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BIOREDUCTION OF HEXAVALENT CHROMIUM BY AQUEOUS FLOWER WASTE EXTRACTS: A SUSTAINABLE APPROACH WITH ANTIOXIDANT AND ANTIMICROBIAL BENEFITS

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ABSTRACT

Hexavalent chromium [Cr(VI)] is a widespread and hazardous pollutant released by several industrial processes, posing severe risks to ecosystems and human health. In this study, aqueous (AFW) and ethanolic (EFW) extracts from waste flowers were evaluated for their potential to reduce Cr(VI) to its less toxic trivalent form [Cr(III)] under environmentally friendly conditions. Phytochemical analysis revealed the presence of phenolics, flavonoids, tannins, and alkaloids in both extracts. Antioxidant assays (DPPH, ABTS, FRAP) demonstrated that AFW possessed superior radical scavenging and reducing potential compared to EFW. GC-MS analysis identified 37 and 33 bioactive compounds in AFW and EFW, respectively. Among the two, AFW exhibited significantly higher Cr(VI) reduction efficiency, achieving a maximum of 67% reduction at 5 mg/mL for 10 ppm Cr(VI) after 30 minutes of incubation at pH 7 and 37 °C. The reduction followed first-order kinetics. Additionally, AFW demonstrated moderate antibacterial activity against *Enterococcus faecalis* and *Staphylococcus aureus*, showing dose-dependent zones of inhibition. These findings highlight that phytochemicals derived from floral waste offer a sustainable, cost-effective, and multipurpose approach for Cr(VI) detoxification, with added antioxidant and antibacterial properties relevant to both environmental remediation and biomedical applications.

KEYWORDS: Water treatment, chromium, phytochemicals, flower waste, eco-friendly.

1. INTRODUCTION

Water pollution has emerged as a major global concern, making it increasingly difficult to access safe drinking water. Hexavalent chromium, a prominent water contaminant released by industries such as tanneries, chrome plating, dye manufacturing, and aerospace, poses a significant threat. Industries without a proper effluent management system risk contaminating water bodies.[1] The lack of efficacy in the electroplating industry in utilization means a considerable amount of chromium ends up wasted.[2] This could significantly increase the accumulation of Cr in the environment and impact public health. Since Cr(VI) is highly toxic, its entry into the body triggers oxidative stress and DNA damage, leading to an increased risk of cancer, genetic mutations, and birth defects.[3] Trivalent chromium [Cr(III)] is a promising option for remediation due to its characteristics, such as lower toxicity and ease of precipitation in water. Therefore, the conversion of Cr(VI) to Cr(III) can be beneficial. Additionally, adsorption and mineralization improve the process of remediation.[4]

There are methods to reduce the chromium accumulated in the environment, including chemical precipitation, membrane process, ion exchange, liquid extraction, and electrodialysis.[5] Such methods have drawbacks, including lower efficacy in removing metal, as well as high energy and reagent requirements. There are also environmentally friendly techniques, such as precipitation, reverse osmosis, ion exchange, coagulation, and adsorption can be used for the removal of environmental pollutants. While utilizing adsorption techniques in the metal removal has the advantage of its

operating flexibility, efficacy and low processing cost. Adsorption utilizes the agricultural and industrial waste.[6] The agricultural waste containing organic compounds made an electrostatic interaction with metal and other pollutants in the water.

With the increasing population in India, the religious rituals result in the accumulation of biomass waste. Flowers and garlands are widely used in such rituals and are dumped in the sacred river.[7] This situation increases the biological oxygen demand in the water bodies, further the release of fertilisers used in the flower degrades the water quality, which would hinder the consumption of the water. For centuries, flowers have been used as a source of color and flavor for meals. The incorporation of edible flowers having phytochemicals such as flavonoids, anthocyanins, carotenoids, and phenolics increases the appeal and nutritional value of the food.[8] Apart from the benefits of the flowers in diet, they play a significant role in various industries such as natural colors, fragrances, textiles, and medicines. The extracts of the flowers can be used as biogas, organic acids, nutraceuticals, and nanoparticles.

This study aimed to evaluate the efficiency of the phytochemicals extracted from the waste flowers in reducing hexavalent chromium Cr(III) in an environmentally friendly way. The experimental work involved in variable parameters such as concentration, pH and operating temperature as well as batch kinetics. In addition to environmental benefits, the extracts from the waste flowers were subjected to antibacterial activity.

2. MATERIALS AND METHODS

2.1. Chemicals

Potassium dichromate (K2Cr2O7), Diphenylcarbazide (DPC), 2, 4, 6-tripyridyl-s-triazine were purchased from Sigma-Aldrich Chemical Corporation, USA. 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were purchased from Sigma Aldrich Chemicals Co, USA. All other chemicals used were of an analytical reagent grade.

2.2. Preparation of aqueous and ethanolic Flower extract

Waste flowers were sourced from the local market (Salem district, Tamil Nadu, India) and were cleaned with water to remove impurities. After cleaning, flowers were shade-dried and ground into a powder. To obtain the aqueous extract (AFW), 15 g of powdered flower was added to 250ml of distilled water, which was subjected to boiling water for 2 h. Using Whatman No. 1 filter paper, the mixture was filtered. The filtered extract was then dried using controlled temperature conditions (40-50 °C) using a hot air oven.

For ethanolic flower extract (EFW), 15 g of flower waste was mixed with 200 mL of ethanol and kept in a shaker for 48 hours. To maximize the extraction, the ethanol was replenished throughout this period. After the extraction, Whatman No. 1 filter paper was used to filter the mixture. Then the filtrate was kept under temperature-controlled environment (40-50 °C) to evaporate the ethanol and stored at room temperature for further studies.

2.3. Qualitative analysis of the flower extract

Qualitative analysis such as, molisch's test for carbohydrates, biuret test for proteins, ferric chloride test for hydrolysable tannins, test for condensed tannins, Liebermann test for phenolic compounds, and dragendorff's & hager's test for alkaloids were performed.

2.4. Quantitative analysis of total phenolic and flavonoid content in AFW and EFW

The estimation of total phenol (TPC) and flavonoid (TFC) content in AFW and EFW was estimated following the previously described method (9). Phenols represented as gallic acid equivalent (µg GAE/g of AFW and µg GAE/g of EFW). While flavonoids are represented as rutin equivalent (µg RE/g of AFW and µg RE/g of EFW).

2.5. Gas chromatography-mass spectrometry analysis

Gas chromatography-mass spectrometry (GC-MS) analysis was performed using an Agilent system (GC 8890/MS 5977C/Autosampler 7693A, USA). The separation was carried out using a DB-5ms column with a length of 30 m, an internal diameter of 0.25 mm, and a film thickness of 0.25 µm. Helium (99.999% purity) was used as the carrier gas at a constant flow rate of 1 mL/min. The oven temperature was initially set at 50 °C for 1 minute, then increased by 10 °C per minute to reach 300 °C, where it was held for 1 minute. The injection port temperature was maintained at 250 °C. The mass spectral scan range was set between 0.6 and 1091 m/z. The identification of compounds was carried out by comparing the mass spectra with the NIST20 library using MassHunter software.

2.6. *In vitro* antioxidant assays

In vitro antioxidant assay for both AFW and EFW was performed using previously described methods. The *in vitro* antioxidant assays of ABTS (10), DPPH (11), and FRAP (12) were carried out.

2.7. Estimation of Cr (VI) and Cr (III)

Hexavalent chromium was estimated by Epock2 multiwell plate reader using colorimetric method. The 1% of S-diphenylcarbazide (DPC) reagent was prepared by adding 250 mg of DPC to 25 mL of methanol and stored in the dark at 4°C. The method includes acidification of Cr (VI) solution by H_2SO_4 followed by the addition of DPC. A standard curve for Cr (VI) was plotted using various concentrations of $K_2Cr_2O_7$.

2.8. Reduction of Cr (VI) by AFW

AFW with a specific concentration (1-5mg/mL) was mixed with Cr (VI) and incubated in room temperature

for 30 min. Then the addition of 1% DPC and H_2SO_4 was followed as described above. Followed by 10 min of centrifugation, the supernatant was collected and read at 540 nm. All the experiments were carried out in two sets of independent duplicates, and the average of the obtained values was taken for analysis.

2.9. Optimization of various conditions for Cr (VI) reduction by AFW

Various factors, such as pH, temperature, and incubation time were optimized for the efficient reduction of Cr (VI) to Cr (III) using AFW. The solution containing different concentrations 1-5 mg/mL of AFW was incubated with Cr (VI). The pH of the mixture was adjusted using sodium hydroxide (0.1 N) or hydrochloric acid (0.1 N) either to 3, 7 and 9. The reaction was carried out at different temperature (10°C, 37 °C, and 45 °C). The reaction mixture was incubated for various periods (15 min, 30 min, and 45 min). The effect of these parameters on the reduction of Cr (VI) was monitored. The reduction of Cr (VI) was measured at 540 nm.

2.10. Kinetics determination of Cr(VI) removal

First-order kinetics would be determined by the equation, $-\ln(c/c_0) = k_1 t$ where c and c_0 are the initial and time-varying Cr(VI) concentrations (t), respectively. k_1 is the rate constant (min^{-1}) for the adsorption of Cr(VI) adsorption. The plot of $-\ln(c/c_0)$ vs t gives a slope which represents the rate constant, k_1. The half-life time is calculated by the equation, $t_{1/2} = \ln 2/k$.

2.11. Antimicrobial activity of AFW

The antimicrobial activity of AFW (stock: 1 mg/mL in distilled water) was evaluated against *Enterococcus faecalis*, and *Staphylococcus aureus* using the agar well-diffusion method. The assay was performed on Mueller Hinton Agar (MHA; Himedia, India) plates. MHA was prepared in distilled water (pH 7.0) and sterilized in an autoclave at 121°C for 15 minutes. The sterilized medium was poured into Petri plates and allowed to solidify. A microbial inoculum containing 10^6 CFU/mL of freshly prepared culture was spread evenly onto the MHA plates using a sterile cotton swab moistened with the microbial suspension. Three wells, each 8 mm in diameter, were punched into the agar. Two wells were filled with 50 µL and 100 µL of the AFW, while the third well was filled with the respective positive control. The plates were kept at room temperature for 4 hours to allow diffusion of the compounds into the medium. Subsequently, the plates were incubated at 37°C for 24 hours. After incubation, the ZOI was measured in millimetres for each plate.

3. RESULT

3.1. Qualitative analysis of phytochemicals in AFW and EFW

The qualitative analysis of both AFW and EFW revealed the presence of carbohydrate, hydrolysable tannin, flavonoids, phenolic acids, and alkaloids and notably absence of protein in both extracts.

3.2. TPC and TFC of AFW and EFW

AFW and MFW were quantified for secondary metabolites such as phenolics and flavonoids. The concentration of TPC in AFW and EFW was found to be 4.7 ± 0.8 mg GAE/g extract and 4.5 ± 0.6 mg GAE/g extract. The concentration of TFC in AFW and MFW was 7.6 ± 1.1 mg RE/g extract and 6.3 ± 0.8 mg RE/g extract.

3.3. GC-MS analysis of AFW and EFW

The GC-MS chromatogram of AFW and EFW is given in Figure 1. The chromatogram of AFW and EFW revealed the presence of 37 and 33 compounds, respectively. The predicted compound in each extract is listed in Table 1 and 2.

3.4. *In vitro* antioxidant activity of AFW and EFW

The antioxidant scavenging and reducing potential of both AFW and EFW were dose-dependent, demonstrating their ability to scavenge radicals and reduce ferric ions, respectively. Throughout the antioxidant assays, ascorbic acid was taken as the positive control. When analysing their antioxidant potential, they reveal a pattern that AFW performs considerably better than the EFW. DPPH scavenging assay (Figure 2) revealed weaker antioxidant activity with EFW (IC_{50} value of 956.12 µg/mL) and moderate scavenging activity of AFW (IC_{50} value of AFW 47.35 µg/mL). While scavenging of ABTS activity (Figure 3), both AFW and EFW showed moderate scavenging potential, AFW demonstrated better ABTS activity (IC_{50} value of 15 µg/mL for AFW; 20.13 µg/mL for EFW). In FRAP activity (Figure 4), increasing the AFW and EFW concentration enhances their reducing power - rising OD value from 0.156 to 0.612 for AFW and from 0.146 to 0.638 for EFW as the concentration increases from 15 µg/mL to 75 µg/mL. However, both extracts had reduced efficacy compared to ascorbic acid, which exhibited strong antioxidant effects.

3.5. Hexavalent chromium reduction by AFW and EFW

Initially, both AFW and EFW were tested to assess their ability to reduce Cr(VI) to Cr(III). Here, AFW demonstrated superior reduction efficiency compared with EFW, leading to further study under various factors such as pH, temperature, and time. The reduction estimation was carried out using different concentrations of Cr(VI) and AFW (Figure 5). At a concentration of 10 ppm, AFW achieved peak reduction of hexavalent chromium. The effective Cr(VI) reduction was observed from 13% to 67% when chromium concentration varied between 5 and 20 ppm. Above 20 ppm, the reduction reached a plateau phase, which indicates that higher concentrations of Cr(VI) impede the reduction efficiency. Hence, the result suggests that AFW is potent in reducing Cr(VI) to Cr(III) when chromium concentrations are lower.

The graphical representation of Cr(VI) reduction at different time intervals (15 to 45 minutes) is shown in Figure 6. The results indicated that the reduction was rapid within the initial period of incubation, with a maximum reduction of 46% observed at 15 minutes. The reduction efficiency increased further, reaching 67% after 30 minutes of incubation when treated with 5 mg/mL of AFW. However, extending the incubation period beyond 30 minutes did not result in a significant increase in reduction efficiency. Therefore, it can be concluded that the optimal time for Cr(VI) reduction using AFW is 30 minutes, where the highest reduction efficiency was achieved.

Figure 7 represents the effect of temperature on the reduction of hexavalent chromium [Cr(VI)] by AFW across a range of temperatures from 10°C to 45°C. Progressive increase in reduction efficiency was observed, which indicates that the reduction of Cr(VI) to Cr(III) was temperature-dependent. The conversion of Cr(VI) to Cr(III) is at maximum (70%) at 37°C, indicating the optimal temperature. A slight decline beyond 37°C suggests that higher temperatures may not significantly enhance the reduction process by AFW. Thus, the result highlights that temperature does play a critical role in chromium reduction efficiency.

Figure 8 shows AFW's ability to reduce Cr(VI), at different pH levels (3, 7, and 9) across various concentrations of AFW (1–5 mg/mL). By analysing the figure, pH 7 was most effective, followed by acidic pH 3, and least effective at alkaline pH 9. At pH 3, the reduction ranged from 12% to 64%, with a maximum reduction observed at 5 mg/mL. Similarly, at pH 7, the reduction was maximum at 5mg/mL, and the efficiency ranged from 13% to 68%, indicating optimal Cr(VI) reduction efficiency. Conversely, at pH 9, the reduction efficiency is significantly lower, ranging from 5% to 39% with the highest reduction observed at 5 mg/mL. These findings demonstrate AFW's reduction of Cr(VI) is pH dependent, and neutral condition is the most favourable environment for maximum reduction efficiency. The reduction of Cr(VI) followed first-order kinetics (Figure 9). Various parameters of first-order kinetics are given in Table 3.

3.6. Antibacterial effect of AFW

The antibacterial effect of AFW was evaluated against *Enterococcus faecalis* and *Staphylococcus aureus* by measuring the zone of inhibition at two concentrations 50 µg/mL and 100 µg/mL. With *E. faecalis* (Figure. 10A), AFW exhibited a ZOI of 11 mm at 50 µg/mL, which increased slightly to 13 mm at 100 µg/mL. In comparison, the standard antibiotic Linezolid showed a significantly higher ZOI of 28 mm. Similarly, AFW demonstrated antibacterial activity against *S. aureus* (Figure 10B), with a ZOI of 15 mm at 50 µg/mL, which increased to 17 mm at 100 µg/mL. However, the inhibition was lower than that of the standard antibiotic Levofloxacin, which exhibited a ZOI of 23 mm. These results suggest that AFW possesses moderate antibacterial activity, with a dose-dependent increase in inhibition, although it is less effective compared to standard antibiotics.

Table 1: List of compounds identified by GC-MS analysis in AFW.

S. No	Name of the Compound	Retention time	Area (%)
1.	Propanoic acid, 2-methyl-, butyl ester	7.236	0.65
2.	Cyclopropanecarboxylic acid, 2,2-dimethyl-3-(2-methyl-1-propenyl)-,(1R-trans)-	11.329	3.34
3.	2-Cyclopentene, 4-(hydroxymethyl)-1,1,2,3-tetramethyl-	12.057	0.42
4.	1-(4-Ethoxyphenyl)propan-1-ol	12.971	0.89
5.	Benzoic acid, 4-ethoxy-, ethyl ester	13.605	1.89
6.	Bicyclo[4.3.0]nonan-7-one, 1-(2-methoxyvinyl)-	14.551	0.48
7.	1,3-Dithiane, 2-benzyl	14.854	0.44
8.	2-Cyclohexen-1-one, 4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl-	15.094	0.81
9.	trans-Z-. alpha. -Bisabolene epoxide	15.312	1.77
10.	2-Cyclohexen-1-one, 4-(3-hydroxy butyl)-3,5,5-trimethyl-	15.753	0.98
11.	Indeno [3a,4-b] oxiren-2-ol, octahydro-4a-methyl-5-[(tetrahydro-2H-pyran-2-yl) oxy]-, (1a. alpha.,2. beta., 4a. beta.,5. beta.,7aS*)-	15.997	0.92
12.	Chamazulene	16.103	0.60
13.	Tetradecanoic acid	16.245	0.85
14.	5-Ethyl-2-furaldehyde	16.583	1.35
15.	2-Cyclohexen-1-one, 4-hydroxy-3,5,5-trimethyl-4-(3-oxo-1-butenyl)-	16.711	1.93
16.	2-Naphthalenemethanol, decahydro-alpha.,.alpha.,4a-trimethyl-8-methylene-, [2R-(2.alpha.,4a.alpha.,8a .beta.)]-	16.991	0.62
17.	1.6-Farnesadiene-3.10.11-triol, acetate	17.312	0.81
18.	Cyclohexanol, 3,3,5-trimethyl-, trans-	17.479	2.13
19.	7-(1,3-Dimethylbuta-1,3-dienyl)-1, 6,6-trimethyl-3,8	17.716	0.52

	dioxatricyclo[5.1.0.0(2,4)]octane		
20.	Uvidin C	17.781	0.48
21.	Hexadecanoic acid, methyl ester	17.916	1.15
22.	n-Hexadecanoic acid	18.291	0.85
23	2(3H)-Benzofuranone, hexahydro-3a, 7a-dimethyl-, cis-	18.459	5.15
24.	2-Oxabicyclo[2.2.2]octan-6-one, 1, 3,3-trimethyl-	18.972	0.96
25.	1,2-Benzenediol, O-(3-cyclopentylpropionyl)-O'-(pentafluoropropionyl)-	19.355	0.56
26.	3-Cyclopentylpropionic acid, 2,3-dichlorophenyl ester	19.413	4.32
27.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	19.558	0.55
28.	8-Oxabicyclo [5.1.0] oct-5-en-2-ol, 1,4,4-trimethyl-	19.933	1.15
29.	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	23.094	1.81
30.	Campesterol	24.307	2.53
31.	Octadecanoic acid, 2,3-dihydroxypropyl ester	24.660	0.63
32.	Stigmasterol	24.930	12.79
33.	1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6 ,7-tetrahydro-, isopropyl ester	25.443	0.57
34.	1H-Indole, 5-methyl-2-phenyl-	25.578	0.61
35.	Stigmasta-5,24(28)-dien-3-ol, (3. beta.,24Z)-	26.066	1.83
36.	beta-Amyrin	26.335	37.11
37.	5(1H)-Azulenone, 2,4,6,7,8,8a-hexa hydro-3,8-dimethyl-4-(1-methylethylidene)-, (8S-cis)-	26.597	5.52

Table 2: List of compounds identified by GC-MS analysis in AFW.

S. No	Name of the Compound	Retention time	Area (%)
1.	Glycerin	4.354	0.92
2.	1,3,5-Triazine-2,4,6-Triamine	5.353	0.19
3.	2-Furancaroxaldehyde, 5- (hydroxymethyl)-	6.964	0.21
4.	9-oxabicyclo [6.1.0] nonane, cis-	7.198	0.37
5.	2,4-decadienal, (E,E)-	7.531	0.32
6.	Aziridine, 2-methyl-3-(1-methylethyl)-, trans-	8.320	0.24
7.	Butanal, 2-methyl-	9.308	6.43
8.	Benzoic acid, 4-ethoxy-, ethyl ester	9.575	0.20
9.	3-Deoxy-d-mannoic lactone	10.553	2.55
10.	d-Glucoheptose	10.797	0.75
11.	d-Glycero-d-galacto-heptose	11.919	0.56
12.	Pentadecanoic acid, 14-methyl-, methyl ester	12.486	0.21
13.	n-hexadecanoic acid	12.752	11.78
14.	10-octadecenoic acid, methyl ester	13.619	0.29
15.	9,12-Octadecadienoic acid (Z,Z)-	13.897	46.56
16.	Octadecanoic acid	14.019	4.43
17.	Phenol, 4,4'-(1-methylethylidene)bis-	14.186	0.51
18.	Carbonic acid, 2-dimethylamino ethyl isobutyl ester	14.708	0.52
19.	Cyclohexene, 1-methyl-3-vinyloxy-	14.974	1.22
20.	Oleic acid	15.052	0.71
21.	Z-2-Octadecen-1-ol	15.119	0.20
22.	E, E-6,11-Tridecadien-1-ol acetate	15.174	0.62
23	8-Hexadecyne	15.285	0.56
24.	Carbonic acid, butyl 2-dimethylamino ethyl ester	15.663	1.34
25.	9,12-Tetradecadien-1-ol, acetate, (Z,E)-	15.752	0.19
26.	Erucic acid	15.885	0.19
27.	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	15.985	2.67
28.	9,17-Octadecadienal, (Z)-	16.919	9.75
29.	1,15-Pentadecanedioic acid	17.030	1.17
30.	Eicosane	17.930	0.19
31.	Chondrillasterol	21.462	2.24
32.	2-Ethylacridine	21.629	0.20
33.	22-Stigmasten-3-one	21.996	0.71

Table 3: Kinetic parameter for Cr(VI) reduction by different concentrations of AFW at various time (pH: 7; °C) temperature: 37.

Kinetic model	AFW (mg/mL)		
	1	2	3
Pseudo-first order K_1 (min^{-1})	0.002	0.003	0.005
Correlation (R^2)	0.991	0.991	0.977
$T_{1/2}$ (min)	346.57	231.05	138.63

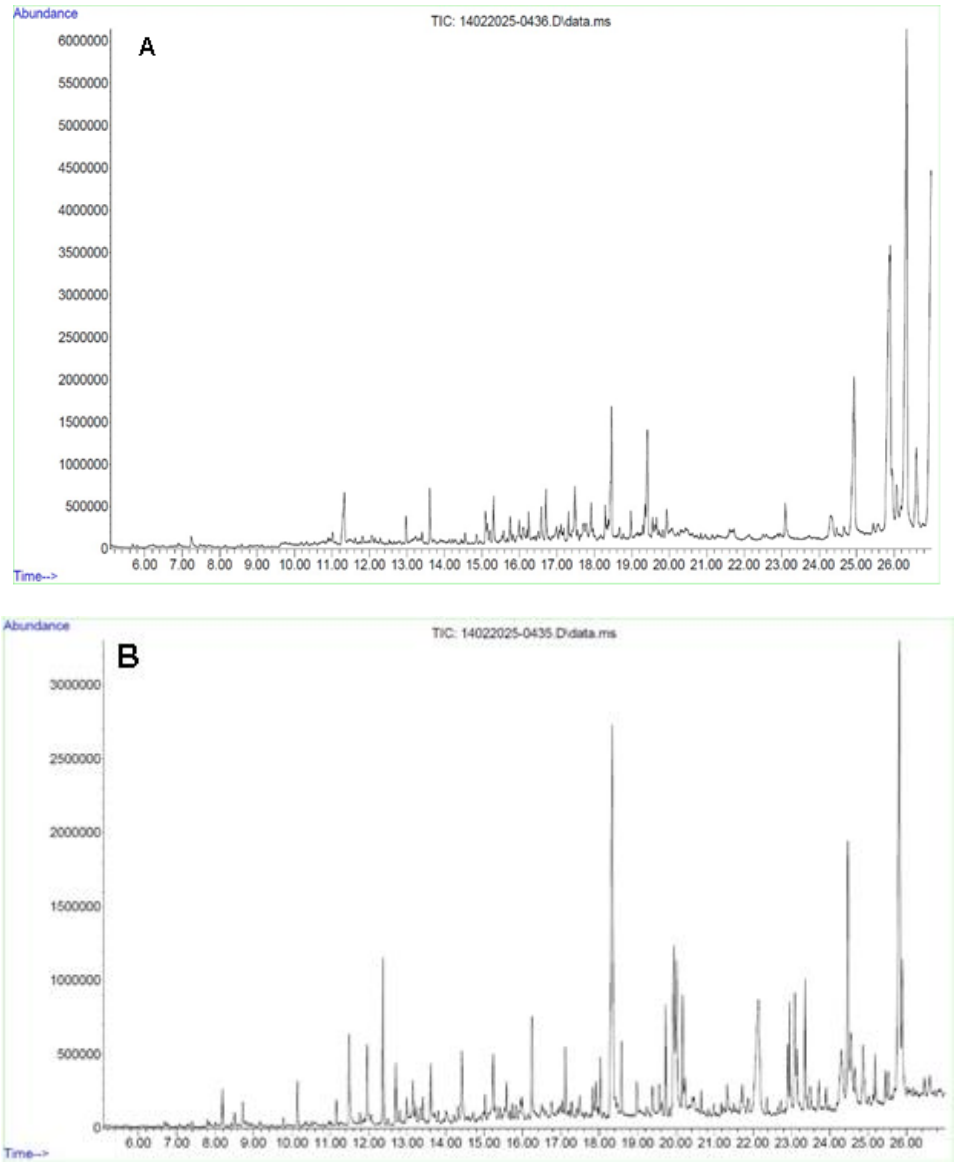


Figure 1: GC-MS chromatogram of (A) AFW, and (B) EFW.

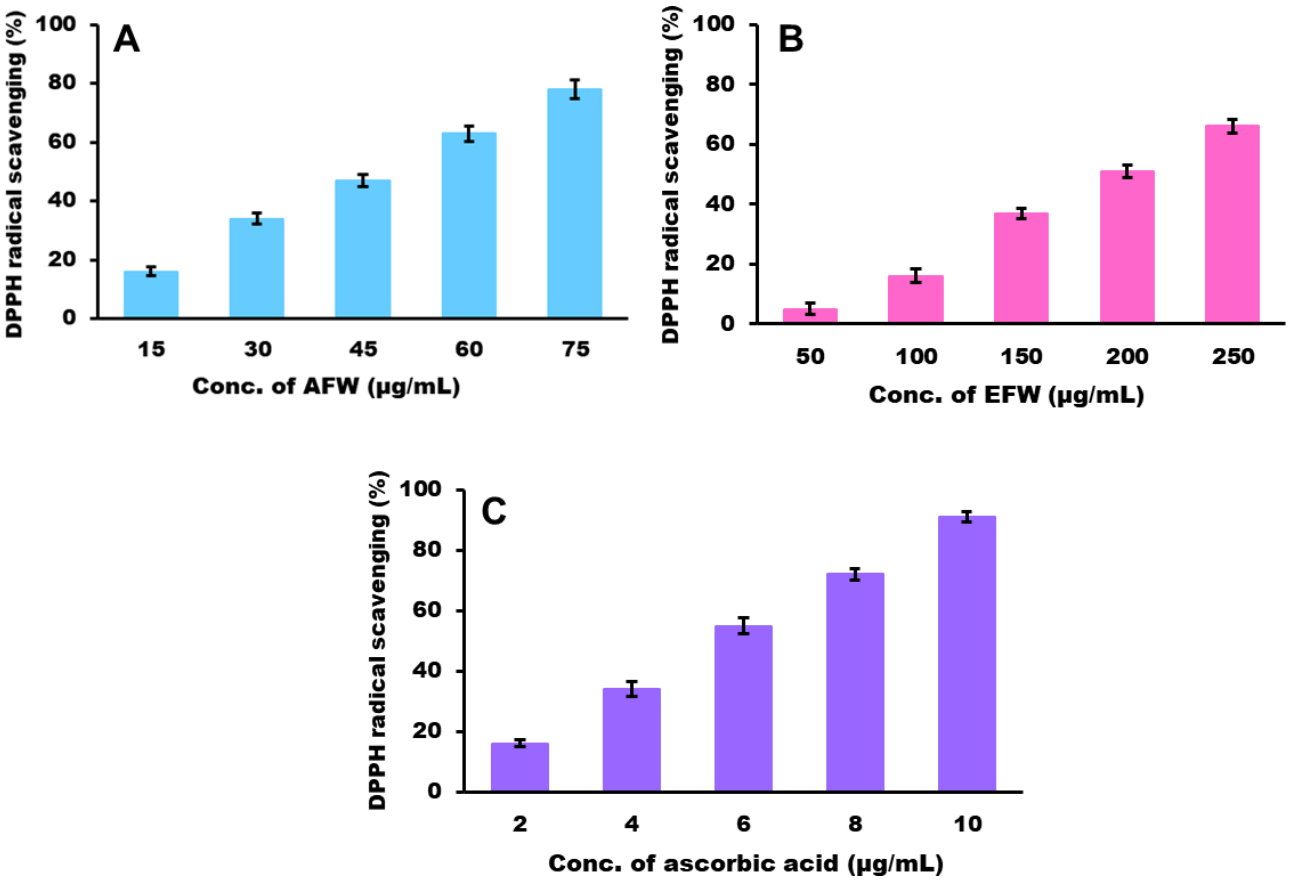


Figure 2: (DPPH radicals scavenging activity of (A) AFW, (B) EFW, and (C) ascorbic acid).

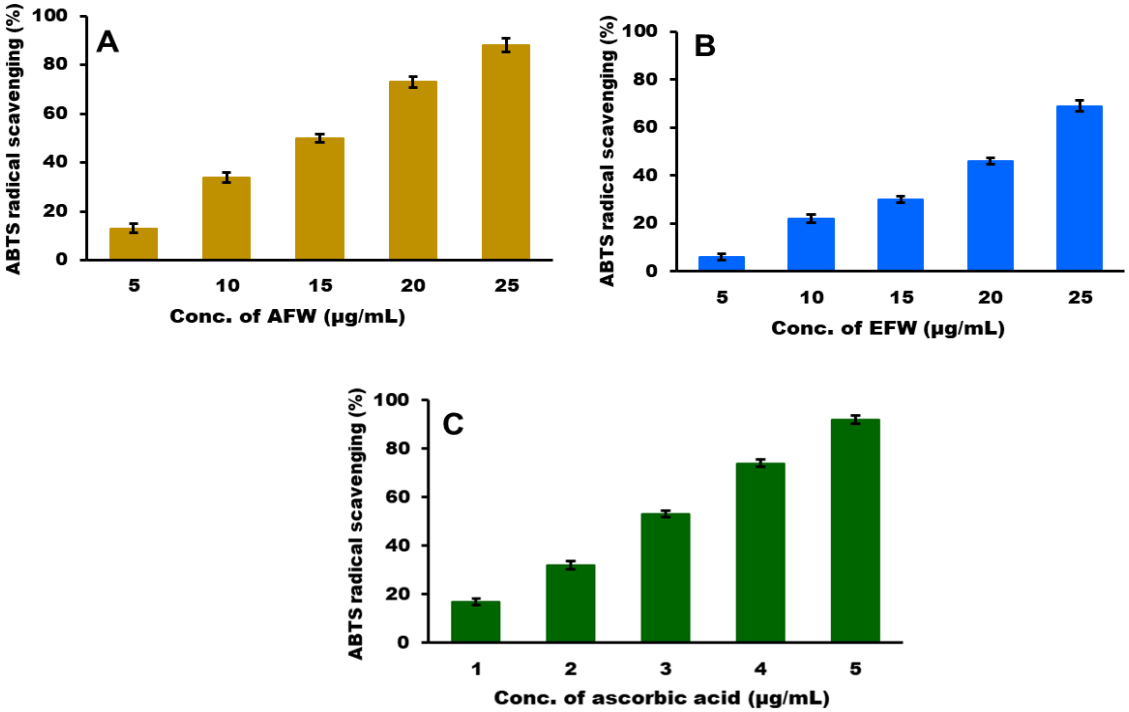


Figure 3: ABTS radicals scavenging activity of (A) AFW, (B) EFW, and (C) ascorbic acid.

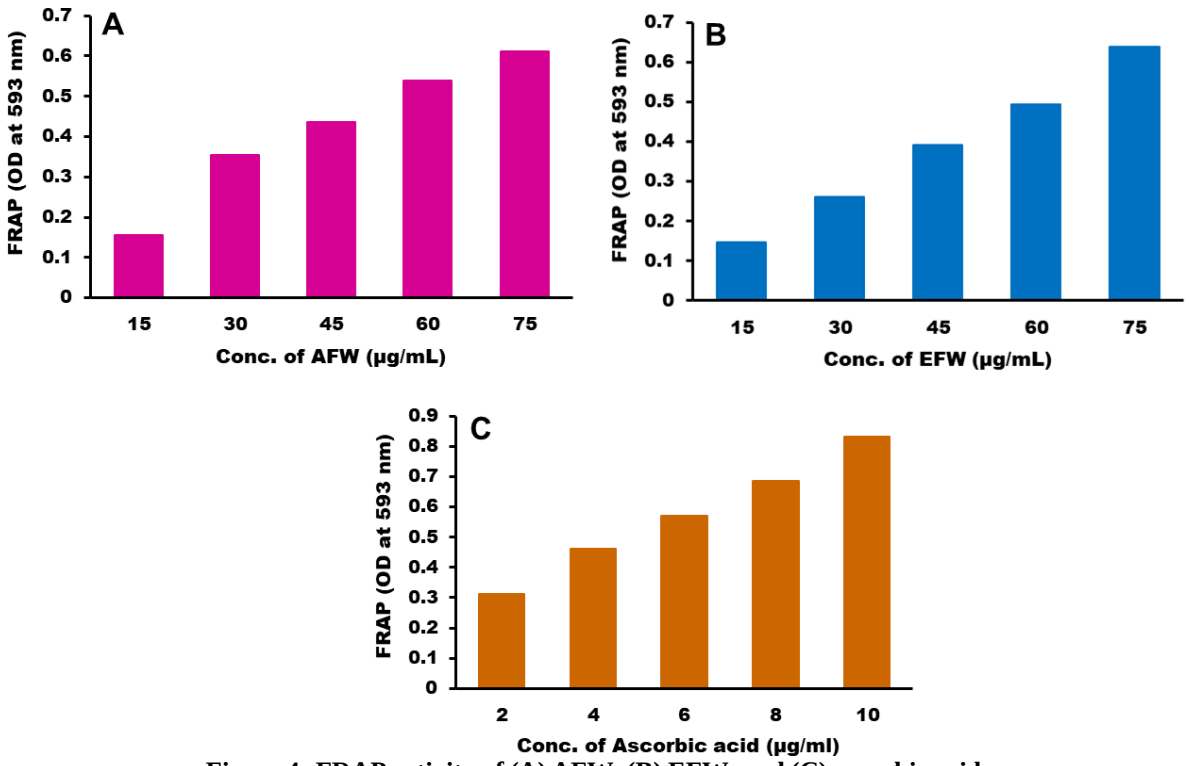


Figure 4: FRAP activity of (A) AFW, (B) EFW, and (C) ascorbic acid.

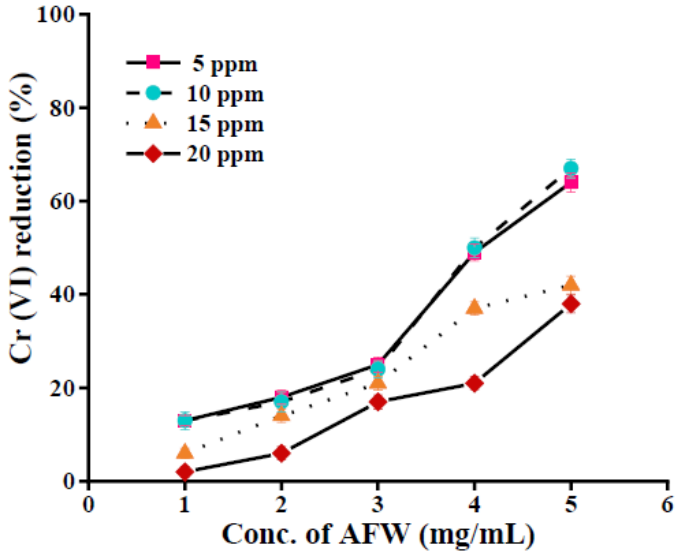


Figure 5: Reduction of Cr (VI) (different ppm) by Different concentrations of AFW.

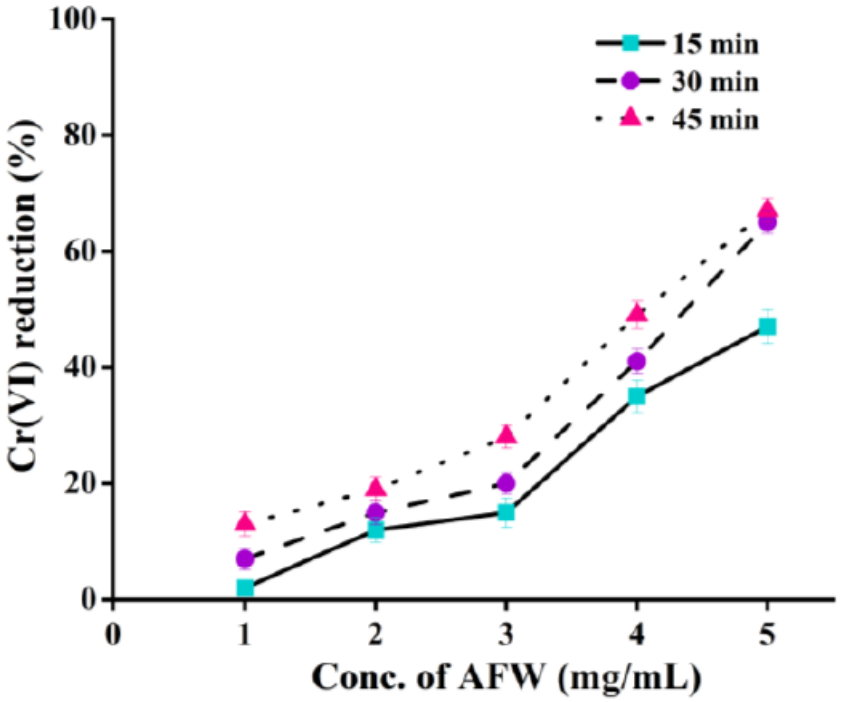


Figure 6: Hexavalent Cr reduction at various time periods.

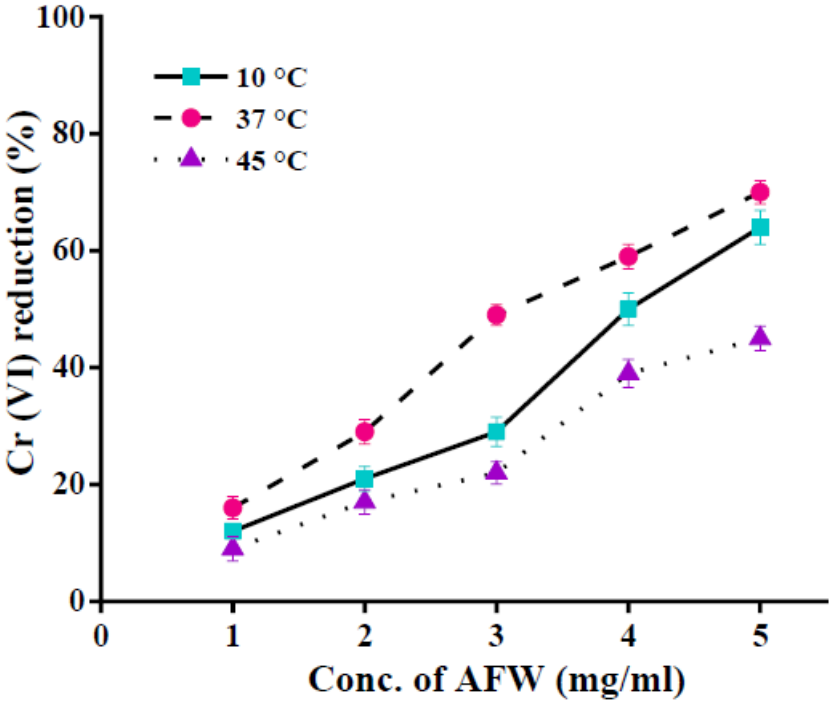


Figure 7: Effect of temperature on Cr reduction.

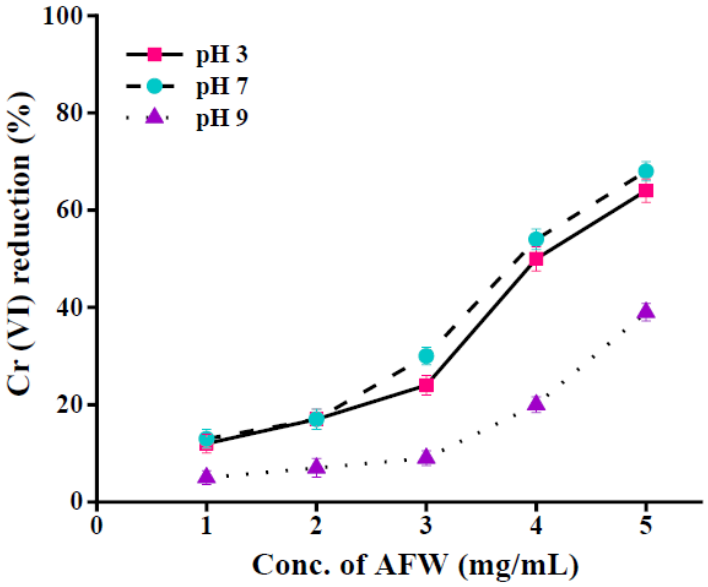


Figure 8: Effect of pH on Cr reduction.

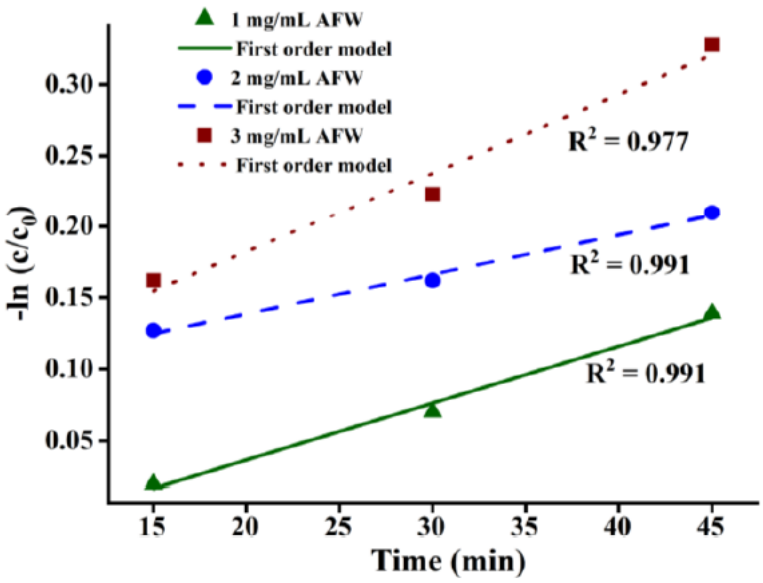
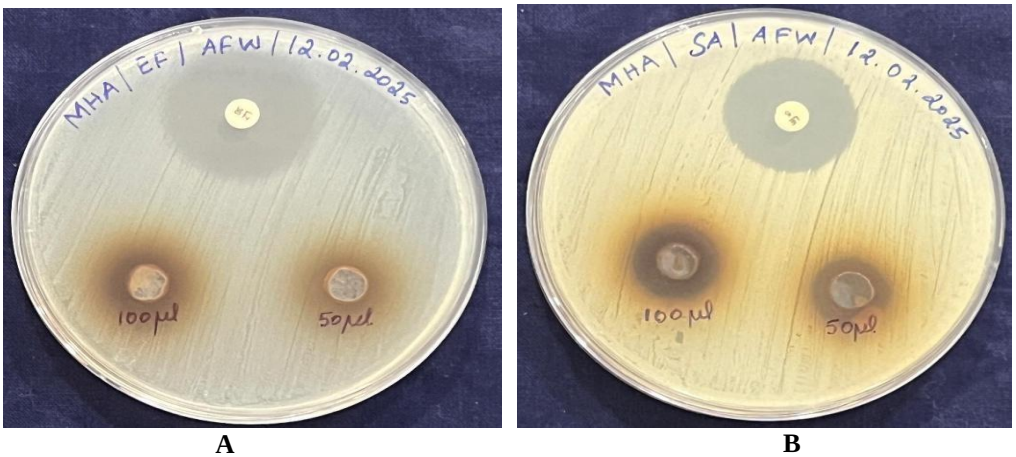


Figure 9: First-order kinetics of Cr (VI) reduction by AFW.



A **B**

Figure 10: Antibacterial effect of AFW against (A) *Enterococcus faecalis* and *(B)* Staphylococcus aureus.

4. DISCUSSION

The reduction of most hazardous hexavalent chromium to trivalent chromium is a key process for alleviating the environmental and health impact related to the chromium contamination. When comparing the Cr(VI) to Cr(III), Cr(III) is considerably less toxic and tends to precipitate, reducing its bioavailability. While Cr(VI) is a carcinogen, it is easily soluble in water, making it a significant pollutant in industries such as leather, electroplating, and pigment manufacturing. Recently, flower-derived phytochemicals gained attention due to their eco-friendly, cost-effective and efficient natural reducing agent. The contents found in the flower extracts, such as phenolics, alkaloids, terpenoids, saponins, and ascorbic acid, can facilitate the reduction of Cr(VI) to Cr(III). This makes waste flower extracts a sustainable solution for tackling chromium pollution.

The key benefit of using phytochemicals for Cr(VI) remediation is the eco-friendliness and sustainable nature. When compared with chemical methods, flower-based remediation utilizes biodegradable natural compounds, thereby minimizing environmental impact, unlike chemical methods, which introduce secondary pollutants. Additionally, the flower extracts offer a sustainable and cost-effective solution for industrial waste management. Floral waste would otherwise be discarded; now, they add value by contributing to sustainable waste management, while also being readily available for a large-scale wastewater treatment system. Flavonoid-rich plants such as *Tagetes erecta*, *Hibiscus rosa-sinensis*, and *Calendula officinalis* contain strong antioxidant activity that contributes to heavy metal detoxification.[13,14] Besides electron transfer, tannins form a complex with the chromium ions, enhancing the efficiency of the reduction.[15] Ascorbic acid found in *Hibiscus sabdariffa* and *Chrysanthemum indicum* is directly involved in redox reaction by donating the electron to Cr(VI).[16] Flavonoids-Cr(III) complex demonstrates superior and more efficient reduction of Cr(VI) than flavonoids alone.[17] Apart from phenols and ascorbic acid, alkaloids from *Catharanthus roseus* contribute through metal ion binding in the reduction of Cr(VI).[18] *Ocimum sanctum and Clitoria ternatea* contain terpenoids and saponins that play a dual role of reducing Cr(VI) and stabilizing Cr(III) through adsorption and precipitation. Polysaccharides and proteins in the same flower extract help stabilise, prevent oxidation and enhance long-term efficacy.[19] A study successfully remediated the Cr(VI) and Cr(III) with the biosorbent derived from *Borassus aethiopum*.[20] Similarly, a study found that flower waste biomass was able to remove chromium ions from tannery effluent by absorbing 70% of the chromium present in the effluent.[21] In another study, rose biomass absorbed 100 ppm of chromium, mercury, and zinc with concentrations of 5.26, 6.76, and 4.07 mg/g, respectively. The study also includes the use of marigold flower powder obtained from the flower waste, which has removed the heavy metals Cr(VI) and cadmium Cd(II) from aqueous solution.[22] Apart from the ability of the phenols and flavonoids to transfer electrons, they generate ROS such as superoxide and hydroxyl radicals contribute to the oxidative degradation of Cr(VI).[23] Further, tannins and alkaloids form a metal complex that enhances the Cr(VI) reduction, followed by precipitation of Cr(III).[24] Further, terpenoids and saponins aid in the absorption of Cr(VI) onto nanoparticles or plant matrices, where reduction and stabilisation occur simultaneously.[25] In our study, qualitative analysis revealed the presence of phytochemicals such as flavonoids, phenols, alkaloids, and tannins. With GC-MS analysis, AFW and EFW were shown to have 37 and 33 compounds. In the antioxidant assay, the AFW and EFW showed radical scavenging activity, although the activity wasn't comparable to standard ascorbic acid; they still have sufficient activity, with a greater number of antioxidant compounds, which was revealed in the GC-MS analysis. The antioxidants are involved in the direct reduction of the Cr(VI) into Cr(III). They act as an electron donor for the reduction of the Cr(VI). Due to the high oxidation state, they are electron-deficient and they accept electrons easily and get reduced into simpler forms such as Cr(III). The hydroxyl group, which is common to the phenols and flavonoids, can donate a hydrogen atom to the electron-deficient Cr(VI).[26,27]

As the initial stage for Cr(VI) reduction process, different concentrations of Cr(VI) were taken (5-20 ppm). Cr(VI) is subjected to the AFW at different concentrations (1 to 5mg/mL), and AFW exhibited maximum reduction efficiency at 10 ppm of Cr(VI) at 5mg/mL. The optimal reduction at 10 ppm Cr(VI) with 5mg/mL of AFW might be due to the ratio of available antioxidants or phytochemicals to the amount of Cr(VI) being optimal for complete reduction. Cr(VI) reduction at different periods, the reduction observed was maximum at 67% after 30 minutes of incubation when treated with 5 mg/mL of AFW. Increasing the incubation time to 45 minutes did not increase the reduction efficiency. This indicates the active compounds present in the AFW are mostly consumed by the time of 30 minutes for the reduction of Cr(VI). Regarding temperature, given AFW is derived from biological material, its optimal Cr(VI) reduction at 37°C indicates that the phytochemicals maintain their conformation, solubility, and stability at this temperature, likely due to their initial environmental adaptations.

Adjusting the pH to obtain the optimal electrostatic interaction of the phytochemicals with the Cr(VI) consequently enhances the formation of Cr(III). pH influences the surface charge by affecting the protonation state of the ionizable functional group in the phytochemicals. At neutral pH, the concentration of H^+ ions is very low. This usually leads to a limitation of reduction when compared with acidic pH. But, in our study, the AFW performed better in a neutral pH environment. This may be because, at a neutral pH, the hydroxyl group in the phenols can deprotonate to form

phenoxide anion. Phenoxide anions are stronger electron donors than the normal phenolic counterpart. This charge difference outweighs the limitation of the proton at neutral pH. The ability of the AFW to perform in neutral pH conditions stands as an advantage as the process removes the chemicals needed for the pH control, leading to cost savings and is much more environmentally friendly. When analysing the Cr(VI) reduction under different environment conditions by AFW, the phytochemicals in floral waste, such as phenolics, flavonoids, and tannins, combine to create a synergistic effect, offering a significant advantage by ensuring effective Cr(VI) detoxification. After detoxification, in reduced form, Cr(III) is less toxic and precipitates as hydroxides, which can be easily removed by aqueous systems, ensuring long-term stability. Also, flower-derived phytochemicals generate minimal secondary waste, easing post-treatment disposal.

Over the past decade, a significant rise in antimicrobial-resistant bacterial infections has been observed in both hospital and community settings. Such increases are solely attributed to the continuous use of various antimicrobials, whether systemic or topical.[28] The escalating threat of multidrug-resistant bacteria like methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *enterococci* (VRE) coupled with bactericidal activity found in gram-negative bacteria such as *Escherichia coli*, and *Klebsiella pneumoniae* shown to surpass certain antibiotics.[29] This condition underscores the need for ongoing research and development of new antimicrobial agents to address bacterial resistance.

Interestingly, flower waste contains bioactive compounds that offer significant medicinal benefits, such as *Rosa indica* (Indian Rose), *Calendula officinalis* (Pot Marigold), *Calendula arvensis* (Field Marigold), *Hibiscus rosa-sinensis* (Hibiscus), and Chrysanthemum. They have potent antibacterial properties against pathogens like *Staphylococcus aureus, Escherichia coli*, and *Streptococcus pneumoniae* due to the rich number of phytochemicals they contain. These properties make them suitable for the development of herbal remedies, pollution-reducing products.

Despite AFW's environmental benefits, it is also tested for antibacterial activity with *E. faecalis* and *S. aureus*. When comparing the ZOI with the standard drug Linezolid, 100 µg/mL of AFW demonstrated a moderate ZOI with 13 mm and 17 mm with *E. faecalis* and *S. aureus*, respectively. Phenolic and flavonoid compounds found in plant extracts are widely documented for their antimicrobial activity. The mechanism is the disruption of the microbial membranes, inhibiting essential enzymes and interfering with nucleic acid synthesis.[30]

5. CONCLUSION

The present study evaluated the efficacy of aqueous flower waste extract (AFW) in reducing toxic hexavalent chromium [Cr(VI)] to its safer trivalent form [Cr(III)]. The comparative analysis indicated that AFW demonstrated superior reduction potential compared to ethanolic flower waste extract (EFW), prompting further optimization studies. The reduction efficiency was highest when 5 mg/mL of AFW was used to treat 10 ppm of Cr(VI), achieving a reduction of approximately 67%. The process was influenced by various factors such as pH, temperature, and time, with optimal conditions observed at pH 7 and 37°C, while the maximum reduction occurred within 30 minutes. In addition to its Cr(VI)-reducing capability, AFW exhibited notable antioxidant activity, as evidenced by its performance in DPPH, ABTS, and FRAP assays, although slightly lower than standard ascorbic acid. Furthermore, AFW demonstrated antibacterial activity against *Enterococcus faecalis* and *Staphylococcus aureus*, showing dose-dependent inhibition, albeit with less potency than conventional antibiotics. AFW effectively detoxifies Cr(VI) due to its bioactive phytochemicals. It's a sustainable, eco- friendly, and cost-effective solution for wastewater treatment, also offering antioxidant and antibacterial benefits for environmental and biomedical uses.

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