement of fluorescence lifetime is the useful technique to distinguish static and dynamic quenching [19].

Almost for all samples, two exponential fit to the fluorescence decay proved to be significantly better than the one exponential fit. The predominant (0.871±0.005 relative amplitude) florescence lifetime of 9-AA in water was found to be 15.8±0.2 ns. However, there is also a minor component in the decay process (0.149±0.0020 relative amplitude) with lifetime of 5.4±0.5 ns. The obtained results found to be in agreement with the values, earlier report in the literature [20, 21]. From Fig. 6 though the decay traces of 9-AA in the absence and presence of quenchers were actually plotted, however, the lifetime of 9-AA remains same in both conditions. Hence the merging of the kinetic traces was observed. For instance, the decay curves of 9-AA in the absence and presence of DPMN were actually plotted, the

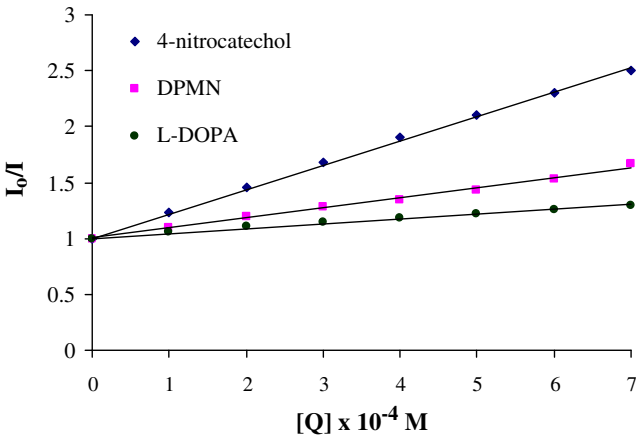



**Fig. 4** Comparison of Stern-Volmer plot for 4-nitrocatechol, DPMN, L-DOPA

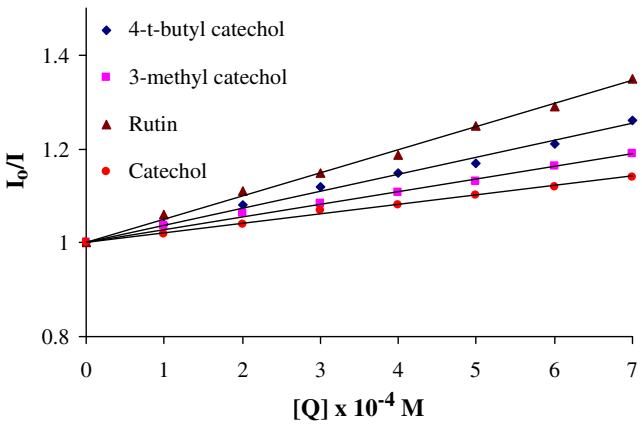



**Fig. 5** Comparison of Stern-Volmer plot for 4-t-butyl catechol, 3-methyl catechol, rutin, catechol

**Table 1** Fluorescence quenching rate constants, and thermodynamic data of 9-Aminoacridine by catechol compounds and rutin

| S.No | Quenchers | $k_q$ ($10^{11}$ $M^{-1}S^{-1}$)[a] | | | $E^{OX}_{1/2}$vs. SCE (V)[b] | $\Delta G^{et}$(eV)[c] |
|---|---|---|---|---|---|---|
| | | 15 °C | 25 °C | 35 °C | | |
| 1 | 4-Nitrocatechol | 18.5 | 15.8 | 12.8 | **** | ***** |
| 2 | DPMN | 4.98 | 4.05 | 3.78 | 0.67 | −3.25 |
| 3 | Rutin | 3.60 | 3.05 | 2.79 | 0.62 | −3.30 |
| 4 | L-DOPA | 3.07 | 2.63 | 2.20 | 0.58 | −3.34 |
| 5 | t-butyl catechol | 2.68 | 2.24 | 1.84 | 0.38 | −3.56 |
| 6 | 3-methyl catechol | 2.51 | 1.82 | 1.56 | 0.26 | −3.66 |
| 7 | Catechol | 2.20 | 1.28 | 0.86 | 0.2 | −3.72 |

[a] determined by steady state fluorescence quenching in water ($\tau_o$=15.6 ns)

[b] Oxidation potential of catechols in V vs SCE

[c] Calculated by Rehm-Weller equation $\Delta G_{et} = E^{ox}(D) - E^{red}(A) - E* + C$,, the reduction potential of 9-Aminoacridine is −1.016 V vs SCE, E* =2.91 eV. Error ± 5 %

plots look like single decay curve. This shows the fluorescence quenching of 9-AA by DPMN was static in nature. It may be noted that similar type of behavior was observed for other quencher molecules present in our study. Hence it follows static quenching and there was no new lifetime formation.

For static quenching, we can deduce the binding constant (K) because static quenching arise from the formation of complex between fluorophore and quencher. Hence the binding constant (K) was calculated by the method given in the following section.

## Binding Constant and Number of Binding Sites

Large $k_q$ beyond the diffusion-controlled limit indicates that some type of binding interaction exists between fluorophore and quencher [22]. For static quenching, the relationship between the intensity and the concentration of quencher can be described by the binding constant formula [23].

The relationship between fluorescence intensity and the quencher medium can be deduced from the following Eq. (4)

$$nQ + B \rightarrow Q_n \ldots..B \tag{4}$$

where B is the fluorophore, Q is the quencher; $Q_n$…..B is the postulated complex between a fluorophore and n molecules of the quencher. The constant K is given by

$$K = [Q_n \ldots.B]/[Q]^n[B] \tag{5}$$

If the overall amount of biomolecules (bound or unbound with the quencher) is $B_0$, then $[B_0] = [Qn \ldots.B] + [B]$, here [B] is the concentration of unbound biomolecules, then the relationship between fluorescence intensity and the unbound biomolecules as $[B]/[B_0] = F/F_0$ that is

$$\log\left[\frac{F_0 - F}{F}\right] = \log K + n \log [Q] \tag{6}$$

Where K is the binding constant and n is the number of binding sites

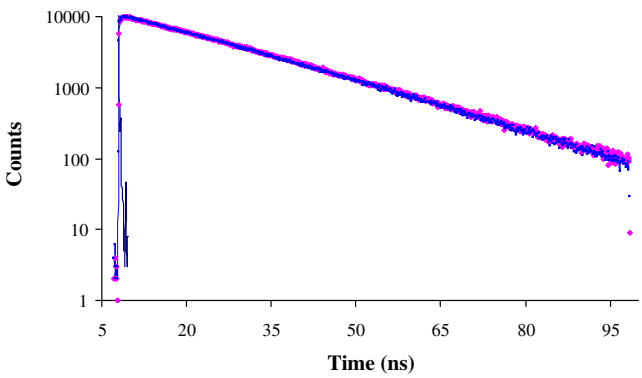



**Fig. 6** Fluorescence decay curves for 9-Aminoacridine ($4\times10^{-6}$ M) in the absence and presence of DPMN ($0–5\times10^{-4}$ M) in water

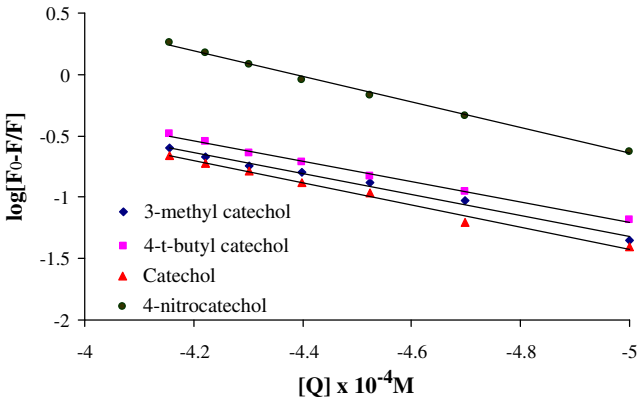



**Fig. 7** Comparison of binding constant plot for 3-methyl catechol, 4-t-butyl catechol catechol,4-nitrocatechol

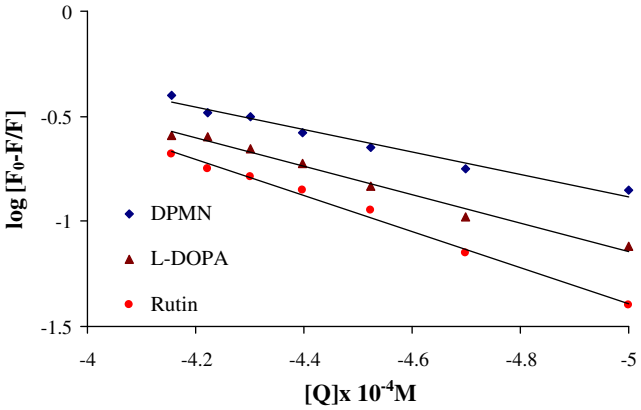



**Fig. 8** Comparison of binding constant plot for DPMN, L-DOPA, rutin

The value of K was determined from the intercept of log [($F_0$—F)/F] versus log [Q] as shown in the Figs. 7 and 8 for various catechols respectively. We calculated the value of binding constant (K) and number of binding sites (n) for all antioxidants which were shown in the Table 2. The correlation co-efficient for all the curves were larger than 0.980, indicating that the interaction of 9-AA with various quenchers agrees well with the site binding model underlying Eq. (6).

## Mechanism of Quenching

The quenching of 9-AA by various quenchers can be explained by a number of possible mechanism such as electron transfer, energy transfer, proton transfer or hydrogen atom transfer. There is no overlap between absorption spectrum of quencher molecules (except 4-nitrocatechol) with the emission spectrum of 9-AA. For instance, Fig. 9, shows the absence of overlap between the absorption spectrum of rutin and emission spectrum of 9-AA. So electronic energy transfer is not operative here. Feasibility of photoinduced electron transfer from a ground state donor such as catechols (except 4-nitrocatechol) and an excited state acceptor (9-AA) mainly depends upon the overall free energy change ($\Delta G_{et}$) for the electron transfer reaction. The thermodynamic feasibility of excited singlet state electron transfer reaction was calculated by employing the well known Rehm-Weller expression [24].

Earlier it was reported in literature that 9-Aminoacridine possess better electron acceptor ability because of its higher reduction potential [1]. Antioxidants such as catechol compounds and rutin are electron donors [25, 26].

$$\Delta G_{et} = E^{ox}(D) - E^{red}(A) - E^{*} + C \quad (7)$$

where

| | |
|---|---|
| $E^{ox}$ (D) | Oxidation potential of the catechols |
| $E^{red}$ (A) | Reduction potential of the 9-Aminoacridine (−1.16V vs SCE) |
| E* | Excitation energy of the 9-Aminoacridine. |
| C | Columbic term. |

The $\Delta G_{et}$ values calculated are listed in Table 1. The negative value of $\Delta G_{et}$ indicates the thermodynamic feasibility of electron transfer processes involved in the present study.

## Energy Transfer

4-nitrocatechol which shows relatively high magnitude of $k_q$ value in the present study ($1.58\times10^{12}$ $M^{-1}S^{-1}$) is attributed to a specific long-range interaction with 9-AA. Thus, the energy transfer process occurs by intermolecular interaction forces between 9-AA and 4-nitrocatechol,when they are in close proximity. The binding action of 4-nitrocatechol with 9-AA proceeds through energy transfer and hence it results in an efficient fluorescence quenching of 9-AA. This mechanism was explained from the overlap of absorption spectra of 4-nitrocatechol with the fluorescence spectra of 9-AA, as shown in Fig. 10. The mechanism of fluorescence quenching of 9-AA by 4-nitrocatechol was shown in the Scheme 2.where, 9-AA=9-Aminoacridine, NC=4-Nitrocatechol, respectively.

**Table 2** Binding constant, binding sites for 9-Aminoacridine with catechols and rutin at various temperatures

| S.No | Quenchers | 15 °C | | 25 °C | | 35 °C | |
|---|---|---|---|---|---|---|---|
| | | log K | n | log K | n | log K | n |
| 1 | 4-nitrocatechol | 6.4 | 1.04 | 5.50 | 1.303 | 4.56 | 1.76 |
| 2 | DPMN | 5.02 | 1.15 | 4.41 | 1.25 | 3.72 | 1.37 |
| 3 | Rutin | 4.44 | 0.85 | 3.94 | 1.067 | 3.15 | 1.12 |
| 4 | L-DOPA | 4.45 | 0.82 | 3.51 | 0.962 | 2.89 | 1.23 |
| 5 | t-butyl catechol | 4.6 | 0.69 | 3.42 | 1.01 | 2.23 | 1.24 |
| 6 | 3-methyl catechol | 3.61 | 1 | 3.00 | 1.3 | 2.16 | 1.50 |
| 7 | Catechol | 3.25 | 0.37 | 2.73 | 0.57 | 2 | 0.92 |

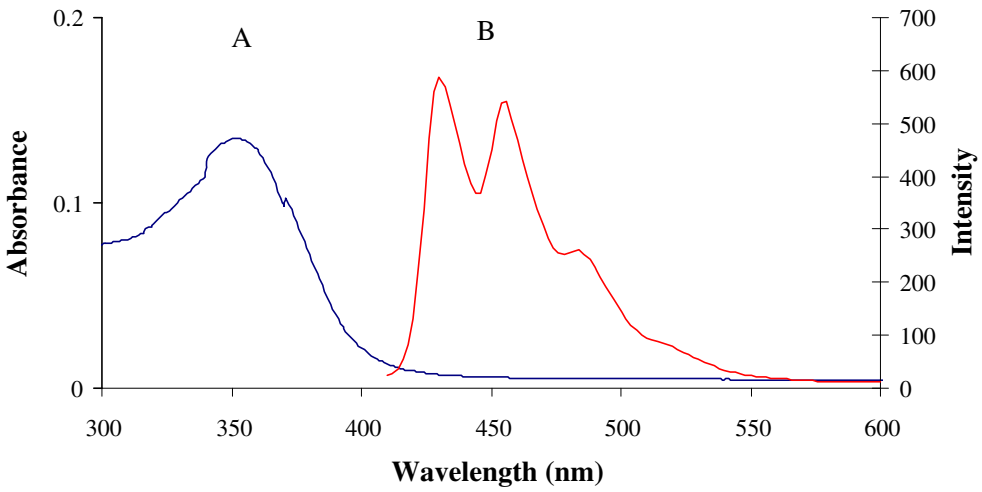



**Fig. 9** The overlap of the (**a**) absorption spectrum of rutin and (**b**) emission spectrum of 9-Aminoacridine

Analysis of Forster non-radiative energy transfer gives a lot of useful informations [18, 27] concerning the molecular details of donor-acceptor. While proper overlap of the emission spectrum of the donor with the absorption spectrum of the acceptor exists, non-radiative energy transfer can be detected. The rate of energy transfer depends on the extent of overlap, the relative orientation of the donor and acceptor transition dipoles and the distance between these molecules. FRET is an important technique for investigating a variety of biological phenomena including energy transfer process [28]. Here the donor and acceptor are 9-AA and 4-nitrocatechol respectively.

According to the Forster non-radiative energy transfer theory [29], the efficiency of energy transfer (E) is reduced to the distance R between donor and acceptor by

$$E = \frac{R_0^6}{R_0^6 + r_0^6} \tag{8}$$

Where, $R_0$ is a characteristic distance, called the Forster distance or critical distance, at which the efficiency of transfer is 50 % and r is the distance between the donor and acceptor. The Forster distance, $R_0$ can be denoted by Eq. 9

$$R_0^6 = 8.8 \times 10^{-25} K^2 N^{-4} \phi J \tag{9}$$

In Eq. 9, $K^2$ is the spatial orientation factor of the dipole of the donor and acceptor, N is the refractive index of the medium, $\phi$ is the fluorescence quantum yield of the donor in the absence of acceptor and the overlap integral J expresses the degree of spectral overlap between donor emission and the acceptor absorption, J can be given by

$$J = \frac{\Sigma F(\lambda)\varepsilon(\lambda)\lambda^4 \Delta\lambda}{\Sigma F(\lambda)\Delta\lambda} \tag{10}$$

where $F(\lambda)$ is the fluorescence intensity of the fluorescent donor at wavelength $\lambda$ and $\varepsilon(\lambda)$ is the molar absorption coefficient of the acceptor at wavelength $\lambda$. The parameter J can be evaluated by integrating spectral parameters in Eq. 10. Under these experimental conditions we found $R_0$ value from Eq. 9 using $K^2=2/3$, $\phi=0.54$, $n=1.3467$ [30] were taken form literature.

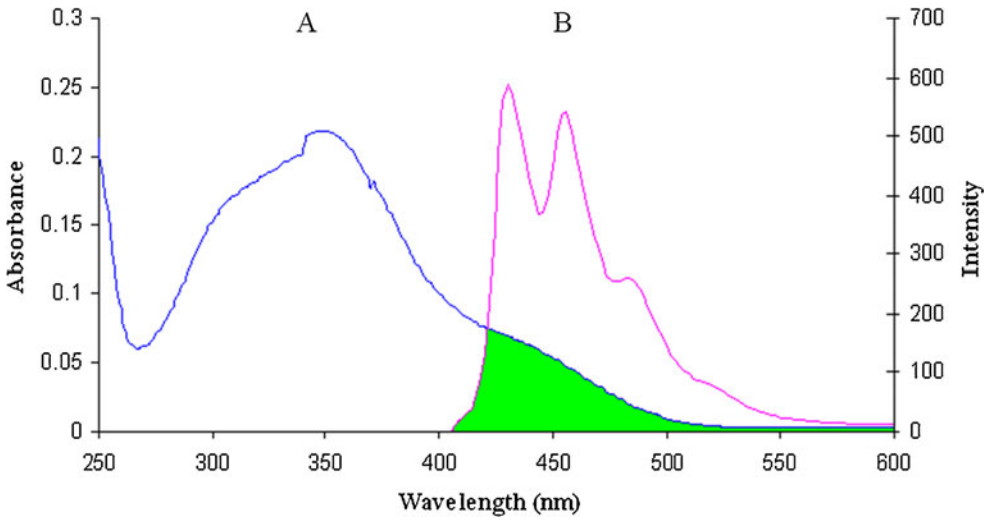
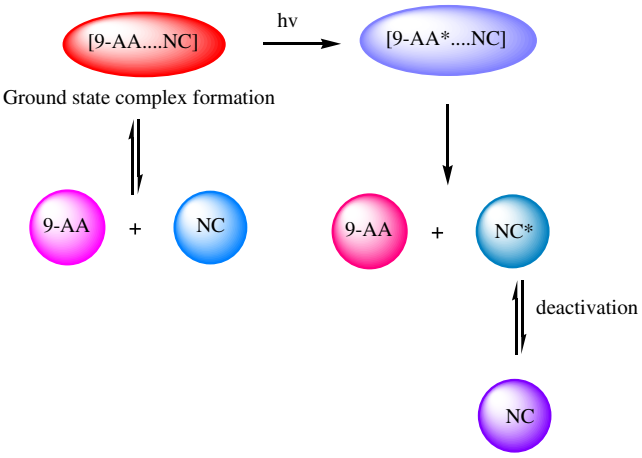



**Fig. 10** The overlap of the (**a**) absorption spectrum of 4-nitrocatechol and (**b**) emission spectrum of 9-Aminoacridine




**Scheme 2** Mechanism of fluorescence quenching

Obviously the calculated value of $R_0$=6.12 nm which is in the range of maximal critical distance ($R_0$=5–10 nm) [31]. This is in accordance with the condition of Forster's non-radiative energy transfer theory, indicating the static quenching interaction between 9-AA and 4-nitrocatechol.

The distance between 9-AA and 4-nitrocatechol can be evaluated from Eq. 8. where $r_0$=6.642 nm. We observed that the value of distance between donor and acceptor ($r_0$) is less than 8 nm suggested that non-radiative energy transfer occurs with high probability and also the obtained value of distance between donor and acceptor is higher than the critical distance ($R_0$) which revealed the static type of mechanism as reported earlier [32, 33].

## Energy Transfer Efficiency

Energy transfer efficiency (E) is given by the following equation Eq. (11)

$$E = 1 - \left(\frac{I}{I_0}\right) \tag{11}$$

Where I is the emission intensity of donor in the presence of acceptor and $I_0$ is the emission intensity of the donor alone. According to Forster's non-radiative energy transfer, the interaction between 9-AA and 4-nitrocatechol is through energy transfer and the energy transfer efficiency is E=60 %. From the above results it is clear that, in the presence of 4-nitrocatechol, the fluorescence intensity of 9-AA is reduced by energy transfer. Based on the Eq. (11) a plot of 'E' with different concentrations of 4-nitrocatechol is shown in Fig. 11. It is seen from this plot, 'E' value steadily increases with increasing concentration of 4-nitrocatechol. This is because while increasing the concentration of 4-nitrocatechol, the amount of energy transfer from 9-AA to 4-nitrocatechol has also been increased.

## Solvent Dependence

In order to get more insight into the quenching mechanism, the fluorescence quenching experiments were carried out in two different solvents of varying polarities ie., water and acetonitrile. We observed the similar trends in both medium as shown in Table 3. It was observed from the Table 3, the quenching rate constant increases with increasing solvent polarity. Generally, in the photoinduced charge transfer reaction the rate of charge transfer reaction increases with solvent polarity. The higher quenching rate constant in polar solvent is attributed to the stabilization of primary reaction intermediates resulting from charge transfer. This phenomenon has been extensively examined by the fluorescence quenching of singlet excited states. Fluorescence quenching need not be due to complete electron transfer. Partial charge transfer states can also cause extensive quenching [34, 35]. Thus we concluded, due to the strong electron accepting ability of 9-AA and electron donating ability of catechols, charge transfer occurs between them and this would be the possible one for fluorescence quenching process.

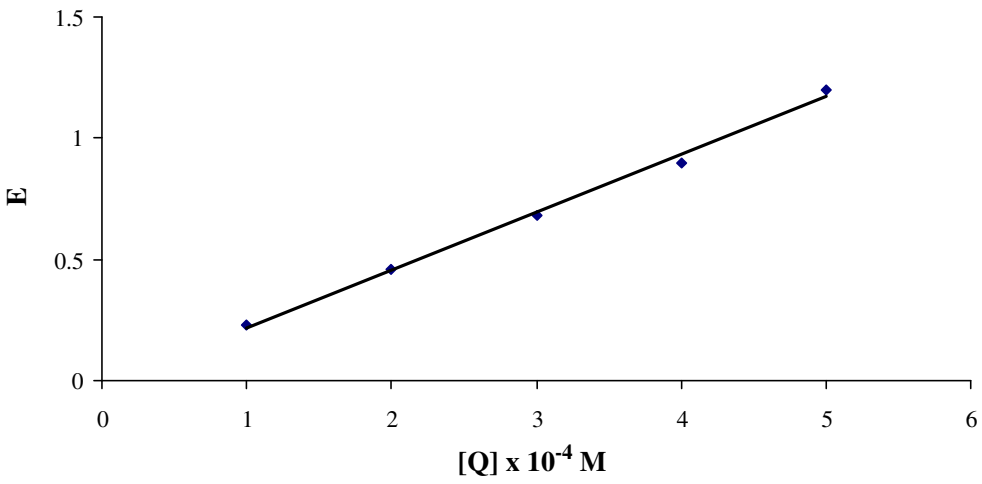



**Fig. 11** Energy transfer efficiency of 9-Aminoacridine with 4-nitrocatechol

**Table 3** Fluorescence quenching rate constants in different solvents at 25 °C

| S.No | Quenchers | $k_q \times 10^{11}$ $M^{-1}S^{-1}$ | |
|---|---|---|---|
| | | A | B |
| 1 | 4-nitrocatechol | 15.8 | 6.5 |
| 2 | DPMN | 4.05 | 2.83 |
| 3 | Rutin | 3.05 | 2.5 |
| 4 | L-DOPA | 2.63 | 2.21 |
| 5 | t-butyl catechol | 2.24 | 1.87 |
| 6 | 3-methyl catechol | 1.82 | 1.70 |
| 7 | Catechol | 1.28 | 1.12 |

[a] determined by (A) steady state fluorescence quenching in water ($\tau_o$= 15.6 ns) and (B) steady state fluorescence quenching in $CH_3CN$ ($\tau_o$= 15.8 ns) [37]

## In vitro-Antioxidant Activity

### Deoxyribose Degradation Assay

Deoxyribose was degraded by incubating with $H_2O_2$ and $Fe^{2+}$ in presence of a reducing agent. Here ascorbic acid was used as reducing agent. The extent of scavenging depends on the concentration of the scavenger relative to deoxyribose [36]. Iron ions interacts with $H_2O_2$ to form a highly reactive tissue-damaging species which was thought to be the hydroxyl radical ($^{\bullet}OH$) which attack the pentose sugar 2-deoxy ribose to yield a mixture of products [37]. At low pH with thiobarbutric acid, these products react to form a pink chromogen which can be measured from the absorbance at 532 nm. Addition of test samples competes with deoxyribose for the produced hydroxyl radical ($^{\bullet}OH$) and diminish the chromogen formation. The antiradical power of the test samples was determined from the decrease in the absorbance at 532 nm. The percentage inhibition was calculated and the results obtained were shown in the Fig. 12. It may seen from the Fig. 12 that all the compounds scavenge hydroxyl radicals.

### Nitric Oxide Scavenging Activity

In aqueous solution, at physiological pH, sodiumnitroprusside generates nitric oxide radicals ($NO^{\bullet}$) which can alter the structure and function of many cellular components. The radicals react with oxygen to form nitrite ions. The amount of nitrite ions produced was measured by using Griess reagent which results in the formation of pink chromogen at 546 nm. On performing the above experiments on test samples at various concentrations, only 4-nitrocatechol exhibits nitric oxide scavenging activity and reduces the nitrite concentration in the assay medium. The nitric oxide scavenging capacity of 4-nitrocatechol at different concentrations (50 μg/ml, 100 μg/ml and 150 μg/ml) was shown in the Fig. 13. The nitric oxide scavenging capacity was dependent on the concentration of 4-nitrocatechol. In the present study we have shown that 4-nitrocatechol has a potent nitric oxide scavenging activity. The percentage of inhibition were increased with increasing concentration of 4-nitrocatechol. Nitric oxide is also implicated in neuronal messenger, antibacterial and antitumor [38].

## Thermodynamic Parameters and Nature of Binding Forces

The forces acting between 9-AA and various catechols are composed by weak interactions of molecules such as hydrogen bond formation, Van der Waals forces, electrostatic interaction and the hydrophobic interaction [39]. The binding mode is confirmed from the thermodynamic parameters, enthalpy change (ΔH) and entropy change (ΔS) of binding

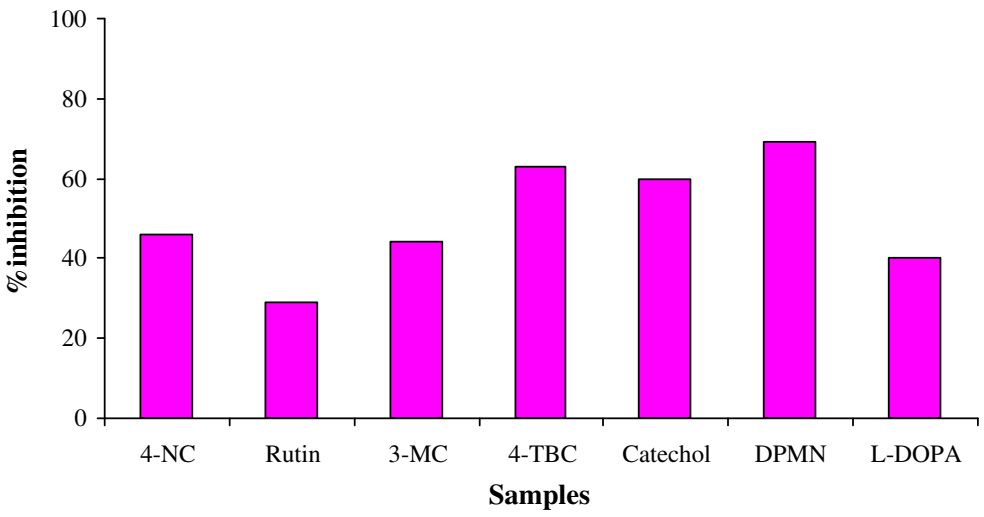



**Fig. 12** Comparison of antioxidant acticity of 4-nitrocatechol (4-NC), Rutin, 3- methylcatechol (3-MC), 4-tertiarybutylcatechol (4-TBC), catechol, Dopamine (DPMN), 3, 4-dihydroxy-L-Phenylalanine (L-DOPA) using deoxyribose degradation assay

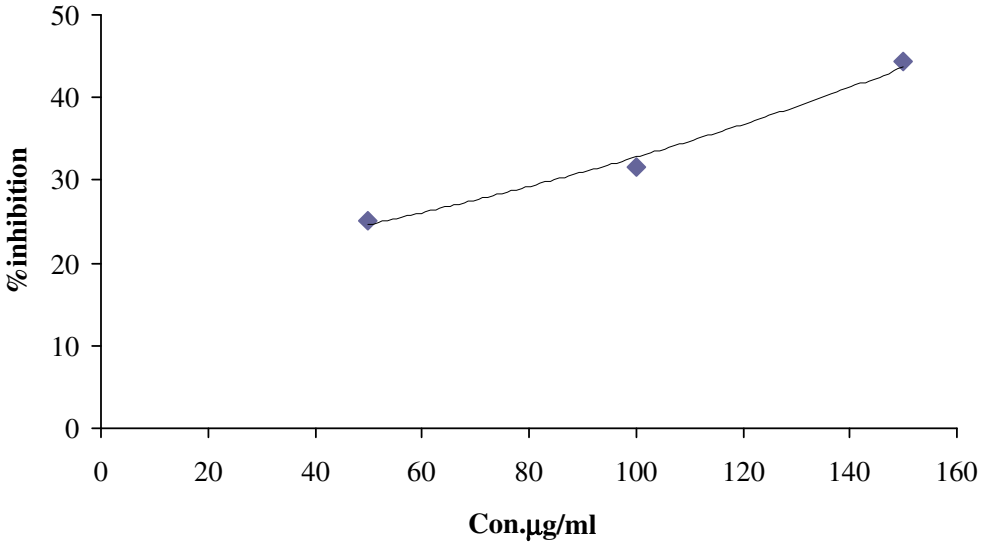



**Fig. 13** Antioxidant activity of 4-nitrocatechol at various concentrations (50 µg/ml, 100 µg/ml and 150 µg/ml) using nitric oxide scavenging assay

reaction. Form thermodynamic point of view, $\Delta H>0$ and $\Delta S>0$ implies a hydrophobic interaction; $\Delta H<0$ and $\Delta S<0$ suggest the Vander Waals forces or hydrogen bond formation and $\Delta H\approx 0$ and $\Delta S>0$ reflects an electrostatic force [40].

According to the following thermodynamic equations

$$\ln K = -\Delta H/RT + \Delta S/R \quad (12)$$

$$\Delta G = \Delta H - T\Delta S = -RT \ln K \quad (13)$$

The free energy change ($\Delta G$) can be estimated from the Eq. (13) based on the binding constant at different temperatures, where R is the gas constant, T is the experimental temperature and K is the binding constant at the corresponding temperature. $\Delta H$ and $\Delta S$ of reaction is determined from the linear relationship between ln K and the reciprocal absolute temperature (1/T).

The thermodynamic parameters for the interaction of 9-AA with catechols and rutin was shown in the Table 4. The negative value of $\Delta G$ signifies that the interaction process is spontaneous. According to the views of Ross and Subramanian [39], the negative enthalpy and entropy values support the non-bonded (Vander Waals) interactions and hydrogen bond formation. Thus, quencher compounds are bound to 9-AA mainly based on Vander Waals interaction and hydrogen bond formation. The magnitude of $\Delta H$ and $\Delta S$ shown in the Table 4, are expected for charge transfer and for hydrogen bonding interaction. In view of the good electron accepting ability of 9-aminoacridine and electron donating ability of antioxidants, charge transfer occurs between them and this would be the possible quenching mechanism for one of the nonradiative processes.

## Conclusion

The fluorescence of 9-AA was quenched by various catechol moieties in both water and acetonitrile solvents. The fluorescence quenching rate constant ($k_q$) decreases with increasing the temperature. The static quenching mechanism was further confirmed from lifetime measurement. Binding constant and number of binding sites were calculated from fluorescence quenching datas. We observed the possibility

**Table 4** Thermodynamic parameters of 9-AA with catechols and rutin

| S.No | Quenchers | $\Delta G^o$ (kcal mol$^{-1}$) | | | $\Delta H^o$ (kcal mol$^{-1}$) | $\Delta S^o$ (cal K$^{-1}$ mol$^{-1}$) |
|---|---|---|---|---|---|---|
| | | 15 °C | 25 °C | 35 °C | | |
| 1 | 4-nitrocatechol | −35.292 | −30.329 | −24.814 | −15.90 | −42.27 |
| 2 | DPMN | −27.571 | −24.263 | −20.403 | −11.41 | −29.10 |
| 4 | Rutin | −24.263 | −21.506 | −17.094 | −10.98 | −30.69 |
| 5 | L-DOPA | −24.539 | −19.355 | −15.936 | −13.82 | −39.16 |
| 6 | t-butyl catechol | −25.366 | −18.75 | −12.297 | −20.85 | −63.24 |
| 7 | 3-methyl catechol | −19.906 | −16.543 | −11.911 | −12.73 | −36.86 |
| 8 | Catechol | −17.646 | −15.058 | −11.028 | −10.98 | −31.50 |

of charge transfer process as the quenching mechanism between 9-AA and catechol moieties, but 4-nitrocatechol quenches the fluorophore via energy transfer mechanism. The negative value of $\Delta G_{et}$ was calculated by Rehm-Weller equation. The thermodynamic parameters showed that the interaction between 9-AA and various quenchers was spontaneous and supports the Vander Waals interaction or hydrogen bond formation. The in vitro antioxidant activity indicates that rutin, catechol and its derivatives are potential antioxidant. The radical scavenging mechanism and binding study are significant in the field of pharmacy, pharmacology and biochemistry.

**Acknowledgements** C.M and K.M.S thanks UGC SAP Fellowship for Meritorious Students. R.R. thanks DST **(Ref. No: SR/S1/PC-12/2011, dt: 20.09.2011)** for research grant. R.R and C.M thanks DST-FIST and UGC-SAP for spectrofluorimeter facility in the School of Chemistry, Bharathidasan University, Trichy. Authors also thank Prof. P. Ramamoorthy, Director, NCUFP, University of Madras, Chennai for lifetime measurements.

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