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Free radical scavenging activity of three different flowers-*Hibiscus rosa-sinensis*, *Quisqualis indica* and *Senna surattensis*

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ABSTRACT

Objective: To evaluate three flowers of *Hibiscus rosa-sinensis* (*H. rosa-sinensis*), *Quisqualis indica* (*Q. indica*) and *Senna surattensis* (*S. surattensis*) for their antioxidant activity by different methods in addition to total phenolic, flavonoid and pigment contents.

Methods: Antioxidant activity of water, ethanol and absolute ethanol extracts of three flowers; *H. rosa-sinensis*, *Q. indica* and *S. surattensis* was evaluated. The antioxidant activity was assessed by 1,1-diphenyl-2-picrylhydrazyl free radical scavenging activity, ferrous chelating activity, reducing power, nitric oxide scavenging activity, hydroxyl radical scavenging activity as well as total antioxidant capacity. Total flavonoids, total phenols and total pigments including chlorophylls and carotenoids were measured for the three flowers.

Results: The results showed that the highest total antioxidant capacity at concentration of 500 mg/L was found in *S. surattensis* as 0.479 ± 0.001. Scavenging activity of *H. rosa-sinensis*, *Q. indica* and *S. surattensis* flower extracts against 1,1-diphenyl-2-picrylhydrazyl radical showed the highest activity of (90.20 ± 0.29)% with 500 mg/L. Phytochemical screening of the three flowers extracts were carried out for alkaloids, flavonoids, saponins, tannins, steroids, glycosides, terpenoids, amino acid and mucilages. *H. rosa-sinensis* showed the total phenolic in water extract of (235.77 ± 14.31) mg/100 g, the other two flowers *Q. indica* and *S. surattensis* had the total phenolic in ethanol extracts of (937.70 ± 25.06) and (850.30 ± 13.81) mg/100 g, respectively. On the other hand total flavonoids were identified in absolute ethanol extracts in the three flowers [(32.83 ± 1.34), (49.24 ± 4.87) and (2.79 ± 0.23) mg/100 g, respectively].

Conclusions: The extracts in the constituents of the three flowers could be used as additives as supplement fractions in foods.

1. Introduction

Reactivity of free radicals is generally stronger than non-radical species though radicals are less stable and disrupt the biological function of biomolecules. Reactive oxygen species and reactive nitrogen species include radicals like hydroxyl ($OH^{\bullet}$), superoxide ($O_2^{\bullet-}$), peroxyl ($RO_2^{\bullet}$), alkoxyl ($RO^{\bullet}$), hydroperoxyl ($HO_2^{\bullet}$), nitrogen dioxide ($NO_2^{\bullet}$), nitric oxide ($NO^{\bullet}$) and lipid peroxyl ($LOO^{\bullet}$); and non radicals like hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), ozone (O_3), singlet oxygen, peroxynitrite ($ONOO^-$), nitrous acid (HNO_2), lipid peroxide (LOOH), dinitrogen trioxide (N_2O_3) as reported by Pham-Huy *et al.* [1]. These radicals destroy a lot of biomolecules such as protein lipids as well as nucleic acid and may cause mutation in living cell which cause diseases. Therefore looking for anti-oxidative and hydroxyl radical, nitric oxide radical scavenger agents from natural products are still ongoing to discover these compounds from plants. Flavonoids, phenolic compounds as well as pigments extracted from flowers petals are a major class of our interest in this investigation.

Hibiscus species are found in tropics and subtropics areas which have a remarkable colour pattern [2]. *Quisqualis indica* (Combretaceae) (*Q. indica*) is indigenous in Africa, Indo–

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Malaysian region and cultivated all over India. The number of its flower is numerous, and at first they are white in colour then they become red [3]. *Senna surattensis* (Fabaceae) (*S. surattensis*) is commonly known as *Glaucous cassia*. It is a small tree or large shrub, distributed throughout India [4]. Plants protect themselves from oxidative damage against ultraviolet exposure by producing antioxidative phenolic compounds and flavonoids in plant tissues [5].

The natural antioxidants may have free-radical scavengers, reducing agents, potential complexes of pro-oxidant metals, quenches of singlet oxygen [6].

Recently, interest has increased considerably in finding natural occurring antioxidants for use in medicinal materials to replace synthetic antioxidants which are being restricted due to their side effects such as carcinogenicity [7].

Crude alcoholic extracts of *Hibiscus rosa-sinensis (H. rosa-sinensis)* flower petals and leaves are used *in vivo* for their antioxidant properties in some studies [8]. *Q. indica* flowers have several beneficial effects by virtue of their antioxidant activity [9]. At the same time flavonoids are recognized as antioxidants with health-promoting properties in human diets. The protective effects of these compounds are attributed to bioactive phytochemicals including antioxidants. On the other hand polyphenols and flavonoids of oregano (*Origanum syriacum* L.) and red grape seed extracts showed hepatoprotective, anti-carcinogenic as well as antioxidant activities [10,11]. The antioxidant activity of water extracts (cold and hot) of six caffeine products was assessed using 1,1-diphenyl-2-picrylhydrazyl (DPPH), ABTS methods and reducing power method at 50 and 100 μg/mL after 15 and 30 min using butylated hydroxyanisole and caffeine as standard compounds [12].

The main purpose of this investigation was to evaluate and study the free radical scavenging activity of three different flowers – *H. rosa-sinensis* (red), *Q. indica* (pink) and *S. surattensis* (yellow). The possibility of using extracts of these flowers in drug preparation was discussed.

2. Materials and methods

2.1. Plant materials

The flowers of *H. rosa-sinensis*, *Q. indica* and *S. surattensis* were collected from the Faculty of Agriculture, Cairo University, Egypt. The plants were identified with the help of available literature and authenticated by the taxonomist at Department of Botany, Faculty of Agriculture, Cairo University, Egypt. The flowers of the plants were washed with water and then stored in tight polythene bags at 4 °C, until use.

2.2. Chemicals

Ascorbic acid, DPPH, sulfanilamide, *N*-(1-naphthyl) ethylenediamine dihydrochloride, 2-deoxyribose and ferrous sulphate were purchased from Sigma Chemical Co., USA. All other chemicals were of analytical reagent grade.

2.3. Preparation of flower extracts

Three different extracts were prepared from flowers of *H. rosa-sinensis*, *Q. indica* and *S. surattensis* using the procedure reported by Ruban and Gajalakshmi [13] with some modifications as follows:

To prepare water extract, 10 g of flowers was grinded with 100 mL of distilled water. After 48 h at 5 °C, the mixture was filtered off using Whatman No. 1. The resulting supernatant was used as water extract in all experiments. The same method was used for the extraction of flowers with ethanol 80% and ethanol absolute. The total solids (%) in all extracts were determined by the dry weights following drying at 100 °C until constant mass was achieved.

2.4. Phytochemical screening of the flower extracts

Phytochemical screening of flower extracts was carried out qualitatively for the presence of alkaloids, flavonoids, saponins, tannins, steroids, glycosides, terpenoids, amino acids and mucilages and gums using the standard methods [14,15].

2.5. Spectrophotometric determinations of pigments

Chlorophylls, xanthophylls, carotenoids and anthocyanins were determined according to the modification of the spectrocolorimetric procedure given by Owayss *et al.* [16]: In a porcelain mortar, 3 g of each flower were well mixed with 30 mL of 80% ethanol then ground and filtered. The filtrate was made up to 100 mL with 80% solvent. The absorbance of the extract was measured against 80% ethanol (as a blank) at 663, 645, 470, 480, 537 and 647 nm. Pigments were expressed as μg/g fresh weight of the flowers according to the following equations:

$$\text{Chlorophyll } a = 12.7 \times OD_{663} - 2.69 \times OD_{645}$$

$$\text{Chlorophyll } b = 22.4 \times OD_{645} - 4.68 \times OD_{663}$$

$$\text{Total chlorophylls} = 8.02 \times OD_{663} + 20.2 \times OD_{645}$$

$$\text{Xanthophylls} = 2\,026.1 \times OD_{470} - 2\,288.6 \times OD_{485} + 0.003\,6\ (a) - 0.06518\ (b)$$

$$\text{Carotenoids} = OD_{480} + (0.114 \times OD_{663} - 0.638 \times OD_{645})$$

$$\text{Anthocyanin} = 0.08173 \times OD_{537} - 0.00697 \times OD_{647} - 0.002228 \times OD_{663}$$

2.6. Determination of total phenolic

Total phenolic content was determined in extracts using Folin–Ciocalteau method as described by Gao *et al.* [17] as follows:

Extract (100 μL) was mixed with 0.2 mL of Folin–Ciocalteu reagent, 2.0 mL of H_2O, and 1.0 mL of 15% Na_2CO_3 solution. The developing colour was measured at 765 nm after 2 h at room temperature using Jenway 6300 spectrophotometer. The concentration was calculated from the standard curve prepared using serial concentrations of standard tannic acid solution.

2.7. Determination of total flavonoids

Total flavonoids content was determined in flower extracts using the method described by Kumaran and Karunakaran [7] by using aluminium trichloride in ethanol in the presence of glacial acetic acid. The absorption was measured at 415 nm and quercetin was used as standard solution. The amount of total

flavonoids content in extracts in quercetin equivalents (QE) was calculated by the following equation:

$$X = (A \times m_0)/(A_0 \times m)$$

where, X is the flavonoids content (mg/mg extract in quercetin equivalents), A is the absorption of extract solution, A_0 is the absorption of standard quercetin solution, m is the weight of flower (mg) in extract and m_0 is the weight of quercetin in the solution (mg).

2.8. Determination of antioxidant activities of flower extracts

2.8.1. Determination of total antioxidant capacity

Total antioxidant capacity of extracts was assayed by the phosphomolybdenum method as described by Kumaran and Karunakaran [7] and using ammonium molybdate. Ascorbic acid solution (0.03%, w/v) was used as positive control.

2.8.2. Determination of reducing power

The reducing power of extracts was determined by the method of Mathew and Abraham [18] by using potassium ferricyanide solution, the absorbance was measured at 700 nm. Ascorbic acid solution (0.03%, w/v) was used as positive control prepared by the same procedure without extract. Increased absorbance of the reaction mixture indicated increased reducing power.

2.8.3. Determination of antioxidant activity by DPPH

The antioxidant activity of extracts, based on the scavenging activity of the stable DPPH free radical, was determined by the method described by Lee *et al.* [19]. The absorbance (A) of the solution was measured at 517 nm. Inhibition of DPPH free radical in percent was calculated from the following equation:

$$\text{Inhibition}\% = [(A_{control} - A_{sample})/A_{control}] \times 100$$

2.8.4. Determination of nitric oxide scavenging activity

The scavenging activity of nitric oxide by extracts was determined by the method described by Kumaran and Karunakaran [7] using Griess reagent (1% sulfanilamide, 2% H_3PO_4 and 0.1% *N*-(1-naphthyl) ethylenediamine dihydrochloride). The absorbance (A) was measured at 546 nm. Ascorbic acid solution (0.03%, w/v) was used as a positive control. Scavenging activity of nitric oxide was calculated from the following equation:

$$\text{Scavenging activity}\ (\%) = [(A_{control} - A_{sample})/A_{control}] \times 100$$

2.8.5. Determination of hydroxyl radical scavenging activity

The scavenging activity of hydroxyl radical by extracts was assayed by deoxyribose method as described by Nagai *et al.* [20]. The absorbance (A) of the solution was measured at 520 nm. Ascorbic acid solution (0.03%, w/v) was used as a positive control. Inhibition of deoxyribose degradation in percent was calculated using the following equation:

$$\text{Inhibition}\% = [(A_{control} - A_{sample})/A_{control}] \times 100$$

2.8.6. Determination of Fe^{2+} chelating activity

The chelating activity of ferrous (Fe^{2+}) ion was determined according to the method described by Oboh *et al.* [21]. In a clean test tube, 0.3 mL of flower extract solution was added to a reaction mixture consisted of 0.336 mL of Tris–HCl buffer solution (pH 7.4) and different volumes of each extract (0.006–0.026 mL). The solution was completed by saline solution (0.9% NaCl, w/v) to a known volume (1.12 mL). The reaction mixture was incubated for 5 min at room temperature before the addition of 0.026 mL of 1,10-phenanthroline solution (0.25%, w/v). The absorbance (A) was measured at 510 nm. Ascorbic acid solution (0.03%, w/v) was used as a positive control. The Fe^{2+} chelating activity (%) was calculated from the following equation:

$$Fe^{2+}\ \text{chelating activity}\ (\%) = [(A_{control} - A_{sample})/A_{control}] \times 100$$

2.9. Statistical analysis

The results were analysed by an analysis of variance ($P < 0.05$) and the means were separated by Duncan's multiple range test. The results were processed by CoStat computer program (1986).

3. Results

3.1. Preliminary phytochemical screening

The phytochemical screening including qualitative as well as quantitative estimation revealed that the three flowers used were rich in phenols, terpenoids and little flavonoids, with antioxidant, hydroxyl radical scavenging and nitric oxide radical scavenging activity. Thus from these biochemical investigations, it is quite evident that three flowers *H. rosa-sinensis*, *Q. indica* and *S. surattensis* are very rich source of secondary metabolites.

The results in Table 1 about the preliminary phytochemical screening for the three flowers; *H. rosa-sinensis*, *Q. indica* and *S. surattensis* revealed the presence of flavonoids, tannins, alkaloids, steroids, terpenoids, amino acids, glycosides while, mucilages and gums could be identified only in the water extract of *H. rosa-sinensis* [22] and saponins was absent in three flowers.

3.2. Total phenols, total flavonoids and total pigments

Total phenols, total flavonoids and total pigments including chlorophylls and carotenoids were determined for the three flowers *H. rosa-sinensis*, *Q. indica* and *S. surattensis* (Tables 2 and 3). The obtained results showed that the total phenolics in water extract of *H. rosa-sinensis* was (235.77 ± 14.31) mg/100 g, while the other two flowers *Q. indica* and *S. surattensis* had the total phenolic in ethanol 80% extracts of (937.70 ± 25.06) and (850.30 ± 13.81) mg/100 g, respectively. On the other hand total flavonoids were identified in absolute ethanol extracts in the three flowers [(32.83 ± 1.34), (49.24 ± 4.87) and (2.79 ± 0.23) mg/100 g, respectively].

3.3. Total antioxidant activity and reducing power

Antioxidant activity of *H. rosa-sinensis*, *Q. indica* and *S. surattensis* extracts showed different antioxidant activity

Table 1
Phytochemical screening of *H. rosa-sinensis*, *Q. indica* and *S. surattensis* flower extracts.

Plant	Extract	Flavonoids	Tannins	Alkaloids	Saponins	Steroids	Terpenoids	Amino acids	Glycosides	Mucilages and gums
H. rosa-sinensis	Water extract	+	+	+	−	+	+	+	+	+
	Ethanol 80% extract	+	+	+	−	+	+	+	+	−
	Ethanol absolute extract	+	+	+	−	+	+	+	+	−
Q. indica	Water extract	+	+	+	−	−	+	+	+	−
	Ethanol 80% extract	+	+	+	−	+	−	+	+	−
	Ethanol absolute extract	+	+	+	−	+	+	+	+	−
S. surattensis	Water extract	+	−	+	−	+	−	+	−	−
	Ethanol 80% extract	+	+	+	−	+	+	+	−	−
	Ethanol absolute extract	+	+	+	−	+	+	+	−	−

+: Presence; −: Absence.

Table 2
Pigments content of *H. rosa-sinensis*, *Q. indica* and *S. surattensis* flowers. μg/g fresh weight.

Plant	Chlorophyll *a*	Chlorophyll *b*	Total chlorophyll	Xanthophylls	Carotenoids	Anthocyanins
H. rosa-sinensis	2.63	1.13	3.81	24.69	162.00	153.20
Q. indica	0.47	0.53	1.02	102.96	47.00	91.50
S. surattensis	0.33	0.52	0.87	36.46	147.00	33.94

Table 3
Total solid, phenolic and flavonoid contents of *H. rosa-sinensis*, *Q. indica* and *S. surattensis* flower extracts.

Plant	Extract	Phenolics (mg/100 g flower)	Flavonoids (mg/100 g flower)	Total solids (%)
H. rosa-sinensis	Water extract	235.77 ± 14.31^{ab}	31.27 ± 2.36^{a}	1.7
	Ethanol 80% extract	281.23 ± 21.68^{a}	29.58 ± 2.34^{a}	6.7
	Ethanol absolute extract	186.17 ± 6.98^{b}	32.83 ± 1.34^{a}	4.8
	Least significant difference	53.74	7.17	–
Q. indica	Water extract	858.77 ± 10.47^{ab}	40.41 ± 2.11^{a}	2.2
	Ethanol 80% extract	937.70 ± 25.06^{a}	24.52 ± 1.81^{b}	8.1
	Ethanol absolute extract	771.47 ± 67.42^{b}	49.24 ± 4.87^{a}	5.0
	Least significant difference	145.22	11.2	–
S. surattensis	Water extract	302.90 ± 4.33^{c}	0.62 ± 0.05^{b}	1.6
	Ethanol 80% extract	850.30 ± 13.81^{a}	1.10 ± 0.11^{b}	5.3
	Ethanol absolute extract	641.47 ± 45.20^{b}	2.79 ± 0.23^{a}	3.3
	Least significant difference	94.82	0.63	–

Values are represented as mean ± SE. Values in the same column followed by the same letter are not significantly different at $P < 0.05$.

depending on species as well as concentration and methods of extraction. The results showed that the highest total antioxidant capacity in water extract was found in *Q. indica* with 500 mg/L as 0.371 ± 0.012. Comparing the potential efficiency of different extracts, the results showed that *H. rosa-sinensis* ethanol 80% extract had significant antioxidant activity followed by ethanol absolute extract and water extract at 500 mg/L (0.443 ± 0.005, 0.423 ± 0.017 and 0.299 ± 0.009, respectively). The same trend could be identified in the other two flower species; *Q. indica* and *S. surattensis* (Table 4).

On the other hand, the reducing power of the compound may serve as a significant indicator of its potential antioxidant activity. Reducing power assay is used to evaluate the ability of natural antioxidant to donate electron [23]. Table 4 shows the reducing power of the extracts of *H. rosa-sinensis*, *Q. indica* and *S. surattensis* flowers compared to ascorbic acid (10 mg/L) as standard. The reducing power of the flower extract was found to be significant and dose dependent.

3.4. DPPH scavenging activity

Scavenging activity of *H. rosa-sinensis*, *Q. indica* and *S. surattensis* flower extracts against DPPH radical were determined and the results are shown in Table 4. The DPPH radical scavenging activity of *H. rosa-sinensis*, *Q. indica* and *S. surattensis* were evaluated and compared with ascorbic acid. The % inhibition of flowers extract were calculated at various concentration (500, 1 000 and 2 000 mg/L) as well as standard ascorbic acid (6 mg/L). The highest scavenging activity of water extracts was identified in *Q. indica* (61.21 ± 2.63)%, followed by *S. surattensis* (21.73 ± 0.26)% and *H. rosa-sinensis* (2.78 ± 0.12)% at concentration of 500 mg/L. While in ethanol 80% extract, the scavenging activity ranked as follows: *S. surattensis* (90.20 ± 0.29)% > *Q. indica* (61.75 ± 2.03)% > *H. rosa-sinensis* (34.80 ± 1.98)% proving that the antioxidant activity depends on the species. The same trend could be identified in the case of extraction with absolute ethanol.

The results were similar to previous reports that phenolic compounds were major antioxidant constituents in medicinal herbs, vegetables, fruits, essential oils and tea leaf [12,24,25].

3.5. Nitric oxide and hydroxyl radical scavenging activity

The results of nitric oxide and hydroxyl radical scavenging activity of *H. rosa-sinensis*, *Q. indica* and *S. surattensis* flower

Table 4
Antioxidant activity of *H. rosa-sinensis*, *Q. indica* and *S. surattensis* flower extracts and ascorbic acid using different methods.

Plant	Extract	Concentration (mg/L)	Total antioxidant capacity (OD_{695})	Total reduction capability (OD_{700})	DPPH scavenging activity (%)	Nitric oxide scavenging activity (%)	Hydroxyl radical scavenging activity (%)	Fe chelation (%)
H. rosa-sinensis	Water extract	500	0.299 ± 0.009^{f}	0.093 ± 0.005^{e}	2.78 ± 0.12^{e}	5.23 ± 0.16^{c}	6.66 ± 0.55^{f}	9.54 ± 1.15^{ef}
		1 000	0.348 ± 0.003^{e}	0.172 ± 0.006^{cd}	17.62 ± 1.62^{d}		12.06 ± 1.83^{e}	15.78 ± 1.50^{c}
		2 000	0.514 ± 0.009^{b}	0.324 ± 0.002^{a}	46.61 ± 4.42^{b}	8.09 ± 0.37^{a}	20.95 ± 1.10^{d}	20.90 ± 1.70^{ab}
	Ethanol 80% extract	500	0.443 ± 0.005^{d}	0.110 ± 0.006^{e}	34.80 ± 1.98^{c}	5.46 ± 0.21^{c}	6.98 ± 0.42^{f}	6.63 ± 0.90^{f}
		1 000	0.474^{c}	0.157 ± 0.006^{d}	51.99 ± 1.97^{b}		23.28 ± 1.69^{d}	13.25 ± 0.40^{cd}
		2 000	0.645 ± 0.004^{a}	0.247 ± 0.003^{b}	78.20 ± 0.37^{a}	7.72 ± 0.15^{a}	36.19 ± 0.52^{b}	22.90 ± 0.63^{a}
	Ethanol absolute extract	500	0.423 ± 0.017^{d}	0.068 ± 0.004^{f}	21.90 ± 1.38^{d}	5.03 ± 0.20^{c}	22.38 ± 0.52^{d}	2.89 ± 0.29g
		1 000	0.468 ± 0.006^{c}	0.100 ± 0.002^{e}	34.00 ± 2.30^{c}		29.86 ± 1.85^{c}	10.77 ± 0.77^{de}
		2 000	0.641^{a}	0.181 ± 0.011^{c}	80.78 ± 0.76^{a}	7.01 ± 0.15^{b}	47.62 ± 1.90^{a}	19.27 ± 1.10^{b}
	Least significant difference		0.024	0.018	6.11	0.69	4.13	3.09
Q. indica	Water extract	500	0.371 ± 0.012^{d}	0.393 ± 0.011^{bc}	61.21 ± 2.63^{c}	5.56 ± 0.16^{e}	22.38 ± 0.55^{c}	12.43 ± 1.28^{de}
		1 000	0.474^{b}	0.301 ± 0.003^{d}	68.10 ± 0.17^{b}		29.84 ± 2.04^{b}	22.88 ± 2.76^{c}
		2 000	0.635 ± 0.003^{a}	0.372 ± 0.024^{c}	80.00 ± 1.36^{a}	7.75 ± 0.09^{c}	52.86 ± 0.82^{a}	33.19 ± 1.01^{a}
	Ethanol 80% extract	500	0.471 ± 0.001^{b}	0.201 ± 0.008^{e}	61.05 ± 2.34^{c}	6.22 ± 0.30^{d}	0.32 ± 0.10^{e}	14.04 ± 0.32^{de}
		1 000	0.474^{b}	0.309 ± 0.009^{d}	61.75 ± 2.03^{c}		1.90 ± 0.36^{e}	16.78 ± 0.63^{d}
		2 000	0.641^{a}	0.423 ± 0.005^{ab}	82.18 ± 0.76^{a}	9.91 ± 0.04^{a}	3.18 ± 0.32^{e}	31.54 ± 2.34^{ab}
	Ethanol absolute extract	500	0.408 ± 0.034^{cd}	0.196 ± 0.006^{e}	57.84 ± 2.46^{c}	6.37 ± 0.09^{d}	8.25 ± 0.96^{d}	12.00 ± 0.54^{e}
		1 000	0.449 ± 0.025^{bc}	0.333 ± 0.002^{d}	58.57 ± 2.55^{c}		23.49 ± 2.20^{c}	16.44 ± 1.21^{de}
		2 000	0.662^{a}	0.427 ± 0.011^{a}	84.52 ± 1.04^{a}	9.01 ± 0.14^{b}	54.76 ± 1.37^{a}	27.54 ± 0.74^{b}
	Least significant difference		0.044	0.032	5.66	0.50	3.58	4.27
S. surattensis	Water extract	500	0.245 ± 0.021^{e}	0.090 ± 0.005^{f}	21.73 ± 0.26^{e}	7.00 ± 0.04^{c}	2.38 ± 0.27^{e}	5.86 ± 1.53^{f}
		1 000	0.441 ± 0.011^{cd}	0.155 ± 0.004^{e}	37.31 ± 1.77^{d}		12.91 ± 1.38^{d}	13.46 ± 0.04^{cd}
		2 000	0.623 ± 0.030^{a}	0.394 ± 0.018^{b}	61.18 ± 1.76^{c}	9.35 ± 0.03^{a}	49.52 ± 1.98^{a}	29.64 ± 1.64^{a}
	Ethanol 80% extract	500	0.479 ± 0.001^{bc}	0.254 ± 0.011^{d}	90.20 ± 0.29^{a}	5.30 ± 0.12^{e}	11.98 ± 1.15^{d}	7.95 ± 0.58^{ef}
		1 000	0.486 ± 0.006^{b}	0.338 ± 0.020^{c}	90.47 ± 0.06^{a}		20.32 ± 0.97^{c}	15.33 ± 0.93^{c}
		2 000	0.648 ± 0.008^{a}	0.427 ± 0.012^{ab}	90.57 ± 0.06^{a}	8.16 ± 0.26^{b}	35.24 ± 4.40^{b}	21.57 ± 1.98^{b}
	Ethanol absolute extract	500	0.412 ± 0.005^{d}	0.163 ± 0.003^{e}	76.66 ± 1.32^{b}	6.09 ± 0.03^{d}	0.00^{e}	11.44 ± 0.37^{de}
		1 000	0.486 ± 0.006^{b}	0.275 ± 0.011^{d}	87.56 ± 0.23^{a}		9.73 ± 0.92^{d}	15.61 ± 1.56^{c}
		2 000	0.648 ± 0.008^{a}	0.440 ± 0.010^{a}	89.17 ± 0.06^{a}	9.17 ± 0.16^{a}	34.60 ± 2.71^{b}	2 7.76 ± 0.205^{a}
	Least significant difference		0.041	0.035	2.83	0.42	5.92	3.55
Standard	Ascorbic acid	6			82.61 ± 0.77			
		10	0.820 ± 0.030	0.229 ± 0.001		1.36 ± 0.15	40.21 ± 2.80	7.41

Values are represented as mean ± SE. Values in the same column followed by the same letter are not significantly different at $P < 0.05$.

extracts were evaluated compared with ascorbic acid and the results are given in Table 4.

The inhibition percentages at various concentrations (500, 1000 and 2000 mg/L) of *H. rosa-sinensis*, *Q. indica* and *S. surattensis* as well as standard ascorbic acid (10 mg/L) were recorded. The high nitric oxide scavenger was detected in *S. surattensis* at 500 mg/L (7.00 ± 0.04)% in water extract and (5.30 ± 0.12)% in ethanol 80% extract, respectively. On the other hand there are no significant differences in nitric oxide scavenging in the two flowers; *Q. indica* and *S. surattensis* when using absolute ethanol extracts. On the other hand, the hydroxyl radical scavenging activity is measured as the percentage of inhibition of hydroxyl radicals generated in Fenton's reaction mixture [26]. The obtained results revealed that the ethanol absolute extract of *Q. indica* flowers (2000 mg/L) was more effective than other flowers in hydroxyl radical scavenging activity. The scavenging activity was increased with increasing of the concentrations of flower extract.

3.6. Metal chelating activity

The results of metal chelating activity of *H. rosa-sinensis*, *Q. indica* and *S. surattensis* flower extracts are shown in Table 4. It is very important to note that ferrous chelating activity depends on two factors; type of flower and extraction methods. In water extracts ferrous chelating activity shows the high value in *Q. indica* followed by *H. rosa-sinensis* and *S. surattensis* at 500 mg/L [(12.43 ± 1.28)%, (9.54 ± 1.15)% and (5.86 ± 1.53)%, respectively]. While in ethanol 80% extract, ferrous chelating activity ranked as follows: *Q. indica* > *S. surattensis* > *H. rosa-sinensis* [(14.04 ± 0.32)%, (7.95 ± 0.58)% and (6.63 ± 0.90)%, respectively] at 500 mg/L. The same trend could be seen in case of extraction with absolute ethanol in Table 4.

4. Discussion

Phytochemical is a natural bioactive compound found in plants, such as vegetables, fruits, medicinal plants, flowers and leaves, to protect against diseases. Some of the most important bioactive phytochemical constituents such as alkaloids, essential oils, flavonoids, tannins, terpenoids possess antioxidant [11,27–30], antiulcer [31], protective effects [32], inhibitory effects [33], in addition to hydroxyl radical scavenging activity, nitric oxide scavenging activity and Fe^{2+} chelating activity. Phytochemical analysis of medicinal plants revealed the presence of major phytocompounds like terpenoids, alkaloids, glycosides, phenolic and tannins as reported [34].

Our results are in agreement with Pillai and Mini [35] who stated that ethyl acetate fraction of *H. rosa-sinensis* had a very high content of total phenolic and flavonoids. This may provide a very good antioxidant capacity of phenolic compounds and flavonoids due to their redox properties against free radicals, quenching singlet and triplet oxygen or decomposing peroxides as well as inhibitory effect on carcinogenesis [36,37]. These compounds possess biological activities during processing of seeds including radical scavenging properties [38,39]. *H. rosa-*

sinensis contains anthocyanins, in addition to carotenoids and chlorophyll which may be responsible for its antioxidant effects as stated by Mak *et al.* [40]. Because the red flowers of *H. rosa-sinensis* have anthocyanin content, radical-scavenging activity, antioxidant capacity, ferric-reducing power and ferrous ion-chelating were approved [41]. Analysis of phenolic content in the three flowering species of *H. rosa-sinensis*, *Q. indica* and *S. surattensis* showed positive correlation with DPPH radical-scavenging activity [42] except for total flavonoids in *S. surattensis* extracts. This observation proved the presence of potent non-flavonoid radical scavengers involved the biochemical reaction.

From the results above flower species with higher total phenolic content and antioxidant capacity values had lower ferrous ion activity, and *vice versa*. This suggests the presence of compounds in the flowers *S. surattensis* with relatively weak radical-scavenging activity but good metal-chelating ability that can prevent the generation of hydroxyl radicals [41].

The strong free radical scavenging activity exhibited by the three flowers could be attributed to high phenols, flavonoids as well as to pigments and chlorophylls. Therefore, they can be considered as a potential source of natural antioxidants to support food as supplements. The three flowers which show antioxidant properties as a result of the bioactive constituents could be used in drugs preparation. Thus these plants have great medicinal potential for the therapy of infection.

Conflict of interest statement

We declare that we have no conflict of interest.

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