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Original Article

Free radical scavenging potential and HPTLC analysis of *Indigofera tinctoria* linn (Fabaceae)<sup>☆</sup>Sakthivel Srinivasan, Wankupar Wankhar, Sheeladevi Rathinasamy, Ravindran Rajan<sup>\*</sup>

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## ABSTRACT

The objective of this study was to evaluate the free radical scavenging potential and high performance thin layer chromatography (HPTLC) fingerprinting of *Indigofera tinctoria* (*I. tinctoria*). Phytochemical analysis was carried out using standard methods, and free radical scavenging activity of the plant was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH), nitric oxide (NO) and superoxide anion ( $O_2^-$ ) radical scavenging capacities. HPTLC plate was kept in CAMAG TLC Scanner 3 and the R<sub>f</sub> values at fingerprint data were recorded by WINCATS software. Aqueous extract of *I. tinctoria* reliably showed the total phenolics ( $267.2 \pm 2.42$  mg/g), flavonoids ( $75.43 \pm 3.36$  mg/g) and antioxidants ( $349.11 \pm 8.04$  mg/g). The extract was found to have DPPH (52.08%), NO (23.12%) and  $O_2^-$  (26.79%) scavenging activities at the concentration of 250  $\mu$ g/mL and the results were statistically significant compared with ascorbic acid standard ( $p < 0.05$ ). HPTLC results confirmed that the extract contained several potential active components such as phenols, flavonoids, saponins and terpenoids as the slides revealed multi-colored bands of varying intensities. This study confirmed that the plant had multipotential antioxidant and free radicals scavenging activities.

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## 1. Introduction

Oxidative damage is a critical etiological factor implicated in several chronic human diseases including cardiovascular dysfunction, inflammation, atherosclerosis, carcinogenesis, drug poisonousness, reperfusion damage and neurodegenerative diseases. Antioxidants play an important role in the prevention of chronic ailments such as heart diseases, cancer, diabetes, hypertension, stroke and Alzheimer's disease by protecting the cells from damage caused by free radicals, highly reactive oxygen compounds [1]. 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 [2]. Antioxidants play an important role in inhibiting and scavenging free radicals, thus providing protection against infections and degenerative diseases [3]. Plants contain several phytochemicals which possess strong antioxidant activities [4]. Medicinal herbs have a long history in improving human health and curing various diseases. India has an extensive rich heritage of herbal medicine since the time of Ayurveda with medicinal properties. Historically, the medicinal values of plants were tested by trial and error, as in the

Doctrine of Signatures [5]. Herbal medicines recently have attracted much attention as alternative medicines. Various medicinal properties have been attributed to natural herbs. Due to enormous propensities of plants which synthesize a variety of structurally various bioactive compounds, plant kingdom is a potential source of chemical constituents with antitumor and cytotoxic activities [6]. Plants have been found useful in medicine in three ways. First, they may be used directly as teas or in other extracted forms for their natural chemical constituents. Second, they may be used as agents in the synthesis of drugs. Third, the organic molecules found in plants may be used as models for synthetic drugs. Extraction of several active phytochemicals from green factories has given birth to some high activity profile drugs [7] and the use of traditional medicine is widely spread in India [8].

The plant *Indigofera tinctoria* (*I. tinctoria*) belongs to the family, Fabaceae, popularly known as Neeli in Tamil and found throughout India. *I. tinctoria* is a shrub, 1.2–1.8 m high and widely cultivated in many parts of the country. *I. tinctoria* is useful in the treatment of cancer, epilepsy, neuropathy, chronic bronchitis, asthma, ulcers and skin diseases, and is used as a hepatoprotective, diuretic and hair growth-promoting agent [9]. *I. tinctoria* leaves extract contains indirubin and indigotone, which were used in the treatment of hydrophobia [10]. Medicinal plants possess immunomodulatory and antioxidant activities [4]. *I. tinctoria* dry powder is used in the treatment of asthma [11]. Decoction of the leaves is used in bites

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and stings by venomous insects and reptiles to relieve the pain [12]. The present study was to evaluate the free radical scavenging activity and make a high performance thin layer chromatography (HPTLC) analysis of aqueous leaf extract of *I. tinctoria*.

## 2. Experimental

### 2.1. Drugs and chemicals

Gallic acid, quercetin, ascorbic acid, sodium nitroprusside, phenazonium methosulphate, reduced form of nicotinamide-adenine dinucleotide (NADH) and DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) were purchased from Sigma Aldrich, Co. (St. Louis, USA). All the other chemicals, which were obtained from Sisco Research Laboratory (Mumbai, India), were of analytical grade.

### 2.2. Collection and identification

The plant *I. tinctoria* was collected (May to November 2013) from the KSG Enterprises (Tindivanam, Tamil Nadu, India) and authenticated by Dr. D. Aravind (Department of Medical Botany, and National Institute of Siddha, Chennai, India). Voucher specimens were deposited at the Herbarium of National Institute of Siddha, Reg no: NIS/MB/83/2013. The collected plants were separated from unwanted materials and dried in shade. The leaves were ground to coarse powder with the help of a suitable grinder. The powder was then stored in an airtight container, kept in a cool, dark and dry place until the analysis.

### 2.3. Extraction procedure

A total of 30 g of the freshly powdered leaves were extracted with 250 mL of sterile distilled water using the Soxhlet apparatus at 100 °C. The aqueous extracts were filtered with Whatman No. 1 filter paper and then freeze dried and stored at 4 °C for further investigation. The extraction efficiency was quantified by determining the weight of the extract and the percentage yield was calculated to be 16%.

### 2.4. Determination of total phenolic content

Total phenolic content of the extract was determined by the Folin–Ciocalteu reagent method [13]. One milliliter of the plant extracts/standard solutions with different concentrations was mixed with 5 mL of Folin–Ciocalteu reagent (previously diluted with water (1:10, v/v)) and 4 mL of sodium carbonate (7.5%). The mixtures were vortexed for a few seconds and allowed to stand for 30 min at 20 °C for color development. Absorbance of samples and standard was measured at 765 nm using a spectrophotometer against blank. The total phenolic content of the plant extract was calculated as the gallic acid equivalent.

### 2.5. Determination of total flavonoid content

Total flavonoid content was determined by aluminum chloride method [14]. One milliliter of the plant extract was mixed with 3 mL of methanol, 0.2 mL of aluminum chloride, 0.2 mL of 1 M potassium acetate and 5.6 mL of distilled water. The mixture was placed at room temperature for 30 min, and the absorbance of the reaction mixture was measured at 415 nm with a spectrophotometer against blank. The total flavonoid content of the plant extract was calculated as the quercetin equivalent.

### 2.6. Total antioxidant capacity

The total antioxidant capacity of the plant extract was evaluated as per the method described by Prieto et al. [15]. The extract was dissolved in a mixture of 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) and incubated at 95 °C for 90 min. After cooled to ambient temperature, the absorbance of the solution was measured at 695 nm against reagent blank containing only the respective solvents. The total antioxidant content of the plant extract was calculated as the ascorbic acid equivalent.

### 2.7. DPPH free radical scavenging assay

The free radical scavenging capacity of the extract was determined using DPPH [16]. The mixture of DPPH (0.1 mM) in methanol was prepared and 4 mL of this solution was added to 1 mL of sample solution in methanol at different concentrations (50 to 250 µg/mL). Thirty minutes later, the absorbance was measured at 517 nm at room temperature using a spectrophotometer Shimadzu UV-1800. Lower absorbance of the reaction mixture indicates a higher free radical scavenging activity. Ascorbic acid was used as a standard.

$$\text{DPPH scavenged (\%)} = \frac{[\text{Abs control} - \text{Abs test}]/\text{Abs control}}{\text{Abs control}} \times 100\%$$

where Abs control is the absorbance of the control reaction, and Abs test is the absorbance of the extract/standard.

### 2.8. NO scavenging assay

The NO scavenging assay was carried out according to the method reported by Sreejayan et al. [17]. In this assay, 200 µL of 10 mM sodium nitroprusside was mixed with 50–250 µg of the plant extract in 1 mL of water and incubated at room temperature for 150 min. Following incubation, 500 µL of Griess reagent (1% sulfanilamide in 5% orthophosphoric acid) and 0.1% N-(1-naphthyl ethylenediaminedihydrochloride) in a ratio of 1:1 were added and incubated for 10 min at room temperature. Ascorbic acid was used as a standard. The absorbance was measured at 546 nm. Controls were run devoid of samples and the inhibition rate is calculated as follows:

$$\begin{aligned} \text{NO scavenging effect (Inhibition rate(\%))} \\ = \frac{\text{O.D (blank)} - \text{O.D (sample)}}{\text{O.D (blank)}} \times 100\% \end{aligned}$$

### 2.9. Superoxide anion scavenging assay

Superoxide anion scavenging was carried out according to the method described by Liu et al. [18]. In this method, superoxide radicals were generated in 3 mL of Tris HCl buffer (16 mM, pH 8.0) containing 1 mL of nitroblue tetrazolium (NBT; 50 µM) solution and 1 mL of nicotinamide adenine dihydrogen salts (NADH; 78 µM) solution and 50–250 µg of the plant extract in 1 mL of water. The reaction started by adding 1 mL of phenazonium methosulphate (PMS) solution (10 µM) to the mixture. The reaction mixture was incubated at 25 °C for 5 min and the absorbance at 560 nm was measured against blank samples. Decreased absorbance of the reaction mixture indicates increased superoxide anion scavenging activity.

$$\begin{aligned} \text{Superoxide anion scavenging effect (Inhibition rate(\%))} \\ = \frac{\text{O.D (blank)} - \text{O.D (sample)}}{\text{O.D (blank)}} \times 100\% \end{aligned}$$

### 2.10. Reducing power assessment

Reducing power was carried out using the method reported by Yildirim et al. [19]. One milliliter of the extract and its sub-fractions (final concentration 50–250 µg/mL) were mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide. The mixture was then incubated at 50 °C for 20 min. To this mixture, 2.5 mL of trichloroacetic acid was added, and centrifuged at 3000 rpm for 30 min. Finally, 2.5 mL of the supernatant solution was collected and mixed with 2.5 mL of distilled water and 0.5 mL of ferric chloride, and the absorbance was measured at 700 nm. Ascorbic acid was used as a standard and phosphate buffer as the blank solution.

### 2.11. HPTLC

For HPTLC fingerprinting analysis, 3, 6 and 9 µL of the plant samples were loaded in pre-coated HPTLC plates [silica gel 60F 254 (E. Merck KGaA) and plate size 5cm × 10 cm]. The samples loaded plate was kept in TLC twin trough a developing chamber (after saturated with solvent vapor) with respective mobile phases (flavonoids and phenols), solvent system [chloroform:methanol:formic acid:acetic acid (80:15:2.5:2.5, v/v/v/v)], solvent front position 50.0 mm and volume 10 mL. The developed plate was dried by hot air at 60 °C to evaporate solvents from the plate. The plate was kept in a photodocumentation chamber (CAMAG TLC Scanner 3) and the images were captured at UV 254 nm and UV 366 nm. The retention factor (Rf) values at fingerprint data were recorded by WINCATS software.

### 2.12. Statistical analysis

Statistical analysis was carried out in triplicates ( $n=3$ ) and standard error (SE) was calculated. All the data were analyzed using analysis of variance (ANOVA) with the statistical software Prism 5.0 version. The analyses were made with 95% confidence. The significance of differences ( $p < 0.05$ ) between mean values obtained from the experiments was determined by Tukey test.

## 3. Results

### 3.1. Total phenolic content

The total phenolic content of the aqueous extract of *I. tinctoria* was determined using the Folin-Ciocalteu reagent and expressed as gallic acid equivalent per gram of plant extract. The total phenolic content of the test fractions was calculated using the standard curve of gallic acid ( $y=0.454x+0.139$ ;  $R^2=0.0997$ ). Aqueous extract of *I. tinctoria* was found to have  $267.2 \pm 2.4$  mg/g phenolic content.

### 3.2. Total flavonoid content

Aluminum chloride colorimetric methods were used to determine the total flavonoid content of the aqueous extract of *I. tinctoria*. Total flavonoid content was calculated using the standard curve of quercetin ( $y=0.494x-0.038$ ;  $R^2=0.977$ ) and expressed as quercetin equivalent per gram of the plant extract. Aqueous extract of *I. tinctoria* was found to have  $75.43 \pm 3.3$  mg/g flavonoid.

### 3.3. Total antioxidant capacity

The total antioxidant capacity of aqueous extract of *I. tinctoria* was evaluated by the phosphomolybdenum method and expressed as ascorbic acid equivalent per gram of plant extract. The

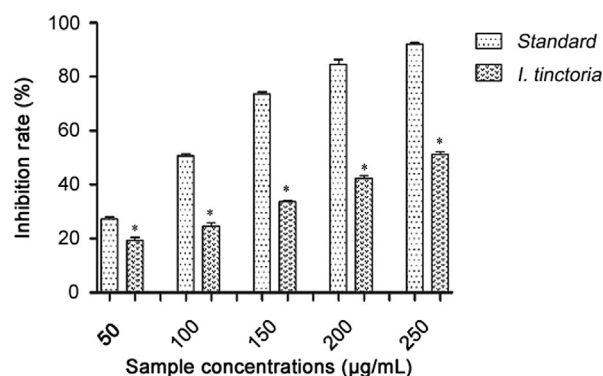


Fig. 1. DPPH scavenging activities of *Indigofera tinctoria* extract and ascorbic acid. \* $p < 0.05$ .

total antioxidant content of the test samples was calculated using the standard curve of ascorbic acid ( $y=0.0014x+0.0114$ ;  $R^2=0.9926$ ), and found to be  $349.11 \pm 8.0$  mg/g.

### 3.4. DPPH free radical scavenging activities

The free radical scavenging activities of aqueous extract of *I. tinctoria* were studied by its ability to reduce DPPH, a stable free radical; and any molecule that can donate an electron or hydrogen to DPPH can react with it and thereby bleach DPPH absorption. DPPH is a purple dye with absorption maxima at 517 nm, and upon reaction with a hydrogen donor the purple color fades or disappears due to conversion of it to 2,2-diphenyl-1-picryl hydrazine, resulting in decrease in absorbance. The DPPH scavenging effect was found to increase with increased concentrations. At 250 µg/mL, aqueous extracts showed 51.08% inhibition and ascorbic acid showed 92.02% inhibition (Fig. 1). IC<sub>50</sub> values were calculated and expressed (Table 1).

### 3.5. NO radical scavenging activities

The extents of NO radical scavenging of *I. tinctoria* aqueous extract at concentrations (50–250 µg/mL) were measured, with ascorbic acid as a standard. The radical scavenging effect was found to increase with increased concentrations. At 250 µg/mL, aqueous extract showed 23.12% inhibition and ascorbic acid showed 91.66% inhibition (Fig. 2).

### 3.6. Superoxide anion scavenging activities

The extents of superoxide anion scavenging of *I. tinctoria* aqueous extract at concentrations (50–250 µg/mL) were measured, with ascorbic acid as a standard. The radical scavenging effect was found to increase with increased concentrations. At 250 µg/mL, aqueous extract showed 26.79% inhibition and ascorbic acid showed 89.04% inhibition (Fig. 3).

Table 1  
IC<sub>50</sub> values of aqueous extracts of *Indigofera tinctoria* in DPPH, nitric oxide and superoxide anion scavenging assay.

Extract	IC <sub>50</sub> (µg/mL)		
	DPPH	NO	O <sub>2</sub>
Aqueous extract	244.71	540.65	466.59
Ascorbic acid	135.14	136.37	140.38

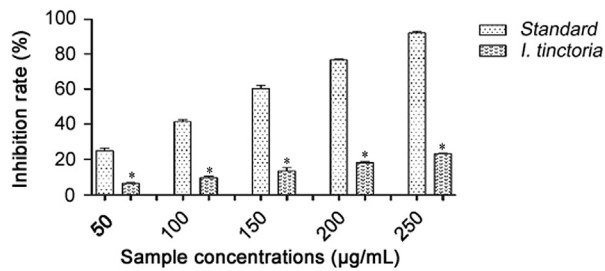


Fig. 2. Nitric oxide scavenging activities of *Indigofera tinctoria* extract and ascorbic acid. \* $p < 0.05$ .

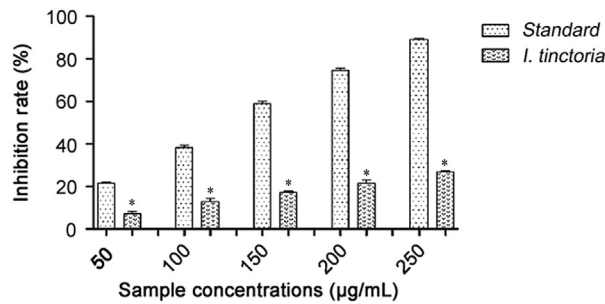


Fig. 3. Superoxide anion scavenging activities of *Indigofera tinctoria* of extract and ascorbic acid. \* $p < 0.05$ .

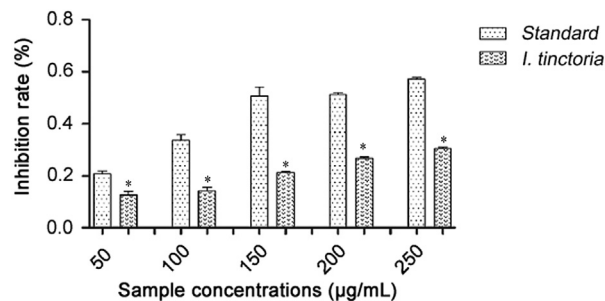


Fig. 4. Comparison of reducing power of *Indigofera tinctoria* extract and ascorbic acid. \* $p < 0.05$ .

### 3.7. Reducing power

Reducing power of the fractions can be assessed by using ferric to ferrous reducing activity as determined spectrophotometrically from the formation of Perl's prussian blue color complex. In the presence of antioxidants in the sample, the absorbance was measured at 700 nm. The reducing power of aqueous extract

(0.305) and ascorbic acid (0.572) at 250 µg/mL is shown in Fig. 4. Higher absorbance indicates more reducing power.

### 3.8. HPTLC

Various solvent compositions of the mobile phase for HPTLC analysis were examined in order to achieve high resolution and reproducible peaks. Chromatogram was developed in *I. tinctoria* aqueous extract under chamber saturation conditions using chloroform:methanol:formic acid:acetic acid (80:15:2.5:2.5, v/v/v/v) as the mobile phase or solvent system. Aqueous extract of *I. tinctoria* was subjected to chromatographic fingerprinting. After chromatographic deprivation with anisaldehyde  $H_2SO_4$ , *I. tinctoria* aqueous extract at different concentrations displayed the chromatogram in 3D at 254 nm (Fig. 5). The HPTLC fingerprinting of *I. tinctoria* revealed several peaks, 16 spots at 3 µL and 6 µL of plant samples (Fig. 6A and B) and 17 spots at 9 µL of plant samples (Fig. 6C). There were 17 polyvalent phytoconstituents and the corresponding ascending order of Rf values ranged from 0.03 to 1.02 (Table 2). The highest area (%) of the phytoconstituents was found to be 19.60% and its corresponding Rf value was 0.53. Exposure of the spotted and developed HPTLC plate to UV 254 nm showed the presence of numerous organic compounds as dark and light bands in a green background (Fig. 7A). HPTLC slides developed at UV 366 nm exposure revealed multi-colored bands with varying intensities (Fig. 7B). It corresponded to several polar and non-polar compounds, which showed light and dark blue, light yellow, bluish purple and fluorescent sky blue color bands, respectively. In the present study, dark blue color bands were observed, which confirmed the presence of phenolic compounds in the plant extract. Light yellow zones were detected from the chromatogram after derivatization, which confirmed the presence of flavanoids and saponins. Bluish purple color indicated the presence of terpenoids.

## 4. Discussion

In the present study, the total phenolic and flavonoid contents of aqueous extract of *I. tinctoria* leaves were estimated. The total phenolic content of aqueous extract was  $267.6 \pm 2.4$  mg/g extract. The result reported by Nagarajan et al. [2] was in agreement that the total phenolic and flavonoid contents of different solvent extracts of *I. tinctoria* leaves were maximum in water extract. The higher content of total phenolic in the highly polar solvent (ethyl acetate, methanol and water) extract of *I. tinctoria* might contribute to the presence of furano-flavones, flavonol glycosides and

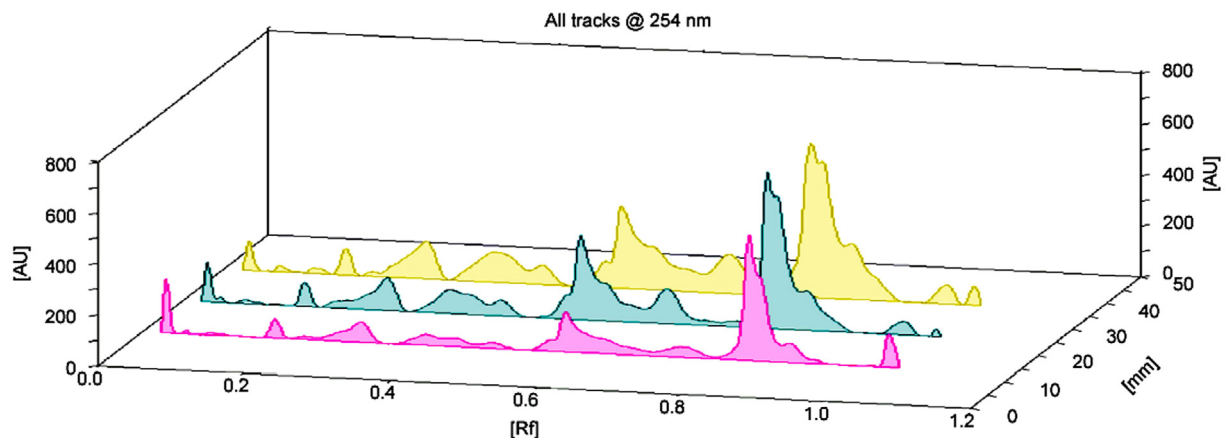


Fig. 5. 3D Chromatogram of *Indigofera tinctoria* extract at different concentrations at 254 nm.



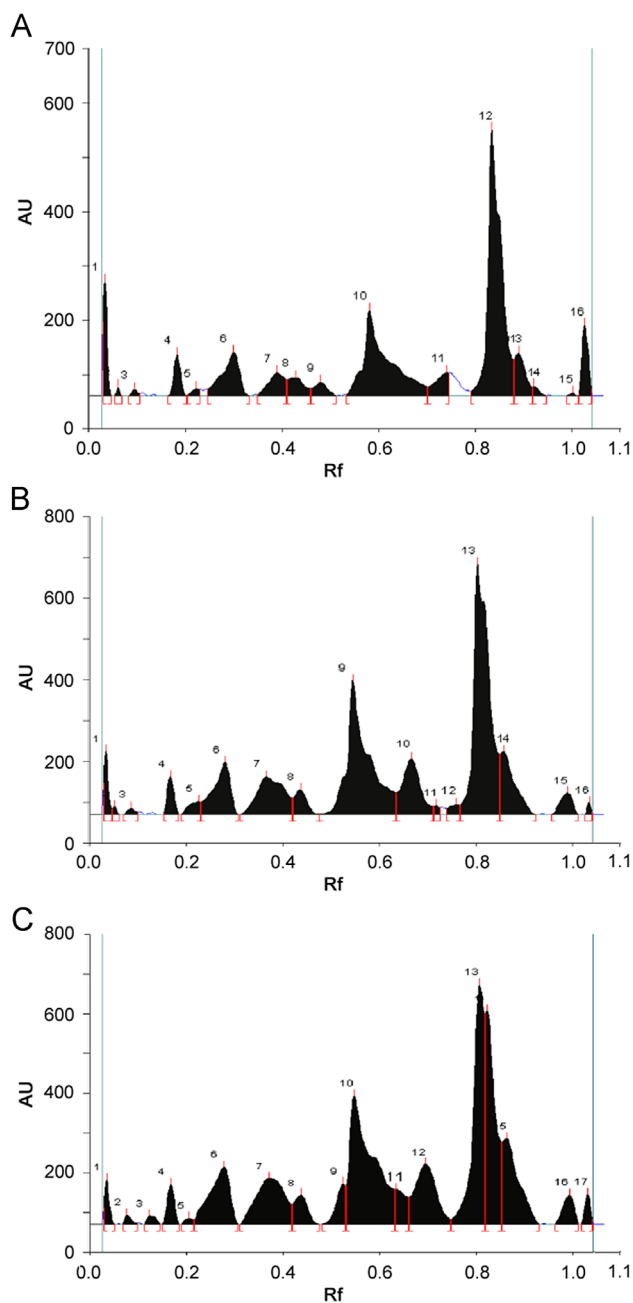


Fig. 6. HPTLC chromatogram of aqueous extract of *Indigofera tinctoria*.

high molecular tannins [20]. Flavonoid is also known to have a wide array of therapeutic activities as antihypertensive, anti-rheumatism, antimicrobial, diuretic, antioxidative [21] and chemopreventive agents [22]. Phenolic compounds are well known as antioxidative and scavenging agents against oxidative damage associated with free radicals [23].

The aqueous extract of *I. tinctoria* plant exhibited some degree of reducing power. The reducing power increased with an increasing sample concentration. The proton-radical scavenging action has been known as an important mechanism of anti-oxidation. DPPH radical is a stable organic free radical which has been extensively used for evaluating the free radical scavenging potential of natural antioxidants. Aqueous extract of *I. tinctoria* showed  $IC_{50}$  of 244.71  $\mu\text{g/mL}$  and ascorbic acid showed  $IC_{50}$  of 135.14  $\mu\text{g/mL}$ . The aqueous extract could scavenge more DPPH radicals as compared with methanolic extract [24]. Nagarajan et al. [2] reported free radical scavenging potential of different solvent

extracts of *I. tinctoria* leaves. Bakasso et al. [1] reported that *I. tinctoria* showed a good antioxidant activity. The DPPH scavenging activity can also be attributed to the electron transfer or the hydrogen donating nature of the polyphenols. Free radical scavenging is the accepted mechanism for antioxidants' inhibiting lipid oxidation [25].

The nitric oxide scavenging assay is based on the principle that sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. In the present study, aqueous extract of *I. tinctoria* showed NO scavenging activity with  $IC_{50}$  of 540.65  $\mu\text{g/mL}$  and ascorbic acid showed  $IC_{50}$  of 136.37  $\mu\text{g/mL}$ . The nitric acid scavenging activity of aqueous plant extract against NO formation was comparable to that of the standard drugs [26]. The aqueous extract of *I. tinctoria* leaves had greater NO scavenging activity. The polyphenolic compounds in *I. tinctoria* might be responsible for the observed scavenging activity. NO scavenging capacities of flavonoid and phenolic compounds are already well known [2].

Aqueous extract of *I. tinctoria* had superoxide anion scavenging activity with  $IC_{50}$  of 466.59  $\mu\text{g/mL}$  and ascorbic acid showed  $IC_{50}$  of 140.38 (Table 1). This statement proved Manju's [24] result that aqueous extract had the superoxide radical scavenging ability. Superoxide anion radical is one of the strongest ROSs among the free radicals and becomes converted to other harmful reactive oxygen species such as hydrogen peroxide and hydroxyl radical, damaging biomolecules which result in chronic diseases [27]. Antioxidants are substances that delay the oxidation process by inhibiting the polymerization chain initiated by free radicals and other subsequent oxidizing reactions [28].

The reducing power of aqueous extract of *I. tinctoria* showed significant changes compared with ascorbic acid (Fig. 4). The aqueous extract of *I. tinctoria* had a free radical scavenging potential and its reducing power was increased with increased concentration [2].

In the present study, phytochemical screening results of *I. tinctoria* leaves revealed the presence of phenols, flavonoids, saponins and terpenoids in aqueous extract, which was confirmed by performing HPTLC separation technique with  $\text{H}_2\text{SO}_4$  spraying reagents. HPTLC showed clear separation of components in the aqueous extract of *I. tinctoria*. This method was applied to identify the plant of *I. tinctoria* from other species. HPTLC fingerprint enables a particular plant to be identified and distinguished from closely related species [29]. The presence or absence of chemical constituent may be useful for identifying the taxonomy of the plant. The Rf values of aqueous extract of *I. tinctoria* were 0.03–1.02 in the absorbance of 254 nm and 366 nm. The highest area (%) of the phytoconstituents was found to be 19.60% and its corresponding Rf value was 0.53 (Table 2). It might be an indigo compound. These results are supported by Francesca et al. [30] who reported that *I. tinctoria* plant had henna commercial product collected from different places and Rf value was 0.55. The developed HPTLC at UV 366 nm exposure of slides revealed multi-colored bands with varying intensities (Fig. 7B). HPTLC developed plates at 366 nm exposure display light and dark blue, light yellow, bluish purple and fluorescent sky blue color bands. HPTLC fingerprint analysis of aqueous extract of *I. tinctoria* leaves revealed the presence of phenols, flavonoids, saponins and terpenoids. It corresponds to several polar and non-polar compounds. *I. tinctoria* methanol extract contained flavonoids, saponins, tannins, steroidal terpenes, phenols and anthroquinonesence [31]. Blue and light brown color zone detected in UV after derivatization in the chromatogram confirmed the presence of polyphenols [32]. Light yellow colored fluorescent zone peak observed in the chromatogram after derivatization confirmed the presence of flavonoid and saponin [31]. Blue-violet colored zones were detected from the

**Table 2**  
Rf values of the peak and peak height.

Peak	Start Rf.	Start height	Max Rf.	Max (%)	End Rf.	Max height	End height	Area	Area (%)
1	0.03	41.7	0.04	4.05	0.05	112.6	0.05	937.9	1.36
2	0.07	1.2	0.08	0.87	0.10	24.2	0.10	275.2	0.40
3	0.11	0.6	0.12	0.78	0.15	21.6	0.15	349.9	0.51
4	0.15	1.1	0.17	3.67	0.19	102.3	0.19	1305.2	1.89
5	0.19	0.0	0.21	0.57	0.22	15.8	0.22	230.6	0.33
6	0.22	12.7	0.28	5.21	0.31	145.1	0.31	5199.4	7.53
7	0.31	0.1	0.37	4.20	0.42	117.0	0.42	6022.5	8.73
8	0.42	50.4	0.44	2.69	0.48	75.0	0.48	1781.7	2.58
9	0.48	0.2	0.53	3.69	0.53	102.7	0.53	1704.3	2.47
10	0.53	99.2	0.55	11.59	0.63	322.9	0.63	13526.3	19.60
11	0.63	88.4	0.63	3.19	0.66	88.7	0.66	1739.2	2.52
12	0.66	69.2	0.70	5.46	0.75	151.9	0.75	5806.2	8.41
13	0.75	13.6	0.81	21.65	0.82	602.9	0.82	12082.9	17.51
14	0.82	530.8	0.82	19.28	0.85	537.0	0.85	9466.5	13.72
15	0.85	207.3	0.86	7.76	0.93	216.2	0.93	6296.7	9.12
16	0.96	0.6	0.99	2.63	1.01	73.2	1.01	1505.3	2.18
17	1.02	1.02	1.03	2.71	1.04	75.6	1.04	781.7	1.13

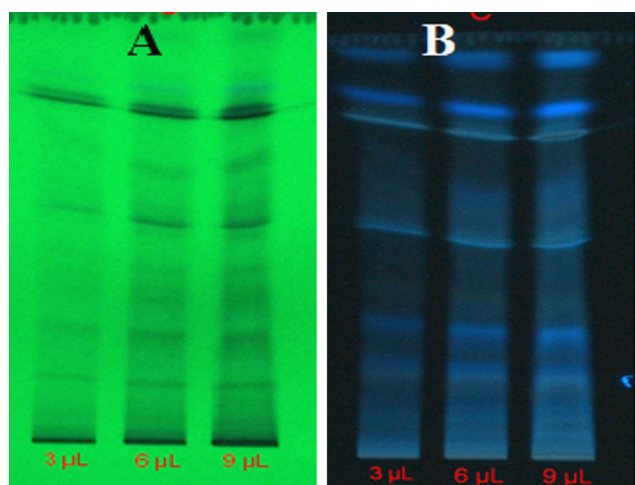


Fig. 7. Photo-documentation of aqueous extract of *Indigofera tinctoria*.

chromatogram after derivatization, which confirmed the presence of flavanoids [32]. Purple and bluish purple observed in the chromatogram after derivatization confirmed the presence of terpenoids [33]. More components were identified from aqueous extract of *I. tinctoria* [34]. The results obtained in this study suggest that the identified phytochemical compounds might be the bioactive constituents responsible for the efficacy of the leaves of the plants studied. Plant extract could be a source for the industrial manufacture of drugs useful in the chemotherapy of some microbial infections.

## 5. Conclusion

In the present study, aqueous extract of *I. tinctoria* showed free radical scavenging activity on NO, O<sub>2</sub><sup>-</sup> and DPPH models. The preliminary study showed the presence of phenols and flavonoids, indicating its potent antioxidant activity, and HPTLC analysis confirmed the presence of phenols, flavonoids, saponins and terpenoids. This investigation on *I. tinctoria* could serve as a primary basis for further pharmacological and drug designing studies.

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