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Free Radical Scavenging Property of *Quisqualis indica*

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ABSTRACT

Antioxidants have been reported to prevent oxidative damage by free radical and reactive oxygen species. Leaves of *Quisqualis indica* are given in a compound decoction for flatulent distension of the abdomen. Since flavonoids have been isolated and characterized from the leaves of *Q. indica*, this study attempted to verify the free radical scavenging property of various leaf extracts when assayed against superoxide anion, hydroxyl and nitric oxide radicals, hydrogen peroxide, metal chelation and reducing power. All *Q. indica* extracts inhibited all these free radicals in a dose-dependent manner.

Keywords: hydrogen peroxide radical, hydroxyl radical, nitric oxide radical, superoxide anion radical

INTRODUCTION

Antioxidants have been reported to prevent oxidative damage by free radical and reactive oxygen species (ROS) in many medicinal plants (Sankhadip *et al.* 2008). They can interfere with the oxidation process by reacting with free radicals, chelating, catalytic metals, and also by acting as oxygen scavengers (Shahidi *et al.* 1992; Buyukokuroglu *et al.* 2001). Recent studies on free radicals have conformed that foods rich in antioxidants play an essential role in the prevention of cardiovascular diseases and cancers and neurodegenerative diseases, like Parkinson's and Alzheimer's diseases. Therefore, there is growing interest in antioxidants as dietary supplements and/or food preservatives (Bhatt and Baek 2007).

Current research is now directed towards finding naturally occurring antioxidants of plant origin due to the safety and toxicity problems of synthetic antioxidants. A great number of plants mentioned in many traditional Indian systems of medicine. *Quisqualis indica* Linn. (Combretaceae) (**Fig. 1**) is one of the potent drugs mentioned in traditional literature. Leaves of the plant are given in a compound decoction for flatulent distension of the abdomen. In China, the ripe seeds are roasted, and given in diarrhea and fever.

Fig. 1 Leaves and flowers of *Quisqualis indica* Linn.

The seeds are a popular anthelmintic (Kirtikar and Basu 2001). Flowers of *Q. indica* are reported to have antioxidant activity (Wetwitayaklung *et al.* 2007). According to a literature survey flavonoids have been isolated and characterized from the leaves of *Q. indica*. Therefore an attempt has been made to check the free radical scavenging property of its various extracts.

MATERIALS AND METHODS

Plant material

The leaves of *Q. indica* were collected from the garden of the University of Burdwan, Golapbag, Burdwan, India. The collected leaves were identified and authenticated from Botanical survey of India, Shibpur, Kolkata, West Bengal. A voucher specimen (Specimen No. CNH/I-I/(201)/2007/Tech.II/2/No. 2) has been deposited at the office of the Central National Herbarium, Botanical Survey of India, Shibpur, Kolkata.

Preparation of extracts

The leaves were shade-dried at room temperature for 15 days, finally powdered and used for extraction. A required quantity of powder (100 g each) was extracted with 50% hydro-alcohol and ethyl acetate separately by continuous hot percolation in a soxhlet apparatus for 72 hrs. Both the extracts were concentrated in reduced pressure below 40°C by using a rotary flash evaporator to get two separate hydro-alcoholic and ethyl acetate extracts. Separately, 100 g of powder was extracted with distilled water (600 ml) by cold maceration and the extract was concentrated under reduced pressure below 40°C to get a separate aqueous extract. All the extracts were stored at 4°C until use.

Superoxide anion scavenging activity assay

The scavenging activity of various extracts of *Q. indica* leaves towards superoxide anion radicals was measured by colorimetric method (Liu *et al.* 1997). Superoxide anion was generated in a non-enzymatic phenazine methosulfate-nicotinamide adenine dinucleotide (PMS-NADH) system through the reaction of PMS, NADH and oxygen. It was assayed by the reduction of nitroblue tetrazolium (NBT). In these experiments the superoxide anion was generated in 3 ml of Tris-HCl buffer (100 mM, pH 7.4) containing 0.75 ml of NBT (300 μM) solution, 0.75 ml of NADH (936 μM)

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Short Communication

solution and 0.3 ml of different concentrations of each extract. The reaction was initiated by adding 0.75 ml of PMS (120 µM) to the mixture. After 5 min of incubation at room temperature, the absorbance at 560 nm was measured by a spectrophotometer. The superoxide anion scavenging activity was calculated according to the following equation:

% inhibition = $((A_0-A_1)/A_0) \times 100$

where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance in the presence of the extract.

Hydroxyl radical scavenging activity assay

The scavenging activity for hydroxyl radicals was measured according to Yu *et al.* (2004). Briefly, the reaction mixture contained 60 µl of 1.0 mM $FeCl_3$, 90 µl of 1 mM 1,10-phenanthroline, 2.4 ml of 0.2 M phosphate buffer (pH 7.8), 150 µl of 0.17 M H_2O_2 and 1.5 ml of extract at various concentrations. Adding H_2O_2 started the reaction. After incubation at room temperature for 5 min, the absorbance of the mixture at 560 nm was measured with a spectrophotometer. The hydroxyl radicals scavenging activity was calculated according to the following equation:

% inhibition = $((A_0-A_1)/A_0) \times 100$

where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance in the presence of the extract.

Nitric oxide scavenging activity assay

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be determined by the use of the Griess lllosvoy reaction (Garrat 1964; Lok and Seung 2007). 2 ml of 10 mM sodium nitroprusside in 0.5 ml of phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of extract at various concentrations and the mixture incubated at 25°C for 150 min. From the incubated mixture 0.5 ml was taken out and added into 1.0 ml of sulfanilic acid reagent (33% in 20% glacial acetic acid) and incubated at room temperature for 5 min. Finally, 1.0 ml naphthylenediamine dihydrochloride (0.1%, w/v) was mixed and incubated at room temperature for 30 min. The absorbance at 540 nm was measured with a spectrophotometer. The nitric oxide radicals scavenging activity was calculated according to the following equation:

% inhibition = $((A_0-A_1)/A_0) \times 100$

where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance in the presence of the extract.

Hydrogen peroxide scavenging activity assay

Hydrogen peroxide scavenging activity of the extract was estimated by replacement titration (Zhang 2000). A 1.0 ml aliquot of 0.1 mM H_2O_2 and 1.0 ml of various concentrations of extracts were mixed, followed by 2 drops of 3% ammonium molybdate, 10 ml of 2 M H_2SO_4 and 7.0 ml of 1.8 M KI. The mixed solution was titrated with 5.09 mM NaS_2O_3 until yellow colour disappeared. Percentage of scavenging of hydrogen peroxide was calculated as:

% inhibition = $((V_0-V_1)/V_0) \times 100$

where V_0 was Volume of NaS_2O_3 solution used to titrate the control sample in the presence of hydrogen peroxide (without extract), V_1 was the volume of NaS_2O_3 solution used in the presence of extract.

Fe^{2+}-chelating activity assay

To 0.5 ml of extract, 1.6 ml of deionized water and 0.05 ml of $FeCl_2$ (2 mM) was added. After 30 s, 0.1 ml ferrozine (5 mM) was added. Ferrozine reacted with the divalent iron to form stable magenta complex species that were very soluble in water. After 10 min of room temperature, the absorbance of the Fe^{2+}-Ferrozine complex was measured at 562 nm (Dinis *et al.* 1994). The chelating activity of the extract for Fe^{2+} was calculated as:

Chelating rate (%) = $((A_0-A_1)/A_0) \times 100$

where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance in the presence of the extract.

Reducing power assay

The extract (0.75 ml) at various concentrations was mixed with 0.75 ml of phosphate buffer (0.2 M, pH 6.6) and 0.75 ml of potassium hexacyanoferrate ($K_3Fe(CN)_6$) (1%, w/v), followed by incubating at 50°C in a water bath for 20 min. The reaction was stopped by adding 0.75 ml of trichloroacetic acid (TCA) solution (10%) and then centrifuged at 800 g for 10 min. 1.5 ml of the supernatant was mixed with 1.5 ml of distilled water and 0.1 ml of ferric chloride solution (0.1%, w/v) for 10 min. The absorbance at 700 nm was measured as the reducing power (Oyaizu 1986). Higher absorbance of the reaction mixture indicated greater reducing power.

Statistical analysis

Tests were carried out in triplicate. The amount of extract needed to inhibit free radicals concentration by 50%, IC_{50}, was graphically determined and means, standard deviation and correlation were computed by using Prism 3.0 software.

RESULTS AND DISCUSSION

Superoxide anion scavenging activity

The superoxide anion scavenging activity of all the extracts of *Q. indica* increased markedly with the concentrations. Now in between three extracts, ethyl acetate extract showed better result than hydro-alcoholic and aqueous extracts. The IC_{50} of ethyl acetate, hydro-alcoholic and aqueous extracts are 15, 70 and 200 µg/ml, respectively (**Table 1**). If we compare this result with some standard antioxidants like BHA (butylated hydroxyanisole) and α-tocopherol, the IC_{50} of BHA is 5 µg/ml and α-tocopherol is 7 µg/ml. If we also compare the results of various extracts of *Q. indica* with the results of *Ixora coccinea* from our previous paper (Bose *et al*. 2008), the IC_{50} of ethyl acetate, hydro-alcoholic and aqueous extracts of *I. coccinea* were 10, 95 and 45 µg/ml, respectively. Thus, the ethyl acetate and hydro-alcoholic ex-

Table 1 Radical scavenging activity of *Quisqualis indica* at different concentrations.

Conc.	Superoxide radical scavenging %			Hydroxyl radical scavenging %		
(µg/ml)	Hydro-alcoholic extract	Aqueous extract	Ethyl acetate extract	Hydro-alcoholic extract	Aqueous extract	Ethyl acetate extract
10	33.12 ± 0.010	36.47 ± 0.010	46.91 ± 0.015	19.22 ± 0.005	20.33 ± 0.017	27.45 ± 0.015
50	35.17 ± 0.015	38.91 ± 0.015	65.17 ± 0.015	23.42 ± 0.000	39.13 ± 0.015	44.19 ± 0.004
100	40.93 ± 0.015	56.76 ± 0.015	74.82 ± 0.005	35.21 ± 0.010	45.24 ± 0.015	56.83 ± 0.016
250	53.82 ± 0.010	66.13 ± 0.015	79.89 ± 0.010	51.31 ± 0.023	51.12 ± 0.005	66.75 ± 0.038
500	72.13 ± 0.005	73.25 ± 0.011	86.43 ± 0.020	59.14 ± 0.011	59.26 ± 0.015	79.53 ± 0.006
750	73.85 ± 0.010	82.48 ± 0.015	90.68 ± 0.011	63.24 ± 0.005	67.43 ± 0.020	85.09 ± 0.027
1000	83.66 ± 0.009	90.66 ± 0.009	96.34 ± 0.015	78.31 ± 0.015	80.16 ± 0.015	96.67 ± 0.015
IC_{50}	200 µg/ml	70 µg/ml	15 µg/ml	230 µg/ml	220 µg/ml	60 µg/ml

Values are means ± SD (n=3), IC_{50} = 50% Inhibition Concentration.

Table 2 Radical scavenging activity of *Quisqualis indica* at different concentrations.

Conc. (µg/ml)	Nitric oxide radical scavenging %			Hydrogen peroxide scavenging %		
	Hydro-alcoholic extract	**Aqueous extract**	**Ethyl acetate extract**	**Hydro-alcoholic extract**	**Aqueous extract**	**Ethyl acetate extract**
10	35.71 ± 0.011	37.76 ± 0.005	30.14 ± 0.005	4.22 ± 0.561	9.12 ± 0.002	9.32 ± 0.663
50	37.17 ± 0.005	41.76 ± 0.011	38.82 ± 0.010	12.13 ± 0.112	16.22 ± 0.553	15.00 ± 0.352
100	44.82 ± 0.025	47.74 ± 0.005	44.66 ± 0.009	21.27 ± 0.432	33.48 ± 0.341	25.21 ± 0.002
250	46.75 ± 0.005	50.64 ± 0.005	49.80 ± 0.005	35.45 ± 0.011	43.11 ± 0.122	30.33 ± 0.001
500	51.65 ± 0.005	61.21 ± 0.010	62.74 ± 0.005	42.00 ± 0.001	45.00 ± 0.001	38.11 ± 1.221
750	58.87 ± 0.005	71.74 ± 0.009	64.81 ± 0.005	49.23 ± 1.231	52.44 ± 0.051	41.00 ± 0.002
1000	64.12 ± 0.009	76.26 ± 0.005	71.65 ± 0.005	53.19 ± 0.002	63.14 ± 0.002	48.47 ± 0.001
IC_{50}	420 µg/ml	230 µg/ml	250 µg/ml	800 µg/ml	720 µg/ml	More then 1000 µg/ml

Values are means ± SD (n=3), IC_{50} = 50% Inhibition Concentration.

tracts of *Q. indica* have better scavenging property against superoxide radicals than the aqueous extract.

Hydroxyl radical scavenging activity

Among all three extracts, ethyl acetate extract showed better result than hydro-alcoholic and aqueous extracts. The IC_{50} of ethyl acetate, hydro-alcoholic and aqueous extracts are 60, 230 and 220 µg/ml, respectively (**Table 1**). When this results are comparing with BHA and α-tocopherol, the IC_{50} were 10 and 15 µg/ml, respectively. The result of various extracts of *I. coccinea* were 20, 120 and 55 µg/ml of ethyl acetate, hydro-alcoholic and aqueous extracts, respectively (Bose *et al*. 2008). Thus, it can be concluded that although ethyl acetate, hydro-alcoholic and aqueous extracts of *Q. indica* have scavenging property against hydroxyl radical that the strength is less than various extracts of *I. coccinea* and standard drugs.

Nitric oxide scavenging activity

Aqueous extract showed better result than hydro-alcoholic and ethyl acetate extracts. The IC_{50} of ethyl acetate, hydro-alcoholic and aqueous extracts are 250, 420 and 230 µg/ml, respectively (**Table 2**). In nitric oxide scavenging activity the IC_{50} of BHA and α-tocopherol are 120 and 150 µg/ml, respectively. The values for the ethyl acetate, hydro-alcoholic and aqueous extracts of *I. coccinea* were 1000, 920 and 620 µg/ml, respectively (Bose *et al*. 2008). Various extracts of *Q. indica* are more effective in scavenging of nitric oxide radicals than various extracts of *I. coccinea*.

Hydrogen peroxide scavenging activity assay

The IC_{50} of hydro-alcoholic, aqueous and ethyl acetate extracts ware 800, 720 and more than 1000 µg/ml, respectively. The aqueous extract showed better results than the hydro-alcoholic and ethyl acetate extracts (**Table 2**). The scavenging property of various extracts of *Q. indica* and *I. coccinea* (Bose *et al*. 2008) are approximately the same but when the results are compared with BHA and α-tocopherol, the IC_{50} are 220 and 170 µg/ml, respectively. All the extracts of *Q. indica* have scavenging property against hydrogen peroxide radicals.

Fe^{+2}-chelating activity

Ethyl acetate extract was better than hydro-alcoholic and aqueous extracts. The IC_{50} of ethyl acetate, hydro-alcoholic and aqueous extracts were 60, 320 and 100 µg/ml, respectively (**Table 3**). Against Fe^{+2} radicals the IC_{50} of BHA and α-tocopherol were 15 and 10 µg/ml, respectively. The values for the ethyl acetate, hydro-alcoholic and aqueous extracts of *I. coccinea* were 80, 225 and 160 µg/ml, respectively (Bose *et al*. 2008). Thus, the ethyl acetate and aqueous extracts of *Q. indica* have better scavenging property against Fe^{+2} radicals than various extracts of *I. coccinea*.

Table 3 Radical scavenging activity of *Quisqualis indica* at different concentrations.

Conc. (µg/ml)	Fe^{2+} chelating activity %		
	Hydro-alcoholic extract	**Aqueous extract**	**Ethyl acetate extract**
10	11.81 ± 0.102	34.63 ± 0.005	37.86 ± 1.000
50	24.47 ± 0.003	42.32 ± 0.000	46.12 ± 0.007
100	39.26 ± 0.005	49.73 ± 0.009	57.32 ± 0.005
250	47.57 ± 0.005	58.38 ± 0.027	69.43 ± 0.009
500	56.12 ± 0.011	67.17 ± 0.000	75.29 ± 0.102
750	70.23 ± 0.010	83.42 ± 0.240	87.14 ± 0.003
1000	81.05 ± 0.005	89.55 ± 0.180	92.03 ± 0.002
IC_{50}	320 µg/ml	100 µg/ml	60 µg/ml

Values are means ± SD (n=3), IC_{50} = 50% Inhibition Concentration.

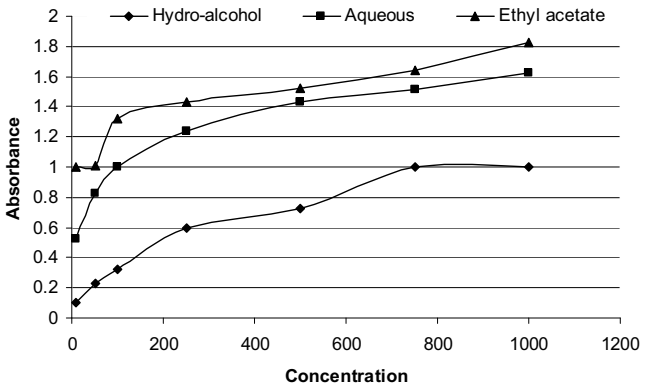

Fig. 2 Reducing power of hydro-alcoholic, aqueous and ethyl acetate extracts of *Quisqualis indica* at different concentrations. ♦: Absorbance of hydro-alcoholic extract, ■: Absorbance of aqueous extract and ▲: Absorbance of ethyl acetate extract.

Reducing power assay

In **Fig. 2** the reductive effect of *Q. indica* is depicted. Similar to the antioxidant activity, the reducing power of *Q. indica* increased with increasing dosage. High absorbance at 700 nm indicates high reducing power.

CONCLUSION

In conclusion, the results indicated that all the extracts of *Q. indica* inhibited all above said free radicals in dose dependent manner. These results clearly indicate that *Q. indica* is effective against free radical mediated diseases. Further investigations should be done on isolation of active constituent(s) and their *in vivo* antioxidant activity and are necessary to determine the specific mechanisms.

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