Antioxidant and Free Radical Scavenging Potential of Different Solvent Extracts of Indigofera tinctoria L. Leaves

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ABSTRACT

Indigofera tinctoria L. (Fabaceae) is traditionally used in Indian and Chinese medicinal systems for various ailments including cancer, liver disorder, inflammation, ulcers and nervous disorders. Since the said curative effects are often related to antioxidant properties, different solvent (petroleum ether, benzene, chloroform, ethyl acetate, methanol and water) extracts of I. tinctoria leaves were evaluated for the antioxidant and free radical scavenging potentials by employing different in vitro assays such as reducing power assay, DPPH, ABTS+, NO and OH radical scavenging capacities, peroxidation inhibiting activity through linoleic acid emulsion system, anthemolysis assay through hydrogen peroxide induced hemolytic cells and metal ion chelating ability. Ethyl acetate, water and methanol extracts exhibited higher phenolic and tannin contents whereas benzene and ethyl acetate extracts showed higher flavonoid contents. Though all the extracts exhibited reducing power, the ethyl acetate extract was found to have more hydrogen donating ability. Ethyl acetate extract further exhibited higher DPPH and NO scavenging activities. All the studied extracts exhibited 22-56% OH scavenging activities at 250 μg concentration in the reaction mixture and strong peroxidation inhibition against linoleic acid emulsion system (87-96%). Methanol extract registered strong ABTS+ scavenging activity (1434.5 ± 236.8 μmol·DM). The potential of multiple antioxidant activity of this plant is evident as the extracts possessed anthemolysic and metal ion chelating activities.

Keywords: Antioxidant; Free radicals; Total phenolics; Indigofera tinctoria; DPPH; ABTS+; Anthemolysic activity

MATERIALS AND METHODS

Chemicals

Potassium ferricyanide, ferric chloride, 2,2'-diphenyl-1-picrylhydrazyl (DPPH), potassium persulfate, 2,2'-azino(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 3-(2-pyridyl)-5,6-bis(4-phenyl-sulfonic acid)-1,2,4-triazine (Ferrozine), linoleic acid, ferrous chloride, ammonium thiocyanate, hydrogen peroxide, ferrous ammonium sulfate, ethylenediaminetetraacetic acid (EDTA) disodium salt, trichloroacetic acid (TCA), naphthylethylendiaminedihydrochloride (NEED), sodium nitroprusside and sulphanilamide were obtained from Himedia, Merck Sigma. All other reagents used were of analytical grade.

Plant material

The fresh leaves of I. tinctoria were collected during the month of June, 2012 from the arid regions around Karur, Tamil Nadu, India. The plant species was authenticated by Botanical Survey of India, southern circle, Coimbatore, and voucher specimen (BUBH2021) was deposited in the Department of Botany Herbarium, Bharathiar University, Coimbatore. Freshly collected plant materials were cleaned to remove adhering dust and then dried under shade. The dried samples were powdered in a Willy Mill to 60-mesh size and used for solvent extraction.

Preparation of extracts

Coarse powder from the shadow dried leaves was extracted in soxhlet extractor successively with different solvents in the increasing order of polarity (petroleum ether, benzene, chloroform, ethyl acetate and methanol). Each time before extracting with the next solvent, the material was dried in hot air oven at 40°C. Finally, the material was macerated using hot water with occasional stirring for 16 h and the water extract filtered. The extracts were concentrated by rotary vacuum evaporator (Yamoto BO410, Japan) and then dried. The dry extract obtained with each solvent was weighed. The percentage yield was expressed in terms of air dried mass (DM). The potential of multiple antioxidant activity of this plant is evident as the extracts possessed anthemolysic and metal ion chelating activities.

In order to understand the highly acclaimed properties of I. tinctoria and its usage in the traditional systems of medicine, we have attempted to evaluate its antioxidant potential through the use of several in vitro assay systems and this will provide insight information with reference to their medicinal value.

INTRODUCTION

Reactive Oxygen Species (ROS) effect oxidative damage to various biomolecules including proteins, lipids, lipoproteins and DNA [1]. This oxidative damage is a critical pathological factor involved in several chronic human diseases including cardiovascular dysfunctions, atherosclerosis, inflammation, carcinogenesis, drug toxicity, reperfusion injury and neurodegenerative diseases. The putative protective effects of antioxidant agents of natural origin against oxidative stress defense and different human have received increasing attention in recent times, especially within biological, medical, nutritional, and agrochemical areas. Thus antioxidant compounds can be used to counteract oxidative damage by reacting with free radicals, chelating free catalytic metals and also by acting as oxygen scavengers.

Indigofera tinctoria L. (Fabaceae), an erect, pubescent shrub has been a part of Indian and Chinese medicinal systems since time immemorial. The plant is known for the natural blue dye (indigo) obtained from its leaflets and branches. It is widely used for the treatment of several nervous disorders, liver ailments, epilepsy, cancer, inflammation, bronchitis and as ointment for sores, old ulcers and hemorrhoids [2,3]. It has a broad range of pharmacological activities including anticancer [4], anti-hyperglycemic [5], anti-diabetic [6], hepatoprotective [7,8], antinociceptive [9], anti-inflammatory [10], antibacterial [11] and anti-epileptic [12] activities. Further, antioxidant activity of aqueous acetone extract of aerial parts [13] and floral extract [14] was also reported. Indirubin, the active constituent from the leaves of this plant is a promising anticancer drug [15]. The furano-flavanoids (pseudosemiglabrin, semiglabrin and glabritephrin) and a rare flavonoid glycoside: kaempferol-4’7-diharmnoside isolated from I. tinctoria were proved as good antidyssplastic agents [16]. Rotenoids (deguelin, dehydrodeguelin, rotenol, rotenone, tephrosin and sumatrol) isolated from I. tinctoria were found to be toxic to larvae of Anopheles stephensi and adults of Callosobruchus chinensis [17]. In order to understand the highly acclaimed properties of I. tinctoria and its usage in the traditional systems of medicine, we have attempted to evaluate its antioxidant potential through the use of several in vitro assay systems and this will provide insight information with reference to their medicinal value.
Antioxidant activity by radical cation (ABTS•⁺)
The total antioxidant activity of the samples was measured by ABTS radical cation decolorization assay according to the method of Re et al. [25] described by Siddhuraju and Manian [19]. ABTS•⁺ was produced by reacting 7 mM ABTS aqueous solution with 24 mM potassium persulfate in the dark for 12-16 h at room temperature. Prior to assay, this solution was diluted in ethanol (about 1:89 v/v) and equilibrated at 30°C to give an absorbance at 734 nm of 0.700 ± 0.02. The stock solution of the sample extracts were diluted such that after introduction of 10µL aliquots into the assay, they produced between 20-80% inhibition of the blank absorbance. After the addition of 1 mL of diluted ABTS•⁺ solution to 10µL of sample or Trolox standards (final concentration 0-15µM) in ethanol, absorbance was measured at 30°C exactly 30 min after the initial mixing. Appropriate solvent blanks were also run in each assay. Triplet determinations were made at each dilution of the standard, and the percentage inhibition was calculated from the blank absorbance at 734 nm and then was plotted as a function of Trolox concentration. The unit of total antioxidant activity (TAA) is defined as the concentration of Trolox having equivalent antioxidant activity expressed as μmol/g sample extracts on dry matter.

Chelating capacity
Chelating property of different solvent extracts was assessed by bipyridyl assay [26]. The reaction mixture contained 0.25 mL of extracts having the concentration of 1 mg, 0.25 mL of 1mM FeSO₄ solution, 1 mL of 0.2 M Tris-HCl buffer (pH 7.4), 1 mL of 22% bipyridyl solution (0.1% in 0.2 M Tris-HCl), 0.4 mL of 10% hydroxylamine-HCl and 2.5 mL of ethanol. The final volume was made up to 5 mL with deionized water and the absorbance was determined at 522 nm. The chelating activity of the extracts was evaluated using EDTA as standard. The results were expressed as mgEDTA equivalent / g sample extracts.

Antimicrobial activity

Antimicrobial activity in linoleic acid emulsion system
The antimicrobial activity of different solvent extracts was determined using the thiocyanide method [28]. Each sample (500µg) in the respective solvent was taken in screw cap tube and the solvent was evaporated to dryness. The tubes were then added with 0.5 mL of absolute ethanol, 0.5 mL of 2.5% linoleic acid in absolute ethanol, 1 mL of 0.05 M phosphate buffer (pH 7.7), and 0.5 mL of distilled water. The reaction mixture was incubated in dark at 40°C in an oven. After incubation the reaction mixture was centrifuged at 1500 rpm for 10 min and the extent of hemolysis was determined by measuring the absorbance at 540 nm corresponding to hemoglobin liberation.
Statistical analysis

The data were subjected to one-way analysis of variance (ANOVA) and the significance of the difference between means was determined by Duncan's multiple range test (P<0.05) using Statistica (Statsoft Inc., Tulsa, USA). Values expressed are means of three replicate determinations ± standard deviation.

RESULTS AND DISCUSSION

Percentage yield, total phenolics, tannin and total flavonoid content

The yield of different solvent extracts during successive solvent extraction was calculated and presented in Table 1. The per cent yield was maximum in methanol extract (18.7%) followed by water extract (14.8%). The ethyl acetate extract gave the minimum yield (1.5%) when compared with other solvent extracts. In the current study, the total phenolics and tannin content of different solvent extracts of *I. tinctoria* leaves were estimated and expressed as tannic acid equivalent. As shown in Table 1, the total phenolic content was maximum in water extract (263.4 ± 10.5 mg/g extract) followed by ethyl acetate extract (257.9 ± 0.63 mg/g extract). Of all the solvents used, the minimum total phenolic contents were recorded in lesser polar solvent extracts. Further, methanol was found to be the most efficient solvent to extract antioxidant tannins as it registered higher levels of tannin content (115.75 ± 1.65 mg/g extract). This is in agreement with the reports of Yen et al. [29] who found that methanol is an effective solvent for extraction of antioxidants. Nonetheless, the flavonoid content was more in benzene extract (7.53 ± 0.22 mg/g extract) followed by ethyl acetate extract (5.22 ± 0.37 mg/g extract). The higher contents of total phenolics in the highly polar solvent (ethylacetate, methanol and water) extracts of *I. tinctoria* might be contributed by the presence of furano-flavones, flavonol glycosides and high molecular tannins [16].

<table>
<thead>
<tr>
<th>Sample</th>
<th>Yield (%)</th>
<th>Total phenolics (mg/g extract)</th>
<th>Tannin (mg/g extract)</th>
<th>Flavonoids (mg/g extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether extract</td>
<td>3.4</td>
<td>97.07± 5.4c</td>
<td>2.20 ± 1.1a</td>
<td>-</td>
</tr>
<tr>
<td>Benzene extract</td>
<td>3.8</td>
<td>138.46 ± 7.6bc</td>
<td>21.24 ± 12.8bc</td>
<td>7.53 ± 0.22a</td>
</tr>
<tr>
<td>Chloroform extract</td>
<td>2.3</td>
<td>176.92 ± 3.5abc</td>
<td>43.22 ± 8.0abc</td>
<td>0.64± 0.07d</td>
</tr>
<tr>
<td>Ethyl acetate extract</td>
<td>1.5</td>
<td>257.87 ± 6.3c</td>
<td>55.31 ± 14.6c</td>
<td>5.22 ± 0.37b</td>
</tr>
<tr>
<td>Methanol extract</td>
<td>18.7</td>
<td>191.94 ± 3.0b</td>
<td>115.75 ± 16.5c</td>
<td>1.27 ± 0.03c</td>
</tr>
<tr>
<td>Water extract</td>
<td>14.8</td>
<td>263.37 ± 10.5a</td>
<td>65.20 ± 56.4h</td>
<td>0.70 ± 0.03d</td>
</tr>
</tbody>
</table>

Values are means of three independent analyses ± standard deviation (n = 3). Mean values followed by different superscript letters indicates significant statistical difference (P<0.05).

Reducing power

The reducing power of different solvent extracts of *I. tinctoria* leaves, which may serve as a significant reflection of the antioxidant activity was evaluated using potassium ferricyanide reduction method. All the extracts exhibited some degree of reducing power (Figure 1). The reducing power increases with increasing concentration. Among the different extracts, ethyl acetate extract exhibited the maximum reductive capability followed by methanol extract; however, their reducing power was inferior to tannic acid standard. Polyphenolic contents of all the sample extracts appear to function as electron and hydrogen atom donors and therefore should be able to terminate radical chain reaction by converting free radicals and reactive oxygen species to more stable products. Similar observation in terms of dose dependant activity between the polyphenolic constituents in terms of dose dependent and reducing power has been reported for several plant extracts [30,31].

Free radical scavenging activity on DPPH•

The proton-radical scavenging action has been known as an important mechanism of antioxidation. DPPH radical is a stable organic free radical which has been extensively used for evaluating the free radical scavenging potential of natural antioxidants. In the present study, concentration of the sample extracts necessary to decrease initial concentration of DPPH• by 50% (IC50) under experimental condition has been calculated and expressed in Table 2. A lower value of IC50 indicates higher antioxidant activity. Higher
Antioxidant activity by radical cation (ABTS extract, Q - quercetin, TA - tannic acid. (P<0.05). PE- petroleum ether extract, BE - benzene extract, CE - chloroform extract, EE - ethyl acetate extract, ME - methanol extract, WE - water extract.

Values are means of three independent analyses ± standard deviation (n=3). Bars having different letters indicate significant statistical difference.

The DPPH• scavenging activity was shown by ethyl acetate extract (87.77±4.94μg/mL) followed by methanol extract (99.53± 0.9μg/mL). However, the DPPH• scavenging activity of all the sample extracts was inferior to the standard antioxidants quercetin (29.0 ± 4.50μg/mL) and tannic acid (37.81±2.52μg/mL). Similar to the reducing power, the extent of DPPH• scavenging activity also appears to depend on the phenolic and tannin concentrations of the extracts, the water extract being the only exception. The DPPH• scavenging activity can also be attributed to the nature of phenolics contributing to their electron transfer/ hydrogen donating ability [32].

The data obtained revealed that the extracts can act as free radical inhibitors and primary antioxidants that react with free radicals.

Nitric oxide radical scavenging activity

The nitric oxide radical scavenging activity of different solvent extracts of *I. tinctoria* was determined, the IC50 under the experimental condition was also calculated and the results are presented in Table 2. The nitric oxide scavenging assay is based on the principle that sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interact with oxygen to produce nitrite ions that can be estimated using Griess reagent. In the present study, all the solvent extracts scavenged the nitric oxide radical and/or inhibited the nitrite formation but at much lower levels when compared with standard antioxidant tannic acid and quercetin. The extracts inhibit nitric oxide formation by competing with oxygen to react with nitric oxide directly and also to inhibit its synthesis. The ethyl acetate extract exhibited higher (11.09±0.19μg/mL) NO• scavenging activity followed by water (13.49±3.32μg/mL) and methanol (16.44±0.82μg/mL) extracts. The polyphenolic compounds present in *I. tinctoria* might be responsible for the observed scavenging activity. NO• scavenging activity of flavonoids and phenolic compounds are already well known [33].

**Table 2: DPPH and nitric oxide radical scavenging activity of different solvent extracts of *I. tinctoria* leaves**

<table>
<thead>
<tr>
<th>Sample</th>
<th>IC50 (μg/mL)</th>
<th>DPPH. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether extract</td>
<td>218.38 ± 17.3a</td>
<td>89.64 ± 5.49b</td>
</tr>
<tr>
<td>Benzene extract</td>
<td>233.31 ± 14.88b</td>
<td>47.45 ± 6.45c</td>
</tr>
<tr>
<td>Chloroform extract</td>
<td>104.35 ± 4.71d</td>
<td>59.46 ± 2.3b</td>
</tr>
<tr>
<td>Ethylacetate extract</td>
<td>87.78 ± 4.86a</td>
<td>11.09 ± 0.19c</td>
</tr>
<tr>
<td>Methanol extract</td>
<td>99.53 ± 10.96a</td>
<td>16.44 ± 0.82b</td>
</tr>
<tr>
<td>Water extract</td>
<td>325.97 ± 3.40d</td>
<td>13.50 ± 3.23d</td>
</tr>
<tr>
<td>Quercetin</td>
<td>29.0 ± 4.50a</td>
<td>3.07 ± 0.01e</td>
</tr>
<tr>
<td>Tannic acid</td>
<td>37.81 ± 2.52f</td>
<td>4.42 ± 0.73a</td>
</tr>
</tbody>
</table>

Values are means of three independent analyses ± standard deviation (n=3). Mean values followed by different superscript letters indicatesignificant statistical difference (P<0.05).

Hydroxyl radical scavenging activity

The hydroxyl radical is the most reactive free radical formed in biological systems and is considered to be one of the quick initiators of the lipid peroxidation process [34]. In the present study, ascorbic acid-iron-EDTA was used to generate hydroxyl radical. The •OH scavenging activity of different solvent extracts is presented in Figure 2. All the extract samples exhibited •OH scavenging activity ranging between 22.41±5.5% and 55.14±7.52% at 25μg concentration in the reaction mixture. Generally, higher levels of •OH scavenging activity exhibited by the non-polar solvent extracts in the present study could be attributed to the active hydrogen donor ability of hydroxyl substitution provided by phenolic flavonoids extracted in the early stages of successive extraction. It may be concluded that the ability of different solvent extracts of *I. tinctoria* leaves to quench hydroxyl radicals seems to be directly related to the prevention of propagation of lipid peroxidation, thus reducing the rate of chain reaction.

![Fig 2: Hydroxyl radical scavenging activity of different solvent extracts of *I. tinctoria* leaves](image)

Values are means of three independent analyses ± standard deviation (n=3). Bars having different letters indicate significant statistical difference (P<0.05). PE- petroleum ether extract, BE - benzene extract, CE - chloroform extract, EE - ethyl acetate extract, ME - methanol extract, WE - water extract, Q - quercetin, TA - tannic acid.

Antioxidant activity by radical cation (ABTS•−)

The ABTS•− radical cation scavenging activity of different solvent extracts of *I. tinctoria* leaves are presented in Table 3. In this assay, total antioxidant activity (TAA) reflects the ability of hydrogen donating antioxidants to scavenge ABTS•−, comparable with that of Trolox. Though all the samples exhibited considerable ABTS•− scavenging activity, the methanol extract exhibited stronger TAA (14348±5.236.8μmol g −1 DM) followed by ethyl acetate and benzene extracts with TAA values of 12111.4±24.26 and 10346.7±60.2 μmol g −1 DM respectively. In the present study, the order of scavenging activity of sample extracts is quercetin > methanol extract > ethyl acetate extract > benzene extract > tanic acid > water extract > petroleum ether extract > chloroform extract. These results suggest that the investigated sample extracts may prevent or retard the *in vitro* formation of radical species related with oxidative stress. The extensive investigations on antiradical and antioxidant activities of small phenolics, including flavonoids and phenolic acids have been reported [35]. Apart from these, Hagerman et al. [36]have reported that the high molecular weight phenolics (tannins) have more ability to quench free radicals (*ABTS•−*) and that effectiveness depends on the molecular weight, the number of aromatic rings and nature of hydroxyl groups substitution than the specific functional groups. In similar lines, the present study also reveals higher *ABTS•−* scavenging activity in the methanol extract of *I. tinctoria* that has registered the highest contents of tannins (Table 1). This is in agreement with the previous reports on the relationship between tannin content and antioxidant activity.
agreement with the reports of Bakasso et al. [13] that the higher ABTS⁺ scavenging activity of acetone extract of I. tinctoria aerial parts is due to its high phenolic content.

Metal ion chelating property

The chelating effects of different solvent extracts of I. tinctoria are shown in Table 3. The final reaction mixture contained 1 mg of extract sample and the values are expressed as mg EDTA/g extract equivalent. All the extracts demonstrated marked ability to chelate ions. Among the samples tested, ethyl acetate (52.63±4.80 mg EDTA/g extract), water (45.16±1.33 mg EDTA/g extract) and methanol (41.02±1.16 mg EDTA/g extract) extracts were found to have higher chelating activity than other samples. The extracts may be able to play a protective role against oxidative damage by sequestering iron (II) ions that may otherwise catalyze Fenton-type reactions or participate in metal-catalyzed hydroperoxide decomposition reactions [37]. The iron (II) chelating properties of the additives may be attributed to their endogenous chelating agents, mainly phenolics. Certain phenolic compounds have properly oriented functional groups, which can chelate metal ions. Chelating agents may serve as secondary antioxidants because they reduce the redox potential thereby stabilizing the oxidized form of the metal ion [38]. Accordingly it is suggested that the low to moderate ferrous ion chelating effects of the different solvent fractions of I. tinctoria would be somewhat beneficial to protect against oxidative damages.

Antihemolytic activity

Antihemolytic activity of different solvent extracts of I. tinctoria leaves is shown in Table 3. In this study, we used a biological test based on free radical-induced erythrocyte lysis in cow blood. Lipid peroxidation of cow blood erythrocyte membrane mediated by H₂O₂ induces membrane damage and subsequently hemolysis. Addition of different solvent extracts of I. tinctoria at the concentration of 500 µg in the final reaction mixture strongly inhibited free radical-induced red blood cell hemolysis. The percentage inhibiting activity of all the extract samples ranged between 61% and 71%. A similar observation of hemolysis inhibition have also been reported from the extracts of Camellia sinensis, Ficus bengalensis, Ficus racemosa and Melothria maderaspatana [31,39]. In the present study, higher antihemolytic potential was exhibited by ethyl acetate extract which might be attributed to the presence of high concentration of phenolic substances (Table 1). The phenolic compounds in the extracts may probably have an important role to participate in the biological pathways in the protection of radical-induced hemolysis.

Table 3: ABTS⁺ scavenging activity, metal chelating activity and antihemolytic activity of different solvent extracts of I. tinctoria leaves

<table>
<thead>
<tr>
<th>Sample</th>
<th>TAA (μmol g⁻¹ extract)</th>
<th>Metal chelating activity (mg EDTA/g extract)</th>
<th>Antihemolytic activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether extract</td>
<td>52.49 ± 1.15</td>
<td>39.76 ± 1.43</td>
<td>50.35 ± 9.3a</td>
</tr>
<tr>
<td>Benzene extract</td>
<td>10.34 ± 0.22</td>
<td>23.30 ± 4.84</td>
<td>64.50 ± 8.0b</td>
</tr>
<tr>
<td>Chloroform extract</td>
<td>20.92 ± 1.42</td>
<td>35.94 ± 1.44</td>
<td>68.27 ± 5.0a</td>
</tr>
<tr>
<td>Ethyl acetate extract</td>
<td>12.11 ± 0.36</td>
<td>52.63 ± 4.80</td>
<td>71.61 ± 5.55</td>
</tr>
<tr>
<td>Methanol extract</td>
<td>14.34 ± 0.29</td>
<td>41.01 ± 1.16</td>
<td>64.62 ± 6.57</td>
</tr>
<tr>
<td>Water extract</td>
<td>7.64 ± 1.94</td>
<td>45.16 ± 1.33</td>
<td>61.46 ± 10.8b</td>
</tr>
<tr>
<td>Quercetin</td>
<td>14.98 ± 4.2</td>
<td>67.6 ± 2.1</td>
<td>69.31 ± 3.49</td>
</tr>
<tr>
<td>Tannin acid</td>
<td>10.25 ± 4.30</td>
<td>61.5 ± 1.9</td>
<td>62.74 ± 4.6</td>
</tr>
</tbody>
</table>

* Total antioxidant activity (μmol Trolox equivalent ABTS⁺ scavenging activity/g extract)

Values are means of three independent analyses ± standard deviation (n = 3). Mean values followed by different superscript letters indicates significant statistical difference (P < 0.05).

Antioxidant activity in linoleic acid emulsion system

The antioxidant potential of different solvent extracts of I. tinctoria was evaluated employing linoleic acid peroxidation system (Figure 3). During the linoleic acid peroxidation, linoleic acid hydroperoxides, generated from the peroxidation of linoleic acid and subsequently decomposed to many secondary oxidation products, or the intermediate products may be converted to stable end products and the substrate was exhausted (Chen et al., 1996). Addition of different solvent extracts of I. tinctoria into the emulsion was able to reduce the formation of hydroperoxide. All the different solvent extracts showed inhibition ability that was comparable with the standard quercetin at 250 µg in the final reaction mixture. At a concentration of 500 µg in the final reaction mixture strongly inhibited free radical-induced red blood cell hemolysis. The percentage inhibiting activity of all the extract samples ranged between 61% and 71%. A similar observation of hemolysis inhibition have also been reported from the extracts of Camellia sinensis, Ficus bengalensis, Ficus racemosa and Melothria maderaspatana [31,39]. In the present study, higher antihemolytic potential was exhibited by ethyl acetate extract which might be attributed to the presence of high concentration of phenolic substances (Table 1). The phenolic compounds in the extracts may probably have an important role to participate in the biological pathways in the protection of radical-induced hemolysis.

Further investigations on the isolation and characterization of individual phenolic compounds will be required to elucidate their different antioxidant mechanism and existence of possible synergism among the compounds.

Fig. 3: Peroxidation inhibiting property of different solvent extracts of I. tinctoria leaves

Values are means of three independent analyses ± standard deviation (n = 3). Bars having different letters indicate significant statistical difference (P < 0.05). PE - petroleum ether extract, BE - benzene extract, CE - chloroform extract, EE - ethyl acetate extract, ME - methanol extract, WE - water extract, Q – quercetin.


