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Full Length Research Paper

Radical scavenging and antioxidant activity of *Hibiscus rosasinensis* extract

Rajesh Mandade[1]*, S. A. Sreenivas[2], D. M. Sakarkar[3] and Avijit Choudhury[4]

[1]Pharmacology Department,S.N. Institute of Pharmacy, Pusad, India.
[2]Guru Nanak Institute of Pharmacy,Ibrahimpattnam, Hyderabad (A.P.), India.
[3]S.N. Institute of Pharmacy, Pusad, India.
[4]Shree Dhanvantary Pharmaceutical Analysis and Research Centre, Kim, Surat, Gujarat, India.

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Free radicals induce numerous diseases by lipid peroxidation, protein peroxidation and DNA damage. It has been reported that numerous plant extracts have antioxidant activities to scavenge free radicals. In the present study, the antioxidant properties of crude extract of *Hibiscus rosasinensis* were examined, using different *in vitro* analytical methodologies, such as total antioxidant activity determination by ferric thiocyanate, hydrogen peroxide scavenging, 1,1-diphenyl-2-picryl-hydrazyl free radical (DPPH) scavenging, 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) ($ABTS^{*+}$ radical cation) radical cation scavenging activity and superoxide anion radical scavenging by riboflavin-methionine-illuminate system. Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and α-tocopherol were used as the reference antioxidant radical scavenger compounds. The crude extract inhibited 94.58% peroxidation of linoleic acid emulsion at 20 µg/ml concentration, while the standard antioxidants BHA, BHT and α-tocopherol indicated an inhibition of 93.75, 96.66 and 83.33% at 60 µg/ml concentration, respectively. The hydrogen peroxide radical, DPPH radical, $ABTS^{*+}$ radical cation(s) and superoxide anion radical scavenging activities of crude extract were also compared to BHA, BHT and α-tocopherol as references antioxidant compounds. The present study shows that the crude extract is an effective natural antioxidant component.

Key words: *Hibiscus rosasinensis*, antioxidant activity, free radical scavenging activity.

INTRODUCTION

Antioxidants help living organisms to deal with oxidative stress, caused by free radical damage. Free radicals are chemical species which contains one or more unpaired electrons due to which they are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability. Reactive oxygen species (ROS) formed *in vivo,* such as superoxide anion, hydroxyl radical and hydrogen peroxide, are highly reactive and potentially damaging transient chemical species. ROS are continuously produced in the human body, as they are essential for energy supply, detoxification, chemical signaling and immune function. ROS are regulated by endogenous superoxide dismutase, glutathione peroxidase and catalase, but due to over-production of reactive species induced by exposure to external oxidant substances or a failure in the defense mechanisms, damage to cell structures, DNA, lipids and proteins (Valko et al., 2006) occur which increases risk of more than 30 different disease processes (Aruoma, 1998).

Hibiscus rosasinensis Linn (Malvaceae) is a glabrous shrub widely cultivated in the tropics as an ornamental plant and has several forms with varying colors of flowers. In medicine, however the red flowered variety is preferred (Adhirajan et al., 2003). The leaves and flowers are observed to be promoters of hair growth and aid in healing of ulcers (Jadhav et al., 2009). Aerial part of *H. rosasinensis* has calcium channel blocking action (Gilani et al., 2005). Recent reports have also shown anti-ammonemic (Essa and Subramanian., 2007), anti-diabetic (Venkatesh et al., 2008), hypolipidemic (Kumar et al., 2009), post-coital anti-fertility, cardio protective and

*Corresponding author. E-mail: raj_mandade@rediffmail.com. Tel: +917233249795 or +918888673088. Fax: 07233247308.

wound healing activities (Gauthaman et al., 2006, Nayak et al., 2007).

The free radical neutralizing property of several plants was reported by previous studies. The extracts from a number of medicinal plants which are known to have some biologically active principles are used in ayurvedic preparations and these extracts are prepared in bulk for commercial purpose. In the present study, the antioxidant property of 80% aqueous-ethanol crude extract of *H. rosasinensis* was examined by different *in vitro* analytical methodologies, such as total antioxidant activity determination by ferric thiocyanate, hydrogen peroxide scavenging, 1,1-diphenyl-2-picryl-hydrazyl free radical (DPPH) scavenging, 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$ radical cation) radical scavenging activity and superoxide anion radical scavenging by riboflavin-methionine-illuminate system.

MATERIAL AND METHODS

Plant

Aerial part of *H. rosasinensis* was collected from the botanical garden of S. N. Institute of Pharmacy, Pusad, India. Identification and authentication of the samples was done by using standard botanical monographs. They were further confirmed at the Department of Botany, R.S.T.M University Nagpur, India.

Preparation of crude extract

The plant material was cleaned off adulterants, shade dried and was coarsely grounded. The powdered material (1 kg) was soaked in 80% aqueous-ethanol for 3 days with occasional shaking. It was filtered through a muslin cloth and then through a filter paper. This procedure was repeated thrice and the combined filtrate was evaporated on a rotary evaporator under reduced pressure (-760 mmHg) to a thick, semi-solid mass of dark brown color, that is, the crude extract with a yield of approximately 10% (Gilani et al., 2005).

Phytochemical screening

The preliminary phytochemical studies (Adhirajan et al., 2003) were conducted for the aforementioned crude extracts of *H. rosasinensis* to find out the presence of sterols, carbohydrates and glycosides, tannins and flavonoid using standard analytical procedures (Gupta et al., 2009) (Table 1).

Estimation of total phenolic compounds

Total phenol

The total phenolic content in the crude extract of *H. rosasinensis* was determined spectrophotometrically with Folin-Ciocalteu reagent using the modified method of Wolfe et al. (2003). An aliquot of the crude extract (0.5 ml) was mixed with 2.5 ml of 10% Folin-Ciocalteu reagent and 2 ml of Na_2CO_3 (75% w/v). The resulting mixture was vortexed for 15 s and incubated at 40°C for 30 min for colour development. The absorbance of the samples was measured at 765 nm using spectrophotometer at UV/visible light. Total phenolic content was expressed as mg/g tannic acid equivalent from the calibration curve using the equation:

$Y = 0.1216x, R2 = 0.936512$

Where x was the absorbance and Y was the tannic acid equivalent (mg/g).

The experiment was conducted in triplicate and the results are reported as mean ± SD values.

Total flavonoid

The method of Ordon et al. (2006) was used to estimate the total flavonoid contents of the crude extract solution based on the formation of a complex flavonoid-aluminium. A volume of 0.5 ml of 2% $AlCl_3$ ethanol solution was added to 0.5 ml of crude extract solution. After 1 h of incubation at the room temperature, the absorbance was measured at 420 nm using UV-VIS spectrophotometer. All determinations were done in triplicate and values were calculated from calibration curve obtained from quercetin using the equation:

$Y = 0.0255x, R2 = 0.9812$

where x was the absorbance and Y the quercetin equivalent (mg/g).

Total flavonols

Total flavonol content was determined by adopting the procedure described by Kumaran and Karunakaran (2007). The reacting mixture consisted of 2.0 ml of the sample, 2.0 ml of $AlCl_3$ prepared in ethanol and 3.0 ml of (50 g/L) sodium acetate solution. The absorption at 440 nm was read after 2.5 h at 20°C. Total flavonoid content was calculated as quercetin (mg/g) equivalent from the calibration curve using the equation:

$Y= 0.0255x, R2 = 0.9812$

where x was the absorbance and Y the quercetin equivalent (mg/g).

Total proanthocyandins

Total proanthocyandins was determined based on Sun et al. (1998) procedure. 3 ml of vanillin-methanol (4% v/v) and 1.5 ml of hydrochloric acid was added to 0.5 ml of 1 mg/ml crude extract solution and then vortexed. The absorbance of resulting mixture was measured at 500 nm after 15 min at room temperature. Total proanthocyandin content was expressed as catechin equivalents (mg/g) using the following equation from the calibration curve:

$Y = 0.5825x, R2 = 0.9277$

where x was the absorbance and Y the catechin equivalent (mg/g).

Total antioxidant activity determination by ferric thiocyanate method (FTC)

The total antioxidant activity of the crude extract and standard antioxidants (BHA, BHT and α-tocopherol) was determined according to the ferric thiocyanate method (Mitsuda et al., 1996) as described by Gulcin (2006b). For stock solutions, 10 mg of the extract was dissolved in 10 ml distillate water. Then, the solution which contains 20 µg/ml concentration of the extract solution in 2.5 ml of sodium phosphate buffer (0.04 M, pH 7.0) was added to 2.5 ml of linoleic acid emulsion in sodium phosphate buffer (0.04 M, pH 7.0). Therefore, 5 ml of the linoleic acid emulsion was prepared by mixing and homogenising 15.5 µl of linoleic acid, 17.5 mg/g of

tween-20 as emulsifier and 5 ml phosphate buffer (pH 7.0).

On the other hand, 5 ml of control was composed of 2.5 ml of linoleic acid emulsion and 2.5 ml sodium phosphate buffer (0.04 M, pH 7.0). The mixed solution (5 ml) was incubated at 37°C in polyethylene flask. The peroxide level was determined by reading the absorbance at 500 nm in a spectrophotometer after reaction with $FeCl_2$ (3.5%) and thiocyanate (30%) at intervals during incubation. During the linoleic acid peroxidation, peroxides are formed and that leads to the oxidation of Fe^{2+}–Fe^{3+}. The latter ions form a complex with ammonium thiocyanate and this complex has a maximum absorbance at 500 nm. This step was repeated every 5 h. The percentage inhibition values were calculated at this point (30 h). High absorbance indicates high linoleic acid emulsion peroxidation (Table 2). The solutions without the extract were used as blank samples. Total antioxidant activity determination was performed in triplicate. The inhibition percentage of lipid peroxidation in linoleic acid emulsion was calculated by following equation:

$$\text{Inhibition of lipid peroxidation (\%)} = 100 - (A_s/A_c \times 100)$$

A_C is the absorbance of control reaction which contains only linoleic acid emulsion and sodium phosphate buffer and A_S is the absorbance in the presence of sample crude extract or standard compounds (Gulcin, 2006a, b).

Hydrogen peroxide scavenging activity

The hydrogen peroxide scavenging assay was carried out following the procedure of Ruch et al. (1989). For this aim, a solution of H_2O_2 (43 mM) was prepared in phosphate buffer (0.1 M, pH 7.4). Crude extract at 20 µg/ml concentration in 3.4 ml phosphate buffer was added to 0.6 ml of H_2O_2 solution (0.6 ml, 43 mM). The absorbance value of the reaction mixture was recorded at 230 nm. Blank solution contains sodium phosphate buffer without H_2O_2. The concentration of hydrogen peroxide (mM) in the assay medium was determined using a standard curve (r^2: 0.9895):

$$\text{Absorbance} = 0.038 \times [H_2O_2] + 0.4397$$

The percentage of H_2O_2 scavenging of crude extract and standard compounds were calculated using the following equation:

$$H_2O_2 \text{ scavenging effect (\%)} = (1 - As/Ac) \times 100$$

where A_C is the absorbance of the control and A_S is the absorbance in the presence of the sample extract or standards (Gulcin, 2006b; Elmastas et al., 2005).

DPPH free radical scavenging activity

The DPPH free radical scavenging activity of crude extract was determined according to the method described by Gulcin (2006b) with slight modifications. In its radical form, DPPH absorbs at 517 nm, but upon reduction by an antioxidant or a radical species its absorption decreases and thereby the bleaching rate of a stable free radical, DPPH in the presence of the sample is monitored at a characteristic wavelength.

Briefly, 0.1 mM solution of DPPH was prepared in ethanol and 0.5 ml of this solution was added 1.5 ml of the extract solution in ethanol at different concentrations (20 to 60 µg/ml). These solutions were vortexes thoroughly and incubated in dark, half hour later, the absorbance was measured at 517 nm against blank samples.

Lower absorbance of the reaction mixture indicates higher DPPH free radical scavenging activity. A standard curve was prepared using different concentrations of DPPH. The DPPH concentration scavenging capacity was expressed as mM in the reaction medium and calculated from the calibration curve determined by linear regression (r^2: 0.9845):

$$\text{Absorbance} = 9.692 \times [\text{DPPH}] + 0.215$$

The capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = (1 - As/Ac) \times 100$$

where A_C is the absorbance of the control which contains DPPH solution and A_S is the absorbance in the presence of the extracts (Gulcin et al., 2004c; Elmastas et al., 2006).

$ABTS^{\bullet+}$ radical cation(s) decolorization assay

The spectrophotometric analysis of $ABTS^{\bullet+}$ radical cation(s) scavenging activity was determined according to Re et al. (1999) method. This method is based on the ability of antioxidants to quench the long-lived ABTS radical cation, a blue/green chromophore with characteristic absorption at 734 nm, in comparison to that of BHA, BHT and α-tocopherol. The $ABTS^{\bullet+}$ was produced by reacting 2 mM $ABTS^{\bullet+}$ radical cation(s) in H_2O with 2.45 mM potassium persulfate ($K_2S_2O_8$), stored in the dark at room temperature for 4 h. Before usage, the $ABTS^{\bullet+}$ solution was diluted to get an absorbance of 0.750 ± 0.025 at 734 nm with sodium phosphate buffer (0.1 M, pH 7.4). Then, 1 ml of $ABTS^{+}$ solution was added 3 ml of the extract solution in ethanol at different concentrations (20 to 60 µg/ml).

After 30 min, percentage inhibition at 734 nm was calculated for each concentration, relative to a blank absorbance. Solvent blanks were run in each assay. The extent of decolorization is calculated as percentage reduction of absorbance. For preparation of a standard curve, different concentrations of $ABTS^{\bullet+}$ radical cation(s) were used. The $ABTS^{\bullet+}$ radical cation(s) concentration (mM) in the reaction medium was calculated from the following calibration curve, determined by linear regression (r^2: 0.9841):

$$\text{Absorbance} = 4.6788 \times [ABTS^{\bullet+}] + 0.199$$

The scavenging capability of $ABTS^{+}$ radical was calculated using the following equation:

$$\text{ABTS scavenging effect (\%)} = (1 - As/Ac) \times 100$$

where A_C is the initial concentration of the $ABTS^{\bullet+}$ radical cation(s) and A_S is absorbance of the remaining concentration of $ABTS^{\bullet+}$ radical cation(s) in the presence of the extract.

Superoxide anion radical scavenging activity

Superoxide radicals were generated by the method of Beauchamp and Fridovich (1971) described by Zhishen et al. (1999) with slight modification. Superoxide radicals are generated in riboflavin, methionine, illuminate and assayed by the reduction of nitroblue tetrazolium (NBT) to form blue formazan (NBT^{2+}). All solutions were prepared in 0.05 M phosphate buffer (pH 7.8). The photo-induced reactions were performed using fluorescent lamps (20 W). The concentration of the extract in the reaction mixture was 20 µg/ml.

Table 1. Preliminary phytochemical screening.

Chemical constituent	Crude extract
Alkaloids	-
Sterols	+
Carbohydrates and glycosides	+
Fixed oil and fats	-
Tannins and phenolic	+
Proteins	+
Triterpenoids	+
Flavonoids	+

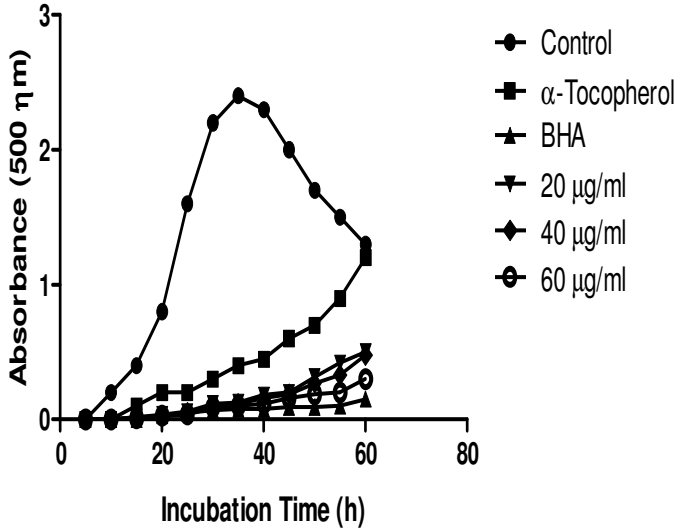

Figure 1. Total ferric reducing power (FRAP) of different concentrations (20 to 60 µg/ml) of extract of *H. rosasinensis* and reference antioxidants; BHA, BHT and α-tocopherol. Total ferric reducing power determined according to the ferric ions (Fe^{3+})-ferrous ions (Fe^{2+}) transformation. The reducing power was estimated based on the absorbance reading at 700 nm with a spectrophotometer. Values are expressed as mean ± standard deviation of three replicate determinations.

The total volume of the reactant mixture was 3 ml and the concentrations of the riboflavin, methionine and NBT was 1.33×10^{-5}, 4.46×10^{-5} and 8.15×10^{-8} M, respectively. The reactant was illuminated at 25°C for 40 min. The photochemically reduced riboflavin generated $O_2^{\cdot-}$ This reduced NBT to form blue formazan. The unilluminated reaction mixture was used as a blank. The absorbance was measured at 560 nm. The extract was added to the reaction mixture, in which O_2 was scavenged, thereby inhibiting the NBT reduction. Decreased absorbance of the reaction mixture indicates increased superoxide anion scavenging activity. The inhibition percentage of superoxide anion generation was calculated by using the following formula:

$$O_2^{\cdot-} \text{ scavenging effect (\%)} = (1 - As/Ac) \times 100$$

where A_C is the absorbance of the l-ascorbic acid and A_S is the absorbance of the extract or standards (Gulcin et al., 2003, 2004b).

RESULTS AND DISCUSSION

A wide variety of *in vitro* methods have been set up to assess radical scavenging ability and antioxidant activity. Antioxidant capacity is widely used as a parameter for medicinal bioactive components. Different artificial free radical species, such as $ABTS^{\bullet+}$ radical cation, DPPH radical, as well as biological radicals or H_2O_2 scavenging activity have been used.

Polyphenol are the major plant compounds with high level of antioxidant activity. This activity could be due to their ability to adsorb, neutralize and to quench free radicals (Duh et al., 1999). Their ability as free radical scavenger could also be attributed to their redox properties, presence of conjugated ring structures and carboxylic group which have been reported to inhibit lipid peroxidation (Rice-Evans et al., 1995).

In the present study, it was found that the crude extract of *H. rosasinensis* contains high level of phenol content that might account for the strong activity observed against $ABTS^{\bullet+}$ radical cation(s) and H_2O_2 radicals. This scavenging activity may be due to the presence of hydroxyl groups attached to the aromatic ring structures and thus, help to quench the radicals (Vinson et al., 1998). On the other hand, the activity depicted in DPPH and superoxide anion may be as a result of the content of flavonoid which has been reported to possess high antioxidant activity.

Total antioxidant activity determination in linoleic acid emulsion system by ferric thiocyanate method

Lipid peroxidation contains a series of free radical-mediated chain reaction processes and is also associated with several types of biological damage. The total antioxidant activity of the crude extract, BHA, BHT and α-tocopherol in the linoleic acid system was determined by the ferric thiocyanate method. This method measures the amount of peroxide produced during the initial stages of oxidation, which is the primary product of lipid oxidation.

Crude extract showed effective antioxidant activity in this system. The effect of different concentration (20 to 60 µg/ml) of the extract on lipid peroxidation of linoleic acid emulsion is as shown in Figure 1.

At these concentrations of the extract caused 94.58, 95.00 and 95.88% lipid peroxidation inhibition of linoleic acid emulsion. Their activities are greater than 60 µg/ml concentration of BHA (93.75%), α-tocopherol (83.33%), but close to BHT (96.66%). Consequently, these results clearly indicate that the extract has an effective and powerful antioxidant activity by ferric thiocyanate method.

Hydrogen peroxide scavenging activity

Hydrogen peroxide has strong oxidizing properties. It can

Table 2. Polyphenol contents of crude extract of *H. rosasinensis*.

Antioxidant compound	Total antioxidant compound
Phenol contents	49.44 mg/g tannic acid equivalent
Proanthocyandins	9.15 mg/g catechin equivalent
Flavonols	5.5 mg/g quercetin equivalent
Flavonoids	4.8 mg/g quercetin equivalent

Table 3. Ability of crude extract to scavenge hydrogen peroxide when compared with BHA, BHT and α-tocopherol.

	H_2O_2 scavenging activity (%)	Superoxide scavenging activity (%)
BHA	38.2 ± 2.8	76.4 ± 5.3
BHT	36.3 ± 3.2	72.2 ± 6.4
α-Tocopherol	41.2 ± 2.7	24.1 ± 3.2
Extract	51.9 ± 4.5	68.2 ± 3.4

be formed *in vivo* by many oxidizing enzymes, such as superoxide dismutase and can cross cellular membranes and may slowly oxidize a number of intracellular compounds. The ability of crude extract to scavenge hydrogen peroxide when compared with BHA, BHT and α-tocopherol is as shown in Table 3.

Hydrogen peroxide scavenging activity of the extract at the used concentration of 20 µg/ml was found to be 51.9 ± 4.5%. On the other hand, BHA, BHT and α-tocopherol exhibited 38.2 ± 2.8, 36.3 ± 3.2 and 41.2 ± 2.7% hydrogen peroxide scavenging activity at the same concentration, respectively. These results showed that the extract had an effective hydrogen peroxide scavenging activity. At the aforementioned concentration, the hydrogen peroxide scavenging effect of the extract and three standard compounds decreased in the order of the extract > α-tocopherol > BHA > BHT. Hydrogen peroxide itself is not very reactive; however, it can sometimes be toxic to cell, because it may give rise to hydroxyl radical in the cells.

Radical scavenging activity

Radical scavenging activities are very important due to the deleterious role of free radicals in foods and in biological systems. Diverse methods are currently used to assess the antioxidant activity of plant phenolic compounds. Chemical assays are based on the ability to scavenge synthetic free radicals, using a variety of radical-generating systems and methods for detection of the oxidation end-point. $ABTS^{\bullet+}$ radical cation(s) or DPPH radical scavenging methods are common spectrophotometric procedures for determining the antioxidant capacities of components.

These chromogens (the violet DPPH radical and the blue green $ABTS^{\bullet+}$ radical cation(s) are easy to use, have a high sensitivity and allow for rapid analysis of the antioxidant activity of a large number of samples. These assays have been applied to determine the antioxidant activity of pure components (Awika et al., 2003, Yu et al., 2002; van den Berg et al., 2000b). DPPH has been widely used to evaluate the free radical scavenging effectiveness of various antioxidant substances (Ozcelik et al., 2003). DPPH is usually used as a reagent to evaluate free radical scavenging activity of antioxidants.

DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares et al., 1997). In the DPPH assay, the antioxidants are able to reduce the stable radical DPPH to non-radical form, DPPH-H. The purple colored alcoholic solution of DPPH radicals changes color to yellow in the presence of a hydrogen-donating antioxidant which could be measured at 517 nm.

Figure 2 illustrates a significant decrease ($P < 0.01$) in the concentration of DPPH radical due to the scavenging ability of the extract and standards. BHA, BHT and α-tocopherol were used as references. The scavenging effect of the extract and standards on the DPPH radical decreased in the order of BHA > Extract > BHT > α-tocopherol which were 86.63, 84.01, 71.54 and 59.55%, at the concentration of 60 µg/m, respectively. DPPH free radical scavenging activity of the extract also increased with an increasing concentration (r^2: 8246).

Generation of the $ABTS^{\bullet+}$ radical cation(s) forms the basis of one of the spectrophotometric methods that have been applied to the measurement of the total antioxidant activity of pure substances solutions, aqueous mixtures and beverages (Miller, 1996). A more appropriate format for the assay is a decolorization technique, in that the radical is generated directly in a stable form prior to reaction with putative antioxidants. The improved technique for the generation of $ABTS^{\bullet+}$ described here involves the direct production of the blue/green $ABTS^{\bullet+}$

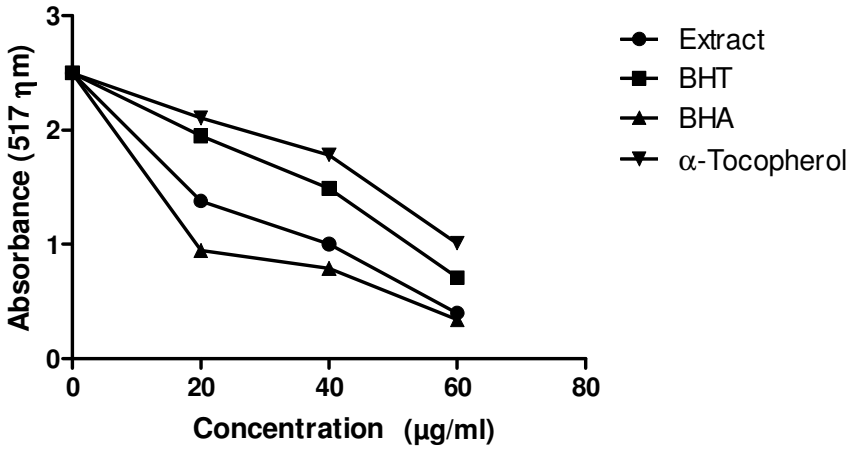

Figure 2. DPPH free radical scavenging activity of different concentrations (20 60 60 µg/ml) of extract of *H. rosasinensis* and reference antioxidants; BHA, BHT and α-tocopherol.

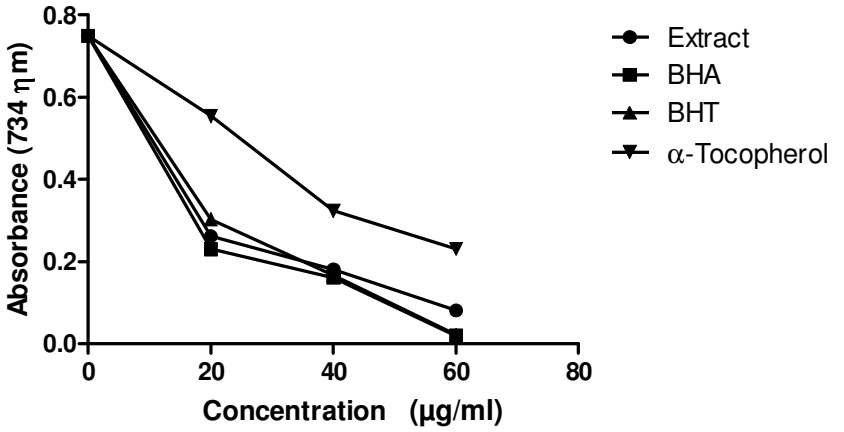

Figure 3. ABTS free radical scavenging activity of different concentrations (20 to 60 µg/ml) of extract of *H. rosasinensis* and reference antioxidants; BHA, BHT and α-tocopherol.

chromophore through the reaction between $ABTS^{\bullet+}$ radical cation(s) and potassium persulfate.

All the tested compounds exhibited affectual radical cation scavenging activity. As seen in Figure 3, the extract had effective $ABTS^{\bullet+}$ radical scavenging activity in a concentration-dependent manner (20 to 60 µg/ml). There is a significant decrease ($P < 0.01$) in all the concentration of $ABTS^{\bullet+}$ due to the scavenging capacity of all the extract concentrations. Also, the scavenging effect of the extract and standards on the $ABTS^{\bullet+}$ radical cation(s) decreased in the order: BHA > BHT > Extract > α-tocopherol, which were 97.33, 97.06, 89.2 and 73.33% at the concentration of 60 µg/ml, respectively.

Superoxide anion are a precursor to active free radicals and plays an important role in the formation of other ROS, such as hydrogen peroxide, hydroxyl radical and singlet oxygen, which induce oxidative damage in lipids, proteins and DNA (Pietta, 2000). Superoxide radical is normally formed first, and its effects can be magnified, because it produces other kinds of free radicals and oxidizing agents. Superoxide anions have the potential of reacting with biological macromolecules and have been implicated in several pathophysiological processes due to its transformation into more reactive species, such as hydroxyl radical that initiate lipid peroxidation. Also, superoxide has been observed to directly initiate lipid peroxidation (Wickens, 2001). In addition, it has been reported that antioxidant properties of some flavonoids are effective mainly via scavenging of superoxide anion radical. Superoxide anion derived from dissolved oxygen

riboflavin/methionine/illuminate system and reduces NBT in this system. In this method, superoxide anion reduces the yellow dye (NBT^{2+}) to produce the blue formazan which is measured spectrophotometrically at 560 nm. Antioxidants are able to inhibit the blue NBT formation (Parejo et al., 2002) and the decrease of absorbance at 560 nm indicates the consumption of superoxide anion in the reaction mixture. Table 3 shows the inhibition percentage of superoxide radical generation by 20 µg/ml concentration of crude extract and antioxidant standards. The inhibition of superoxide radical generation results of extract and standards were found to be similar statistically. As shown in Table 3, the percentage inhibition of superoxide anion radical generation by 20 µg/ml concentration of the extract was found as 68.2 ± 3.4 %.

On the other hand, at the same concentration, BHA, BHT and α-tocopherol exhibited 76.4 ± 5.3, 72.2 ± 6.4 and 24.1 ± 3.2% superoxide anion radical scavenging activity, respectively. According to these results, crude extract had similar superoxide anion radical scavenging activity to BHA and BHT; however, it had higher superoxide anion radical scavenging activity than α-tocopherol.

Conclusion

According to data obtained from the present study, 80% aqueous-ethanol crude extract *of H. rosasinensis* extract was found to be an effective antioxidant in different *in vitro* assays, including total antioxidant activity determination by ferric thiocyanate, DPPH radical, $ABTS^{\bullet+}$ radical cation(s) radical, superoxide anion radical scavenging and hydrogen peroxide scavenging when it is compared to standard antioxidant compounds, such as BHA, BHT and α-tocopherol.

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