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ASSESSING THE CHELATING ABILITY OF *AERVA LANATA*: ADSORPTION OF CHROMIUM FROM TANNERY EFFLUENT AND ITS TOXICITY MEASUREMENT

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The chelating ability of aqueous leaf extract of Aerva lanata was assessed in vitro. The aqueous leaf extract showed a dose dependent decrease in chelating ability using $FeCl_2$. The highest chelating ability of aqueous leaf extract was observed at $2 \cdot 10^{-5}$ g/mL (100 ± 0.00). The antioxidant activity of the aqueous leaf extract ranged from 42.13% to 88.66%. At $2 \cdot 10^{-5}$ g/mL concentration, a strong positive significant correlation was observed between chelating ability and total phenolics concentration (R = 0.94; P = 0.001). The chelating ability of aqueous leaf extract ($2 \cdot 10^{-5}$ g/mL) showed a high positive significant correlation with antioxidant activity (R = 0.78; P = 0.001). Aqueous leaf extract removed the chromium from tannery effluent by 43 mg/g. Allium cepa toxicity test was performed on tannery effluent treated with aqueous leaf extract that increased the root length of onion.

Keywords: *Aerva lanata*, chelating ability, chromium, resin, toxicity assessment.

Introduction

A wide variety of industries such as petroleum refining, chemical manufacturing, metal finishing and circuit manufacturing create waste water streams that are polluted with heavy metals. Recent federal amendments to the clean water act limit the metal concentrations that can be discharged from these industries [1]. Therefore, there is a need to develop cost-effective and environmental friendly processes to recover the heavy metals from waste water streams.

The environmental impact and building up of heavy metals in flora and fauna at soil ecosystem has been a cause of great concern in recent years. Chromium is introduced in ecosystem as a result of different industrial activities such as iron and steel manufacturing, tannery, chromium plating and other anthropogenic sources [2]. In particular, Cr(III) is widely used in tannery industries in Tamil Nadu. Heavy metals are usually removed from aqueous waste streams by chemical precipitation and electrical deposition [3 – 5]. The

physical-chemical methods for removing Cr(VI) from industrial wastewater are available. Cost effective and more environmental friendly techniques need to be developed to remove Cr(VI) ions in addition to already existing techniques such as adsorption on activated carbon, precipitation under alkaline conditions, ion exchange and reverse osmosis [6]. Bio-treatment technologies have been studied based on the biosorption (heavy metal removal capacity) features of biological materials such as filamentous fungi, bacteria, leavenings, seaweed and biomasses due to some functional group in the cell surface [7 – 9]. The phytoremediation by chelating property has become an alternative method to remove heavy metal and other pollutants from efluent [10].

Aerva lanata is a woody, prostrate or succulent, perennial herb in the Amaranthaceae family of the genus *Aerva* and it is a common weed which grows wild everywhere in plains of India. The root has camphor like aroma. The dried flowers which look like soft spikes are sold under the commercial name as Buikallan or Boor. Decoction of the flowers is said to cure stones in any part of the stomach and that of the root is diuretic and cure for kidney stones [11].

This study was carried out to evaluate (i) the chelating ability of aqueous leaf extract of *Aerva lanata* against Fe^{2+} and (ii) to investigate the Cr(VI) removal using aqueous leaf extract from tannery effluent generated from industry.

Experimental

Aerva lanata was collected from in and around areas of Salem, Tamil Nadu, India. The leaves were shade dried and powdered with electric grinder. Aqueous leaf extract of the leaf sample was prepared as follows; 1 g of *Aerva lanata* leaf powder was dissolved in 100 ml of distilled water (1% aqueous leaf extract [w/v]). The mixture was heated at 100 °C and concentrated 3 fold. The filtrate was stored in a refrigerator and then used for analysis of interested parameters. The aqueous leaf extract filtrate was then kept in vaccum dryer at 25 °C.

According to the method of Minotti and Aust [12] the chelating ability of plant extract was determined. The filtrate of various volumes ($1 \cdot 10^{-5}$, $1.5 \cdot 10^{-5}$, $2 \cdot 10^{-5}$, $2.5 \cdot 10^{-5}$ and $3 \cdot 10^{-5}$ g/mL) was mixed with 0.1 ml of 0.2 M $FeCl_2$ and 0.2 ml of 5 mM ferrozine solution. After reaction for 10 min, the absorbance of the solution was read at 562 nm. A 1 cm path length quartz cuvette was used for the UV Vis spectrophotometric assay. A complex of Fe^{2+}/ferrozine has a strong absorbance at 562 nm. Catechol was used as a standard. The higher the ferrous ion chelating ability of sample, lower absorbance obtained:

$$CA = \left(\frac{A_{control} - A_{sample}}{A_{control}}\right)100,$$

where CA – chelating ability expressed in percent, %.

According to the method of Blois [13], antioxidant activity of the filtrate was determined. Various concentrations of filtrate ($1{\cdot}10^{-5} - 3{\cdot}10^{-5}$ g/mL) were added to 100 μM 2, 2-diphenyl-1-picrylhydrazyl (DPPH) in methanol. The reaction mixture was shaken vigorously and incubated at 37 °C for 30 min in dark. The absorbance of the solution was read at 517 nm on a UV Vis spectrophotometer (Perkin Elmer-Lambda 650). A 1 cm pathlength quartz cuvette was used for the UV Vis spectrophotometric assay. Catechol was used as standard reference antioxidant. Antioxidant activity (AA,%) was expressed in terms of inhibition of DPPH free radical in percent and was calculated by the formula

$$AA = \left(\frac{A_{control} - A_{sample}}{A_{control}}\right)100$$

Total phenolic content of filtrate from *Aerva lanata* leaf was determined using catechol as standard ($1{\cdot}10^{-5} - 3{\cdot}10^{-5}$ g/mL) as described by Slinkard and Singleton [14] with some minor modifications. Various volumes of filtrate or standard (Catechol) was mixed with 2.0 ml of 2% (w/v) Na_2CO_3 vortexed vigorously and after 3 min mixed with 0.1 ml of (1N) Folin- ciocalteu's phenol reagent. The mixture was incubated at room temperature for 30 min and the absorbance was measured at 685 nm using UV Vis spectrophotometer.

Cr(VI) standard chromium solution was prepared by dissolving 50 mg/L from calculated amount of $K_2Cr_2O_7$ salt in deionized water. Chromium tannery effluent was used as the test sample. The effluent contains chromium sulphate (33% basic) in trivalent state. In order to convert it to hexavalent, hydrogen peroxide was added to oxidize trivalent chromium to hexavalent chromium by keeping it in a hot plate until all hydrogen peroxide gets evaporated and filtered through whatman No1 filter paper. Serial dilutions of tannery effluent (90%) with chromium concentration of 50 mg/L were prepared. 50 ml of chromium tannery effluent was treated with aqueous leaf extract in the same manner as standard solutions. The mixture was shaken in a thermostatic shaker at a speed of 200 rpm at room temperature for 1 h and the filtrate was analysed for chromium. A 0.25% solution of diphenylcarbazide in 50% acetone was prepared. To the different concentrations of the standard solution, 1 ml of

0.25% diphenylcarbazide was added for complexation. The final activity of the test solution (0.4 N) was made with sulfuric acid. Absorbances were recorded at 540 nm using UV Vis spectrophotometer [15].

The adsorption experiments were carried out using 50 ml of tannery effluent containing initial chromium concentration (50 mg/L) at pH 4. The mixture was shaken in a thermostatic shaker at a speed of 200 rpm at room temperature and the filtrate was analysed for chromium. The influence of different contact time (15, 30, 45, 60, 75 and 90 min) and pH (3, 5, 7, 9 and 11) on the removal of chromium was investigated.

The chromium removed effluent was used for toxicity study. Equal sizes of onions were obtained from local vegetable market, Salem, Tamil Nadu, India. *Allium cepa* was exposed for 96 h to chromium effluent treated with aqueous leaf extract. The base of the onion bulbs was grown on plant extract treated chromium effluent (50 mg/L) in the 15 ml boiling test tubes after which the number of roots and its length were measured [16].

Student's t-test was used for statistical analysis. Pearson correlation values less than or equal to 0.1, 0.05, 0.01 or 0.001 were considered as significant.

Results and discussion

The chelating ability of the aqueous leaf extract of *Aerva lanata* shows a dose-dependent inhibition with ferrous ion generated in vitro using $FeCl_2$ as Fe^{2+} donor. The chelating ability values were 67.40 ± 10.34, 88.60 ± 6.36, 100 ± 0.00, 100 ± 0.00, 100 ± 0.00%, respectively for various concentrations of aqueous leaf extract examined ($1 \cdot 10^{-5}$, $1.5 \cdot 10^{-5}$, $2 \cdot 10^{-5}$, $2.5 \cdot 10^{-5}$ and $3 \cdot 10^{-5}$ g/mL). The aqueous leaf extract has the highest chelating ability at $2 \cdot 10^{-5}$ g/mL itself, with 100% loss in Fe^{2+} activity, when compared with a standard phenolic compound (catechol) which has the highest chelating ability at $2.5 \cdot 10^{-5}$ g/mL (84.25%) shown in Table 1, 2.

The observation shows the chelating ability of filtrate of *Aerva lanata* leaf extract. Chelating ability of plant extract provides a strategy to avoid free-radical generation and iron-overload by chelation of metal ion. Phenol is a very important constituent present in medicinal plants and it can scavenge a wide range of reactive oxygen and nitrogen species owing to their hydroxyl groups [17]. These phytochemicals act as free radical terminators and it play critical role in chelating iron like transition metal ions [18]. The chromium rich effluent from tanneries cause various environmental and health problems to people who are residing in and around the tannery industries. Our results

prompted us to remove the chromium from effluent using aqueous leaf extract of *Aerva lanata*.

Table 1. Changes in the levels of chelating ability, antioxidant activity and total phenolic content of aqueous leaf extract of Aerva lanata

Concentration of aqueous leafextract (g/mL)	Chelating ability (%)	Antioxidant activity (%)	Total phenolic content (μg/mL)
$1 \cdot 10^{-5}$	67.40 ± 10.34	42.13 ± 9.99	1500 ± 300.00
$1.5 \cdot 10^{-5}$	88.60 ± 6.36	71.29 ± 5.02	2106 ± 415.11
$2 \cdot 10^{-5}$	100.00 ± 0.00	84.96 ± 1.56	2034 ± 932.50
$2.5 \cdot 10^{-5}$	100.00 ± 0.00	87.90 ± 1.56	2941 ± 569.00
$3 \cdot 10^{-5}$	100.00 ± 0.00	88.66 ± 1.56	2992 ± 321.23

Note.Values are mean ± S.D of 5 analyses per concentration.

Table 2. Changes in the levels of chelating ability, antioxidant activity and total phenolic content of catechol

Concentration of catechol (g/mL)	Chelating ability (%)	Antioxidant activity (%)	Total phenolic content (μg/mL)
$1 \cdot 10^{-5}$	72.85 ± 4.56	54.00 ± 5.37	456.00 ± 19.77
$1 \cdot 5.10^{-5}$	76.88 ± 6.36	70.20 ± 1.16	545.00 ± 21.98
$2 \cdot 10^{-5}$	79.14 ± 3.45	75.34 ± 1.23	748.20 ± 12.33
$2.5 \cdot 10^{-5}$	84.25 ± 4.98	79.09 ± 1.44	907.00 ± 6.77
$3 \cdot 10^{-5}$	85.00 ± 5.01	82.45 ± 1.97	1122.00 ± 1.57

Note. Values are mean ± S.D of 5 analyses per concentration.

The chelating and total phenolic content of aqueous leaf extract of *Aerva lanata* was found to be higher than that of catechol. The value of chelating ability and total phenolic content was 100% and $3.034 \cdot 10^{-3}$ g/mL respectively for *Aerva lanata* and 84.25% and $1.122 \cdot 10^{-3}$ g/mL for catechol. The antioxidant activity of aqueous leaf extract was dose independent. $3 \cdot 10^{-5}$ g/mL content of aqueous leaf extract has the highest antioxidant activity with 88.66% which was higher than the antioxidant activity of catechol (79.09% at $2 \cdot 10^{-3}$ g/mL). The

increasing order of antioxidant activity at different concentrations of aqueous leaf extract and standard are shown in Table 1.

In recent years, natural products have been the most successful source of new biodegradation agent [19]. Antioxidant activity of many plants is useful in unravelling its bioactivity [20]. Polyphenols are the major rich plant compounds with antioxidant activity, which can play an important role in adsorbing and neutralizing free radicals, quenching of singlet and triplet oxygen or decomposing peroxides [21]. DPPH, a stable free radical with a characteristic absorption at 517 nm, was used to study the radical scavenging effects of extract. The mechanism for DPPH scavenging activity was provided by the proton-donating ability of chemical components in the extract, while the inhibitory effect on metals could have come from synergistic mechanisms, including chelation of metal catalysts, scavenging of initiating radicals, chain breaking reactions and reductions in the concentration of reactive oxygen species [22]. The position and degree for hydroxylation of phenolic compounds especially β-ring also play a major role in antioxidant activity [23].

A strong positive significant correlation was observed between the chelating ability and total phenolic content of aqueous leaf extract at $2 \cdot 10^{-5}$ g/mL (R = 0.94; P =0.001) as shown in Table 3.

Table 3. Pearson correlations between chelating ability and total phenolic content of Aerva lanata aqueous leaf extract

Concentration of aqueous leaf extract (g/mL)	Correlation (R)	T value	P(0.1)	P(0.5)	P(0.01)	P(0.001)
$1 \cdot 10^{-5}$	0.41	1.26	NS	NS	NS	NS
$1.5 \cdot 10^{-5}$	0.56	0.78	NS	NS	NS	NS
$2 \cdot 10^{-5}$	0.94	7.87	SG	SG	SG	SG
$2.5 \cdot 10^{-5}$	0.28	2.44	SG	SG	NS	NS
$3 \cdot 10^{-5}$	0.35	2.97	SG	SG	NS	NS

Note. SG – Significant, NS – Non significant.

The correlation between antioxidant activity and the total phenolic content showed negative correlations at all concentrations of the aqueous leaf extract (For $1 \cdot 10^{-5}$ g/mL concentration, R = – 0.24, P = 0.1, not significant and weak; for $2 \cdot 10^{-5}$ g/mL concentration, R = 0.01, P = 0.01,

high significant correlation) (Table 4). Table 5 shows a high spositive high significant correlation was observed between the chelating ability and antioxidant activity of aqueous leaf extract at $2 \cdot 10^{-5}$ g/mL (R = 0.78; P = 0.001).

The removal of chromium from effluent using *Aerva lanata* filtrate was performed at room temperature for 1 h. Table 6 shows aqueous leaf extract removel of chromium from tannery effluent by 42 mg/g when compared to the control. The chromium (VI) removed (50%) from soil in presence and absence of compost amendment using Pterocarpus indicus and Jatropha curcas [24]. The chromium was removed effectively from tannery effluent in our study. To our knowledge this is the first report for demonstrating the ability of aqueous leaf extract of *Aerva lanata* to remove chromium.

Table 4. Pearson correlations between antioxidant activity and total phenolic content of Aerva lanata aqueous leaf extract

Concentration of aqueous leaf extract (g/mL)	Correlation (R)	T value	P(0.1)	P(0.05)	P(0.01)	P(0.001)
$1 \cdot 10^{-5}$	−0.24	1.22	NS	NS	NS	NS
$1.5 \cdot 10^{-5}$	−0.98	1.68	NS	NS	NS	NS
$2 \cdot 10^{-5}$	−0.01	5.87	SG	SG	SG	NS
$2.5 \cdot 10^{-5}$	−0.35	7.56	SG	SG	NS	NS
$3 \cdot 10^{-5}$	−0.56	1.90	SG	NS	NS	NS

Table 5. Pearson correlations between chelating ability and antioxidant activity of Aerva lanata aqueous leaf extract

Concentration of aqueous leaf extract (g/mL)	Correlation (R)	T value	P(0.1)	P(0.05)	P(0.01)	P(0.001)
$1 \cdot 10^{-5}$	−0.45	2.06	NS	NS	NS	NS
$1.5 \cdot 10^{-5}$	−0.56	1.72	NS	NS	NS	NS
$2 \cdot 10^{-5}$	0.78	7.12	SG	SG	SG	SG
$2.5 \cdot 10^{-5}$	0.44	8.23	SG	SG	SG	NS
$3 \cdot 10^{-5}$	−0.40	3.99	SG	SG	NS	NS

Table 6. Removal of chromium from tannery effluent using Aerva lanata aqueous leaf extract

Types of tannery effluent	Cromium removal (mg/g)
Tannery effluent (Control)	50
Aqueous leaf extract treated tannery effluent (Test)	42

Cr(VI) can exist in several forms such as $Cr_2O_7^{2-}$, $HCrO_4^-$, $HCr_2O_7^-$ and CrO_4^{2-} and the relative abundance of particular complex depends on the concentration of the chromium ion and pH of the solution [25]. The removal of Cr(VI) on aqueous leaf extract has been investigated for function of pH in the range of 3 to 11 with 50 mg/L as initial chromium concentration at room temperature. Therefore the chromium removal by aqueous leaf extract was studied at five different initial pH levels viz., 3, 5, 7, 9 and 11 by keeping all other parameters like contact time, dose and chromium concentration from tannery effluent as constant. The pH of the working solution was controlled by adding HCl/NaOH solution. Fig. 1 shows the chromium removal capacity of aqueous leaf extract as a function of pH. The effect of pH on the removal of Cr(VI) by aqueous plant extract was higher at low pH levels and decreases with increase in solution pH.

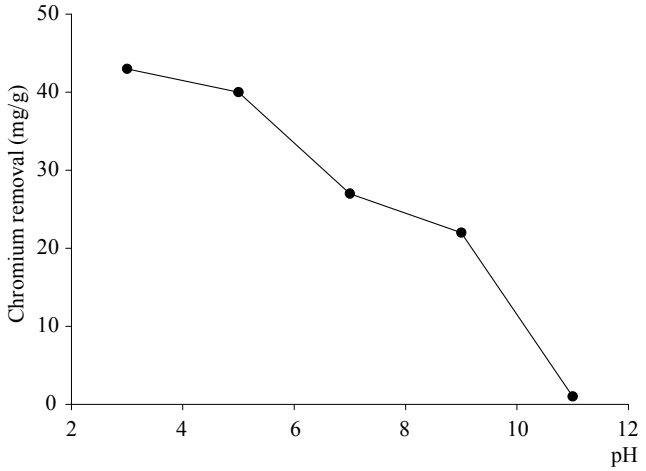

Fig. 1. Chromium removal from tannery effluent on influence of pH at 60 min.

The reason may be at lower pH levels, plant extract resin has acquired positive charge due to the exothermic reactions of phenolic groups and the chromium has been adsorbed through electrostatic attraction which may be reduced into Cr(III) and complexed by the aqueous plant extract [26, 27] which results in an increased adsorption at low pH ranges.

The root length of *Allium cepa* was measured in the presence of chromium effluent treated with aqueous leaf extract (Fig. 2).

There was no root growth of *Aerva lanata* observed in the chromium tannery effluent (control-2 roots, length-1 mm). The increased *Aerva lanata* root length was observed in the presence of filtrate of *Aerva lanata* treated effluent (aqueous leaf extract-11 roots, length-35 mm). The study indicates that chromium effluent treated using filtrate of *Aerva lanata* prepared from its filtrate can be successfully employed for agricultural purpose.

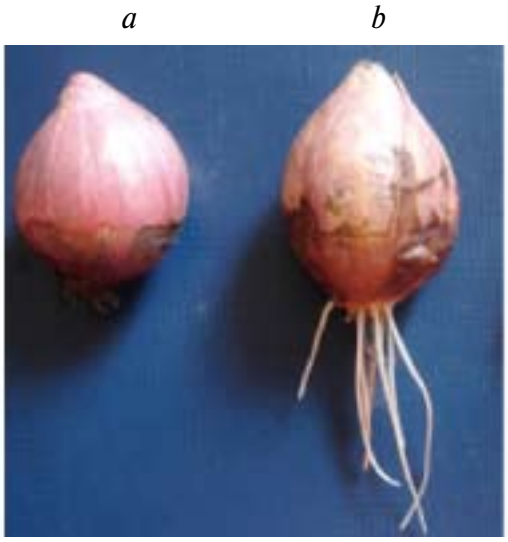

Fig. 2. Effects on Allium cepa using chromium tannery effluent (a) and Aerva lanata aqueous leaf extract treated chromium effluent (b).

Conclusion

The aqueous leaf extract of *Aerva lanata* exhibit highest chelating ability. The positive correlations between chelating ability, antioxidant activity and total phenolics content were depending on concentration of the filtrate used. The plant used in this study provides good opportunities for bio-treatment development against heavy metals. As a result, it can be concluded that this

plant can be used successfully in the removal of hexavalent chromium from tannery effluents.

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