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Phytoremediation of Arsenic by *Withania somnifera* and Aloe-vera

Ashok Kumar Jha[1*], Rajendra Kumar[2], Raghbendra Thakur[3]

[1]*University Department of Chemistry, T.M.B.U., Bhagalpur, Bihar, India.*

[2]*Department of Chemistry, P.B.S. College, Banka, Bihar, India.*

[3]*Atomic Minerals Directorate for Exploration and Research Department of Atomic Energy, Bangalore, Karnataka, India*

Abstract

Arsenic, a metalloid, has unfavorable impact on human health because of its presence in groundwater. This paper deals with the phytoremediation of arsenic (III) by *Withaniasomnifera* and Aloe barbadensis miller (Aloe–vera) along with the percent uptake by roots, stems and leaves. The analysis of arsenic by ICP -OES has shown that maximum uptake of arsenic takes place by roots of the plant. The behavior of arsenic uptake by roots, stems and leaves of plants are different. The HPLC of *Withaniasomnifera* and *Aloe-vera* extract after being sonicated for four hours confirms uptake of arsenic. HPLC of *Withaniasomnifera* extract with methyl alcohol and *Aloe-vera* with dilute methyl alcohol characterizes the plant. The uptake of arsenic at 24 h, 48 h, and 72 h by 10 g of Ashwagandha (*Withaniasomnifera)* live plant and *Aloe-vera* has been studied and different masses of the plants have also been treated up to a fixed time. The kinetic data reveal that the uptake follows pseudo-second order kinetic model. The initial concentration and pH have also been varied to fix the optimum condition. It has been established that maximum phytoremediation by plants takes place at a pH of 2 and initial concentration of 5 ppm.

Keywords: Phytoremediation, uptake, HPLC, ICP – OES.

Full length article **Corresponding Author*, e-mail:*ashokjha39@gmail.com*

1. Introduction

Arsenic is one of the most widespread pollutants in the Gangetic plain of the country amongst Cr, Ni, Cu, Cd and Pb. Soils, formed as a result of degradation of rocks, has become a sink of heavy metals due to anthropogenic as well as geochemical reactions[1-3]. Prolong ingestion of heavy metals causes neurological, liver and lung cancer whereas arsenic causes hyperpigmentation and cancer. The most common oxidation state of arsenic(III) is present in drinking water whereas soil containsbothAs(V) and As(III)[4]. Owing to its toxicity and non-biodegradability, it has adverse impact on the health of the inhabitants exposed to arsenic contamination either through food chain or water[5-6]. Arsenic enters food chain such as rice, maize, wheat and vegetables where biomagnifications take place[7-8]. Though oxidation, coagulation and biological methods are employed for arsenic remediation, phytoremediation has emerged as an alternative technique. Few bacterial biomass and agricultural wastes have also been found effective in removal of arsenic[9-12]. Aquatic weeds such as *Eichhorniacrassipes, Lemna minor, and Azolla* have also been tried for removal ofAs(III) from aqueous medium but these species are not very effective[13].Inaddition, few worth mentioning reported accumulator of arsenic through phyto remediation are *Baccharisneglecta, Atriplexlentiformis, Leptospermumscoparium, Anthoxanthumodoratum, Viola Macedonia.* A few fern species such as *Pterislongifolia, Pteriscriteca*and*Pterisumbrosa*have been reported as arsenic accumulators. 400 hyperaccumulators have been reported till date. Bacteria have also been found efficient for arsenic removal. The reported bacteria are Pseudomonas, psychrobacter, Citrobacter, Bacillus, Bosea, Vibrio and Enterobacter. Plants such as*Medicagosativa L.Pterisvittata,*and *Breyundimonas sp.* have also been established as an accumulator[14-16].Live plants and herbs also bioaccumulate arsenic through their roots, stems and leaves. The property of accumulation of toxic elements by the plants help mitigate arsenic in a natural way. The accumulation of arsenic by plants is supposed to take place through inorganic phosphate transport. Arsenate movement to the xylem vessel thus takes place through transport mechanism[17]. The accumulation of arsenic by plants is supposed to take place through inorganic phosphate transport. Arsenate movement to the xylem vessel thus takes place through transport mechanism[18]. Plant roots are the best accumulator of arsenic than stem and leaves.

Arsenate reduction to arsenite and its sequestration into the vacuole in the roots has been proved to be one of the

possible mechanisms of arsenic removal through roots.Medicinal plants utilized as folk medicine have also been reported to remove arsenic through roots and leaves.Thispaper deals with the phytoremediation of arsenic through roots, stem and leaves of a plant species of the genus Aloe barbadensis miller (Aloe – vera). The important constituents are vitamins, minerals, lignin, salicylic acids, amino acids and saponins. *Withaniasomnifera*known as ashwagandha has also been put under experimental study of arsenic detoxification in the present paper. *Withaniasomnifera*contains alkaloids, anahygrine, withaferins and saponins. Saponins have also been used as natural surfactants for increasing the surface area of bentonites despite modifications of bentonites with other compounds have also been reported for removal of arsenic[19-21].Inductivelyplasma-optical emission spectroscopy (ICP – OES) has been used toanalyze arsenic.

2. Materials and Methods

Live plants of *Withaniasomnifera*and *Aloe – vera* have been collected from the Acharya P.C. Ray Garden of University Department of Chemistry, TilkaManjhi Bhagalpur University, Bhagalpur and 10 g of the live plant after washing with double distilled water, was dipped into 100 ml 10 ppm sodium arsenite solution with and without nutrient support up to 24 h, 48 h, and 72 h. The residual concentration of arsenic has been measured by Merckoquant arsenic kit and further confirmed by ICP – OES at wavelength 193.695 nm. Arsenic uptake by different parts of the live plants such as root, stem and leaves has been analysed by Inductively Coupled Plasma – Optical Emission Spectroscopy (ICP – OES). Plant samples were first ashed at 100^{o}C, kept for 2 hours in a porcelain crucible. Sample residue was fused with phosphate /alkali fluoride flux and estimations were made by ICP – OES at a wavelength 193.695.HPLC of *Aloe – vera*and *Withaniasomnifera*has been done before and after treatment with As(III) ion. The extract of *Aloe – vera*was prepared by repeatedly washing with double distilled water and dissolved in a mixture of methanol and water. Similar method was adopted for the preparation of extract of *Withaniasomnifera*. The detection wavelength for HPLC was fixed at 254 nm, 280 nm and 329 nm as several components absorbed light at these wavelengths and consequently analysis became easy. UAL and AL stand for untreated and treated *Aloe – vera*samples for HPLC whereas US and S_2(L) represent untreated and treated *Withaniasomnifera* samples respectively. C-1 to C-6 samples stand for treatment with *Withaniasomnifera*whereas D-1 to D-6 samples stand for treatment with *Aloe-vera*. L, R, and S stand for leaf, root and stem, respectively.5 ml micronutrient of Murashige and Skoog basal solution (10X) of Himedia was added to the medium to overcome the plant stress.

3. Results and Discussion

The residual concentrations of 100 ml 10ppm As(III) solution after treatment with 10 g of *Withaniasomnifera* and *Aloe – vera* up to 72 h are 5.5 and 8.2 ppm respectively(Table1). Maximum percentage removal has been recorded at 72 hours with *Withaniasomnifera*. Table 2 shows the arsenic content in stem, root and leaves.

Table 3 shows the residual concentration of As(III) with an initial concentration of 2 ppm atpH 7, whereas Table 4 shows residual concentration with an initial concentration of 5 ppm.Different initial concentrations and pH values have been chosen for study to arrive at a suitable condition.Arsenic uptake by different parts of the plants such as root, stem and leaves has been shown in Table 2. It was clear from the Table 2 that maximum uptake of As(III) took place by the root of the plant.Figure 1 shows that maximum uptake of As(III) by *Aloe-vera*and *Withaniasomnifera* takes placeat a pH 2. Figure 2 shows that percentage removal of As(III) at an initial concentration of 5 ppm is maximum. Values of q_t, c_t/q_t, log q_t and log C_t at different contact times at pH 2 for As(III) removal are shown in Table 5.Linearity in Figure 4 clearly indicated that phytoremediation of As(III) by *Aloe-vera*and *Withaniasomnifera* follows pseudo second order kinetic model. From Figure 5, it appears that intra-particle diffusion model is not applicable.HPLC results shown in Fig.8,9, 10 &11 clearly show the different peaks before and after adsorption of As(III) by *Aloe-vera*and *Withaniasomnifera*. HPLC of untreated and treated *Aloe-vera* in (Figures 8 and 9) clearly showed the change in peaks. The change in peaks explained that As(III) ions was adsorbed on the surface either through penetration into the inner matrix or surface complexation. Similar change in peaks was observed in HPLC of untreated and treated *Withaniasomnifera*(Figures 10 and 11).The uptake of As(III) by plants during phytoremediation appeared more complex[22-23].The transport of arsenic from the root into xylem takes place and translocated to the shoot where it gets sequestered in the vacuole[24-26].ACR3 gene in the plant species may be responsible for arsenic tolerance. Though ACR3 is explained in all parts of the plant, the root of the plant accumulates greater amount of arsenite. The roots of *Withaniasomnifera*contain steroidal alkaloids and lactones. Arsenite complex is formed through S-containing ligands such as glutathione and phytochelatins which resists in the transportation of As(III) –thiol complexes into vacuoles[27]. Thus, free As(III) ion concentration is reduced. Experimental data show that roots accumulated more As(III) ions than stem and leaves.One of the possiblemechanisms is the entry of arsenite through the root nodulin 26-like intrinsic proteins, a group of aquaporin channel.Other mechanisms are yet to be explored. The pathways and mechanisms can only be unveiled by intrinsic research. The maximum removal of 98.4% takes place at 5 ppm on pH 2 and initial concentration by *Withaniasomnifera*and *Aloe- vera*at 48 hours. So, optimum condition of removal of As(III) with *Aloe – vera*and *Withaniasomnifera*is as follows: Initial concentration = 5 ppm, pH = 2, Time = 48 hours.At 72 hours some release of As(III) takes place due to occupation of all the vacant sites leading to saturation. The occupied sites start process of desorption till the equilibrium is attained.

Table 1 (a): Residual concentration of 100 ml of 10 ppm As(III) after treatment with *Withaniasomnifera*.

Sample no.	Plant name	Weight oflive plantin g	Timeintervalin hours	Medium	Residual concentrationof Arsenic in ppm	pH
C-1	*Withaniasomnifera*	10	24 h	Distilledwater	6.3	7
C-2	*Withaniasomnifera*	10	48 h	Distilledwater	8.5	7
C-3	*Withaniasomnifera*	10	72 h	Distilledwater	5.5	7
C-4	*Withaniasomnifera*	10	24 h	Distilledwater withmicronutrients	8.4	7
C-5	*Withaniasomnifera*	10	48 h	Distilledwater withmicronutrients	7.9	7
C-6	*Withaniasomnifera*	10	72 h	Distilledwater withmicronutrients	8.5	7

Table 1 (b): Residual concentration of 100 ml of 10 ppm As(III) after treatment with *Aloe – vera.*

Sample no.	Plant name	Weight ofliveplantin g	Time interval in hours	Medium	Residual concentration of Arsenic in ppm	pH
D-1	*Aloe –vera*	10	24	DistilledWater	8.3	7
D-2	*Aloe -vera*	10	48	DistilledWater	9.1	7
D-3	*Aloe –vera*	10	72	DistilledWater	8.2	7
D-4	*Aloe –vera*	10	24	Distilledwater withmicronutrients	8.4	7
D-5	*Aloe –vera*	10	48	Distilledwater with micronutrients	8.5	7
D-6	*Aloe –vera*	10	72	Distilledwater with micronutrients	8.5	7

Table 2 (a): Arsenic content in root, and leaves of *Aloe – vera*after treatment with As(III) solution.

Serial no.	Sample No.	Weight of plant in g	Plant name	Plant Part	Time interval in hours	Residual concentration of As in ppm	Medium	pH
1	D1-L	10	*Aloe-vera*	Leaves	24	17.4	Distilled water	7
2	D1-R	10	*Aloe-vera*	Root	24	63.0	Distilled water	7
3	D2-L	10	*Aloe-vera*	Leaves	48	53.8	Distilled water	7
4	D2-R	10	*Aloe-vera*	Root	48	84.1	Distilled water	7
5	D3-L	10	*Aloe-vera*	Leaves	72	30.3	Distilled water	7
6	D3-R	10	*Aloe-vera*	Root	72	164.2	Distilled water	7
7	D4-L	10	*Aloe-vera*	Leaves	24	27.4	Distilled water+ micronutrients	7
8	D4-R	10	*Aloe-vera*	Root	24	55.7	Distilledwater+ micronutrients	7
9	D5-L	10	*Aloe-vera*	Leaves	48	68.3	Distilled water+ Micronutrients	7
10	D5-R	10	*Aloe-vera*	Root	48	77.0	Distilled water+Micronut rients	7
11	D6-L	10	*Aloe-vera*	Leaves	72	50.2	Distilled water+ Micronutrients	7
12	D6-R	10	*Aloe-vera*	Root	72	103.5	Distilled water+ Micronutrients	7

Table 2 (b): Arsenic content in root,stem and leaves of*Withaniasomnifera*after treatment with As(III) solution.

Sample name	Weight of plantin g	Plantname	Plant part specification	Timeinterval in hours	Residualconcentration of As in ppm	Medium	pH
C1-L	10	*Withaniasomnifera*	Leaves	24	5.00	Distilled water	7
C1-R	10	*Withaniasomnifera*	Root	24	44.00	Distilled water	7
C1-S	10	*Withaniasomnifera*	Stem	24	22.2	Distilled water	7
C2-L	10	*Withaniasomnifera*	Leaves	48	71.7	Distilled water	7
C2-R	10	*Withaniasomnifera*	Root	48	49.4	Distilled water	7
C2-S	10	*Withaniasomnifera*	Stem	48	29.8	Distilled water	7
C3-L	10	*Withaniasomnifera*	Leaves	72	15.7	Distilled water	7
C3-R	10	*Withaniasomnifera*	Root	72	55.5	Distilled water	7
C3-S	10	*Withaniasomnifera*	Stem	72	17.7	Distilled water	7
C4-L	10	*Withaniasomnifera*	Leaves	24	11.3	Distilled water+ Micronutrients	7
C4-R	10	*Withaniasomnifera*	Root	24	37.1	Distilled water+ Micronutrients	7
C4-S	10	*Withaniasomnifera*	Stem	24	10.0	Distilled water+ Micronutrients	7
C5-L	10	*Withaniasomnifera*	Leaves	48	20.7	Distilled water+ micronutrients	7
C5-R	10	*Withaniasomnifera*	Root	48	70.0	Distilledwater+ Micronutrients	7
C5-S	10	*Withaniasomnifera*	Stem	48	14.8	Distilledwater+ Micronutrients	7
C6-L	10	*Withaniasomnifera*	Leaves	72	12.6	Distilled water + Micronutrients	7
C6-R	10	*Withaniasomnifera*	Root	72	54.8	Distilled water + Micronutrients	7
C6-S	10	*Withaniasomnifera*	Stem	72	28.9	Distilled water + Micronutrients	7

Table 3 (a): Residual concentration of 100ml of 2 ppm As (III) after treatment with*Withaniasomnifera*at pH 7.

SampleNo.	Plant name	Weight oflive plantin g	Timeintervalin hours	Medium	Residualconcentration ofAs (III) in ppm	pH
E1	*Withaniasomnifera*	10	24	Distilled water	0.25	7
E2	*Withaniasomnifera*	10	48	Distilled water	0.25	7
E3	*Withaniasomnifera*	10	72	Distilled water	0.25	7
E4	*Withaniasomnifera*	10	24	Distilled water + Micronutrients	0.25	7
E5	*Withaniasomnifera*	10	48	Distilled water + Micronutrients	0.25	7
E6	*Withaniasomnifera*	10	72	Distilled water + Micronutrients	0.25	7

Table 3 (b): Residual concentration of 100 ml of 2 ppm As (III) after treatment with *Aloe- vera* at pH 7.

Sample No.	Plant name	Weight oflive plantin g	Timeinterval in hours	Medium	Residualconcentration of As (III) in ppm	pH
F1	*Aloe-vera*	10	24	Distilled water	0.30	7
F2	*Aloe-vera*	10	48	Distilled water	0.30	7
F3	*Aloe-vera*	10	72	Distilled water	0.25	7
F4	*Aloe-vera*	10	24	Distilled water + Micronutrients	0.30	7
F5	*Aloe-vera*	10	48	Distilled water + Micronutrients	0.30	7
F6	*Aloe-vera*	10	72	Distilled water + micronutrients	0.25	7

Table 4 (a): Residual concentration of 100 ml of 5ppm As(III) at different pH values after treatment with*Withaniasomnifera*.

Sample No.	Weight oflive plant *(Withaniasomnifera)*	Time intervals in hours	Medium	Residual concentration in ppm	pH
BC1	10 g	24	Distilledwater	0.40	7
BC2	10g	48	Distilled water	0.40	7
BC3	10g	72	Distilledwater	0.30	7
BC4	10g	24	Distilled water+Micronutrients	0.50	7
BC5	10g	48	Distilled water+Micronutrients	0.45	7
BC6	10g	72	Distilled water+Micronutrients	0.30	7
BC7	10g	24	Distilled Water	0.10	2
BC8	10g	48	Distilled Water	0.08	2
BC9	10g	72	Distilled Water	0.40	2
BC10	10g	24	Distilled water +Micronutrients	0.10	2
BC11	10g	48	Distilled water +Micronutrients	0.08	2
BC12	10g	72	Distilled water +Micronutrients	0.18	2

Table 4 (b): Residual concentration of 100 ml 5ppm As(III) at different pH values after treatment with*Aloe-vera.*

Sample No.	Weight oflive plant (*Aloe-vera)*	Time interval in hours	Medium	Residual concentration in ppm	pH
BC13	10g	24	Distilled water	0.40	7
BC14	10g	48	Distilled water	0.40	7
BC15	10g	72	Distilled water	0.30	7
BC16	10g	24	Distilled water+Micronutrients	0.50	7
BC17	10g	48	Distilled water+Micronutrients	0.45	7
BC18	10g	72	Distilled water+Micronutrients	0.40	7
BC19	10g	24	Distilled water	0.10	2
BC20	10g	48	Distilled water	0.08	2
BC21	10g	72	Distilled water	0.18	2
BC22	10g	24	Distilled water+Micronutrients	0.10	2
BC23	10g	48	Distilled water+Micronutrients	0.08	2
BC24	10g	72	Distilled water+Micronutrients	0.10	2

Table 5: Value of q_t, C_t / q_t, log q_t and log C_t at different contact times at pH 2 for As(III) removal.

Initial concentration in ppm	Plantspecies	Timein hours	Residual concentration mg/L (C_t)	As(III)adsorbedmg/L	q_t	C_t/q_t	logC_t	logq_t
5	*Withaniasomnifera*	24	0.10	4.90	0.490	0.204	-1	-0.309
5	*Withaniasomnifera*	48	0.08	4.92	0.492	0.163	-1.096	-0.3080
5	*Withaniasomnifera*	72	0.40	4.60	0.460	0.870	-0.3979	-0.3372
5	*Aloe-vera*	24	0.10	4.90	0.490	0.204	-1	-0.3095
5	*Aloe-vera*	48	0.08	4.92	0.492	0.163	-1.096	-0.3080
5	*Aloe-vera*	72	0.18	4.82	0.482	0.373	-0.744	-0.3169

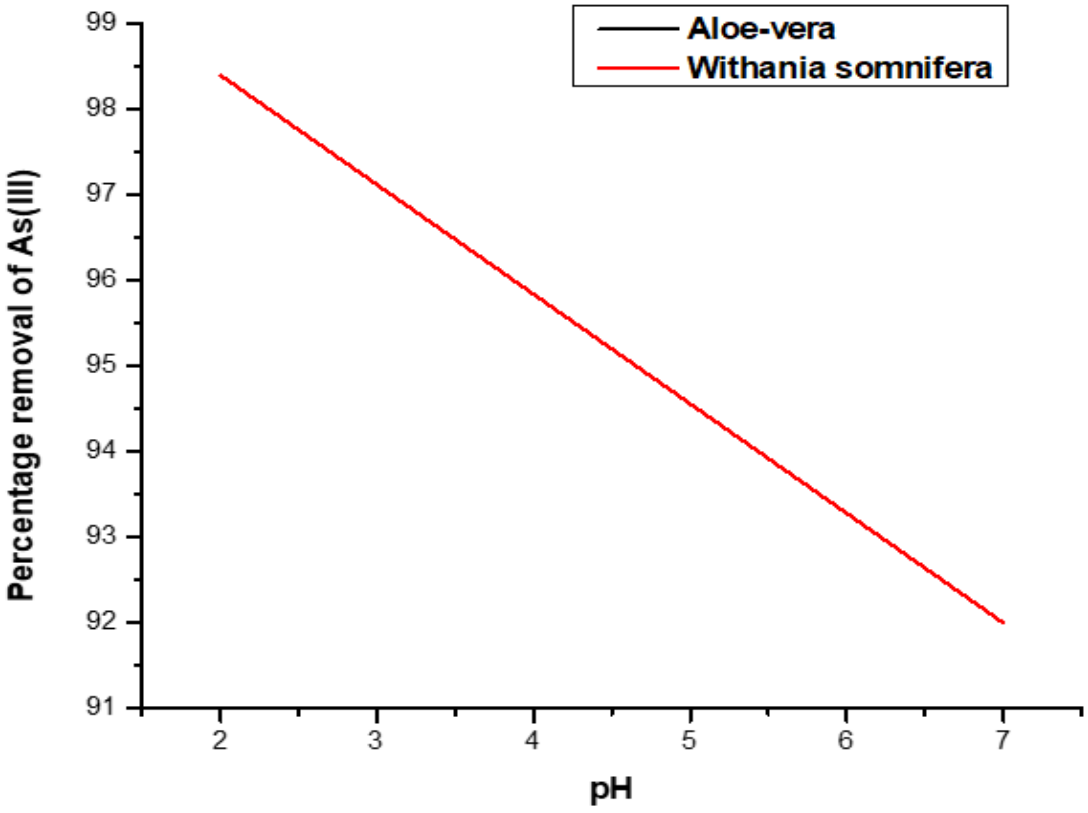

Figure 1: Percentage removal of As (III) versus pH.

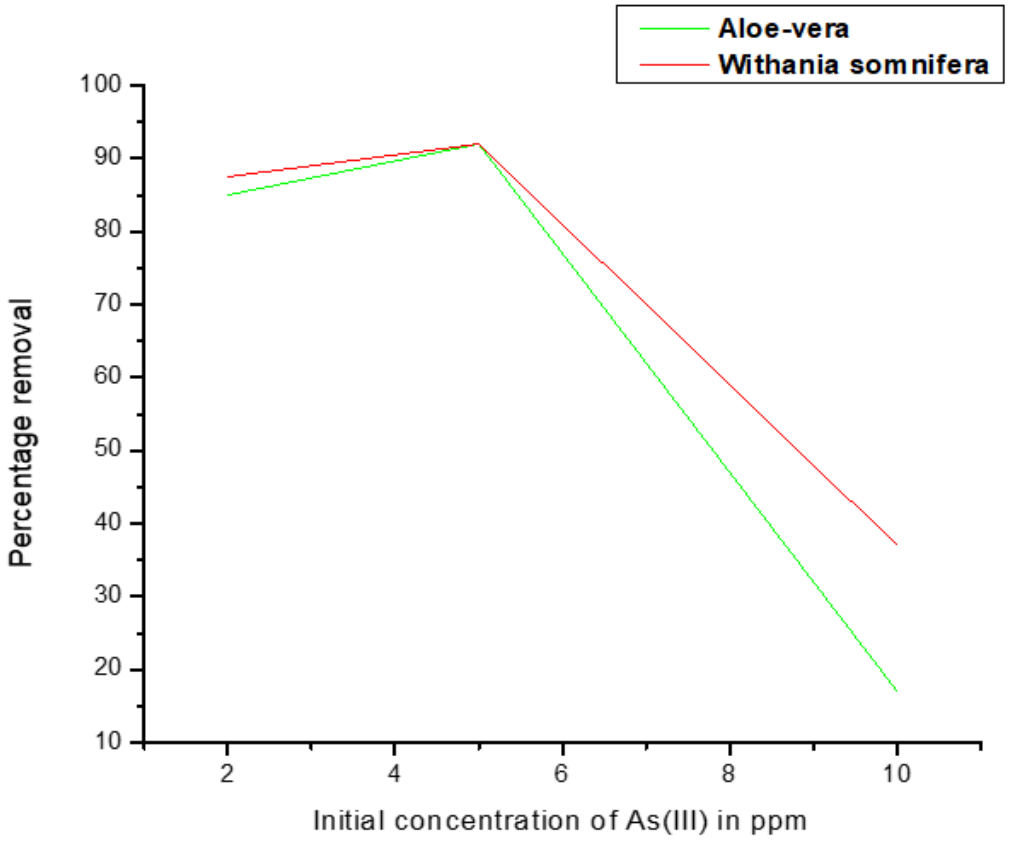

Figure 2: Percentage removal of As (III) versus initial concentration in ppm.

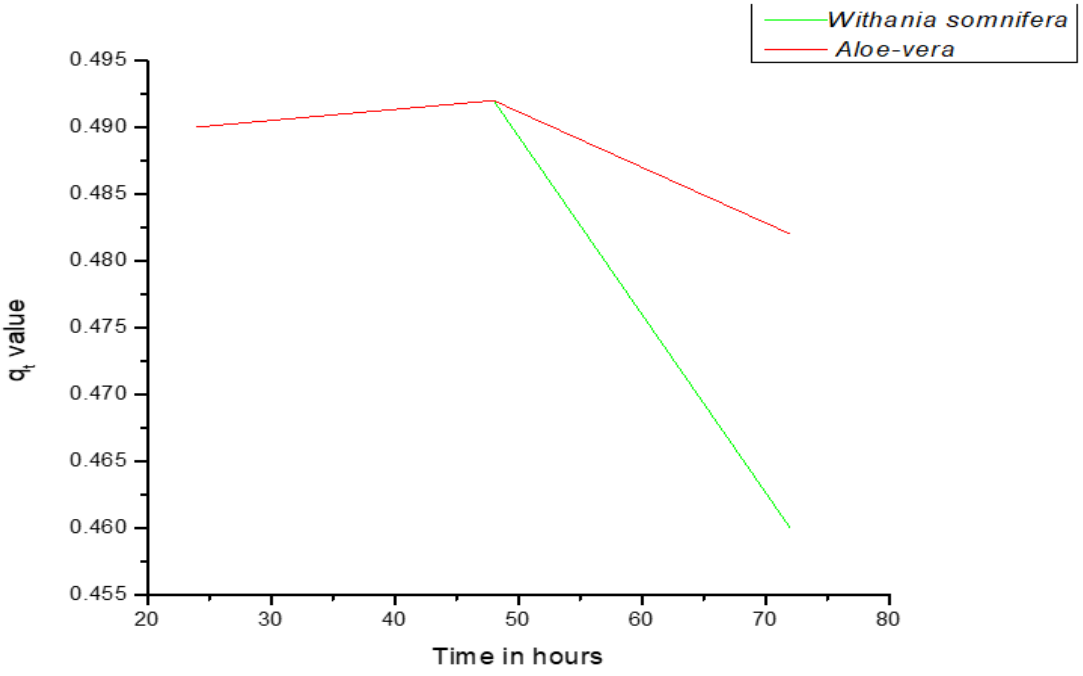

Figure 3: q_t versus time in hours.

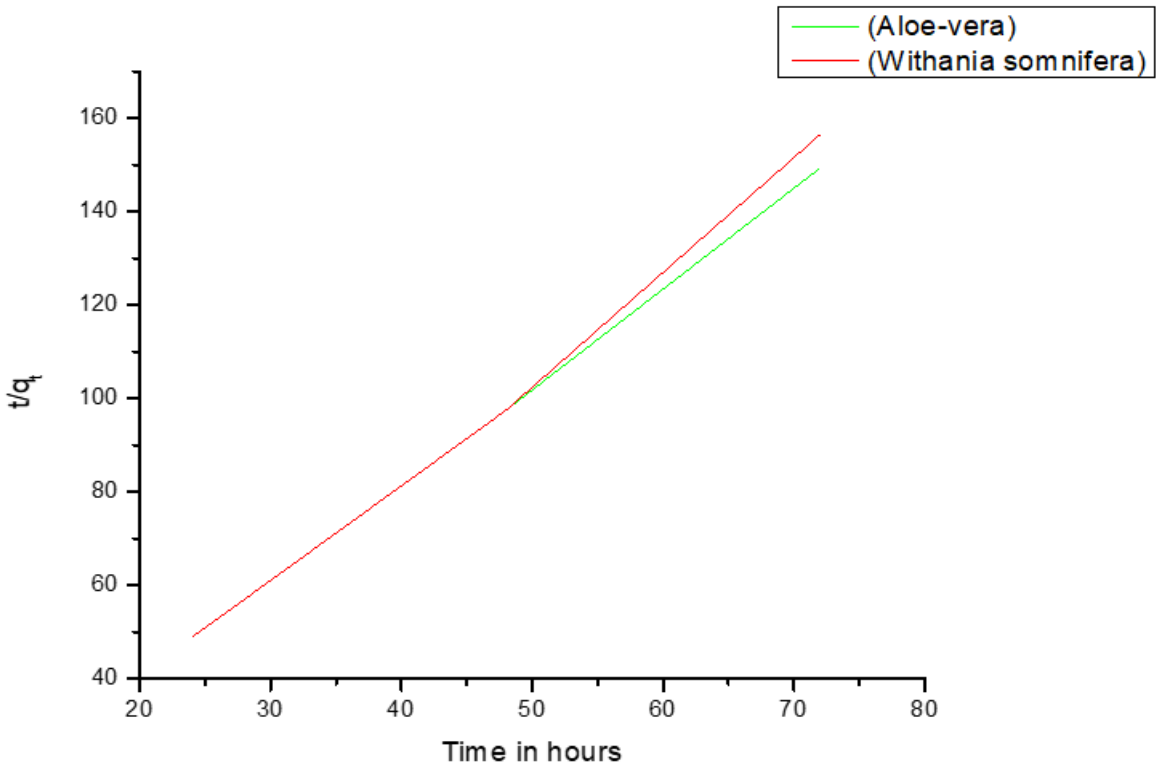

Figure 4: t/q_t versus time in hours.

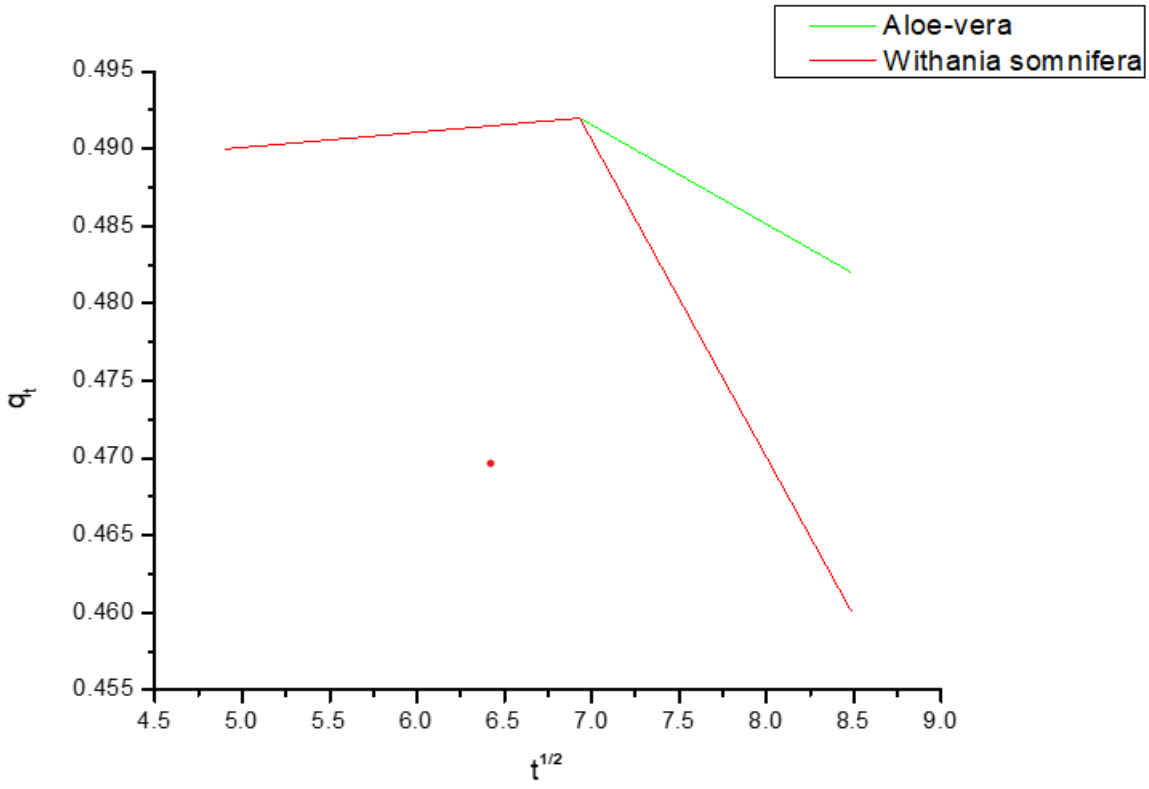

Figure 5: $q_t$versus $t^{1/2}$.

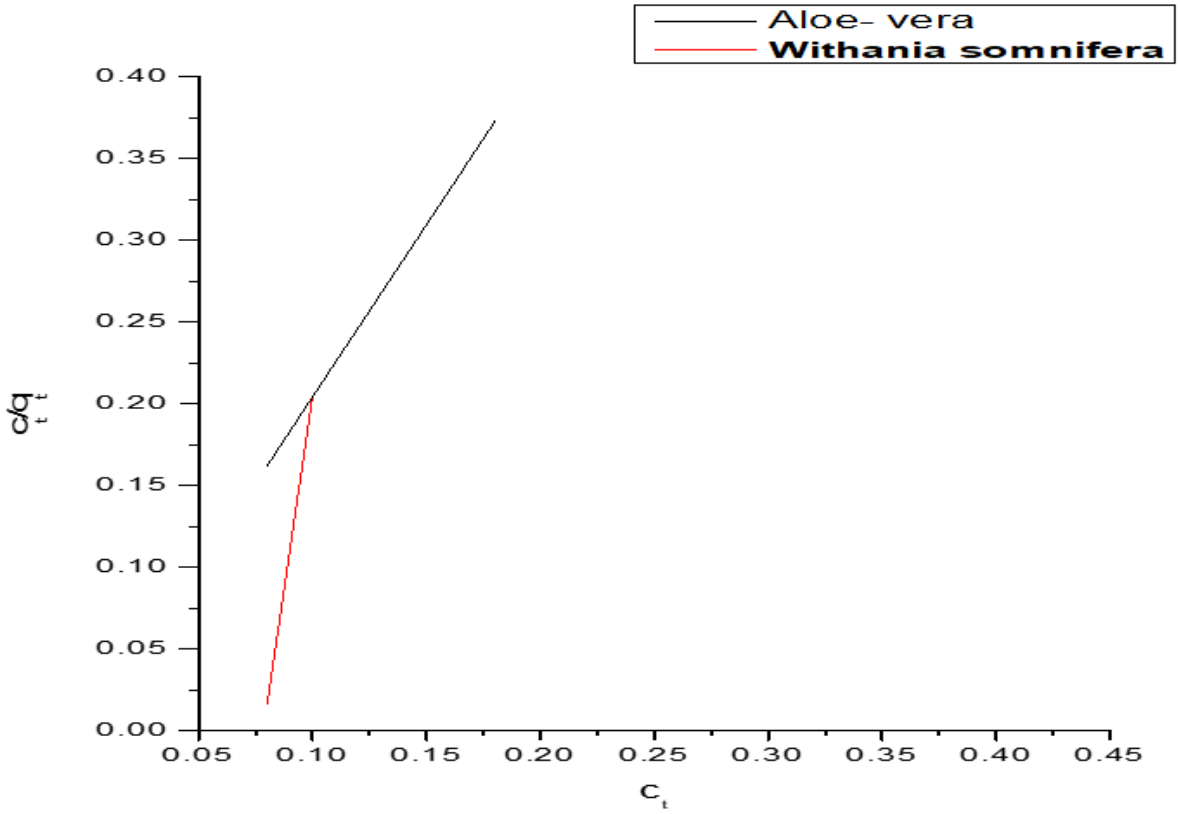

Figure 6: C_t/q_t versus C_t.

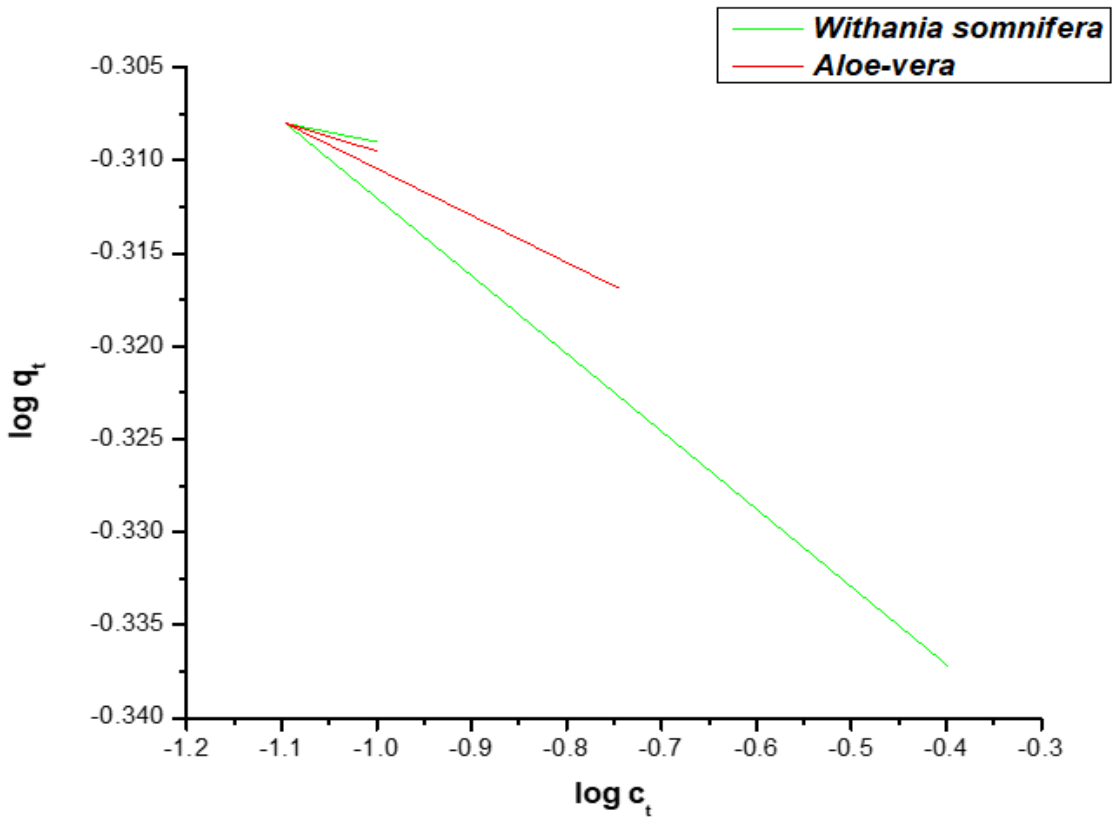

Figure 7: log C_t versus log q_t.

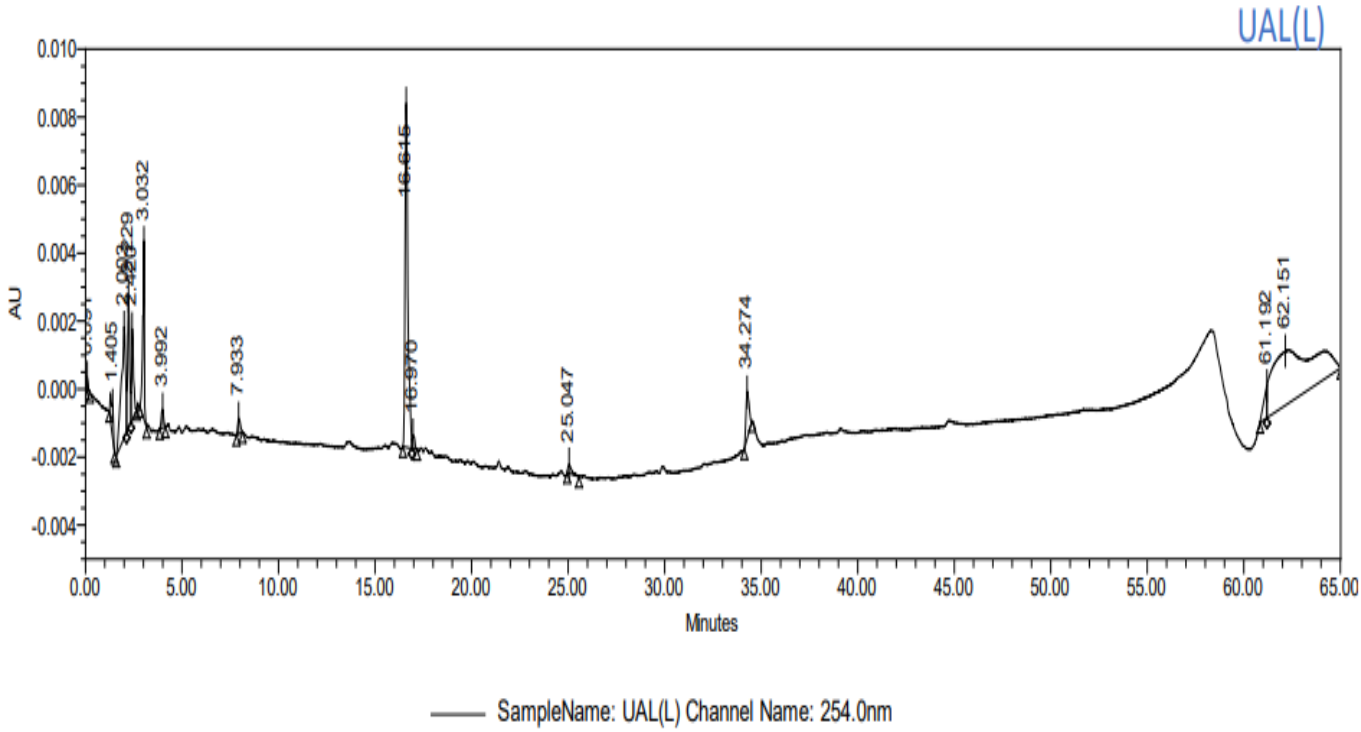

Figure 8(a):UAL(L) at 254 nm.

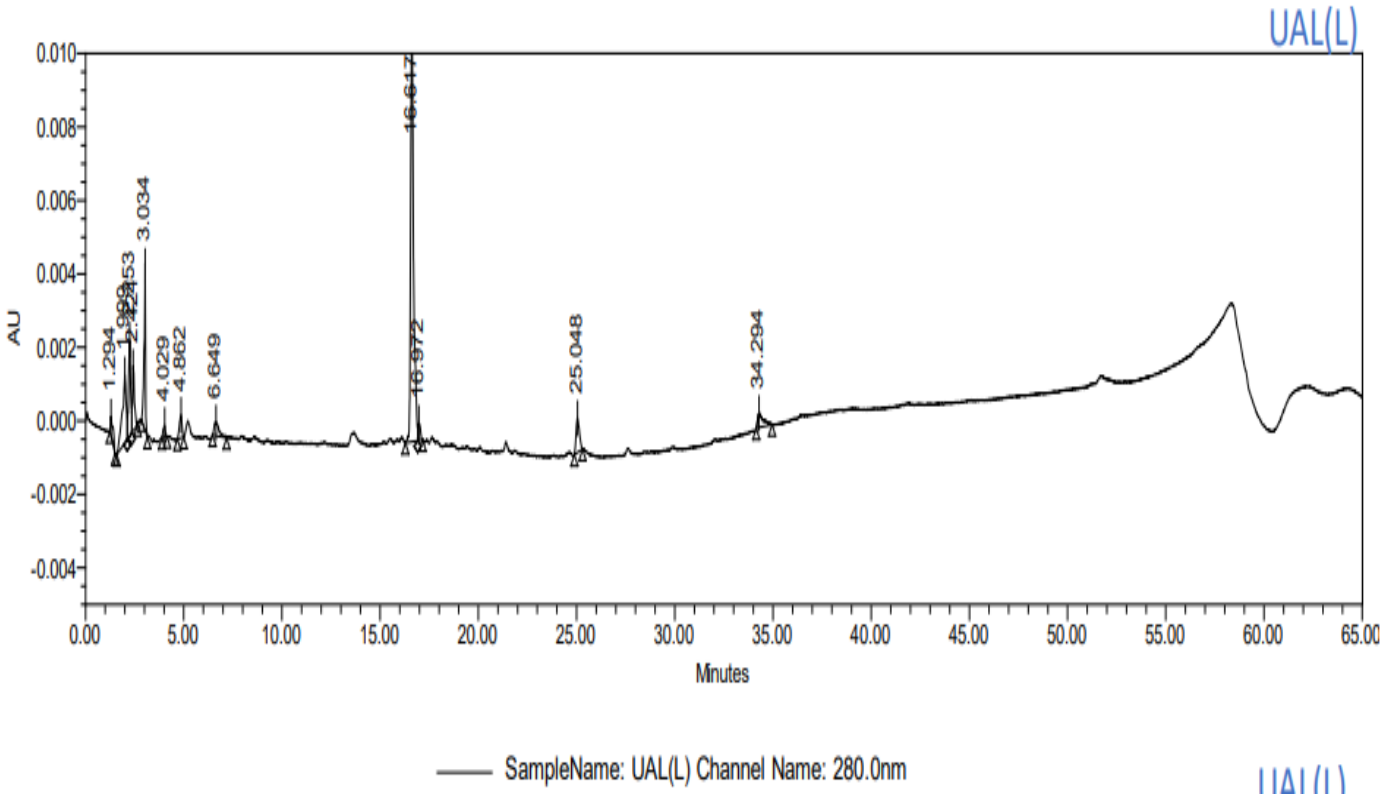

Figure 8 (b):UAL(L) at 280 nm.

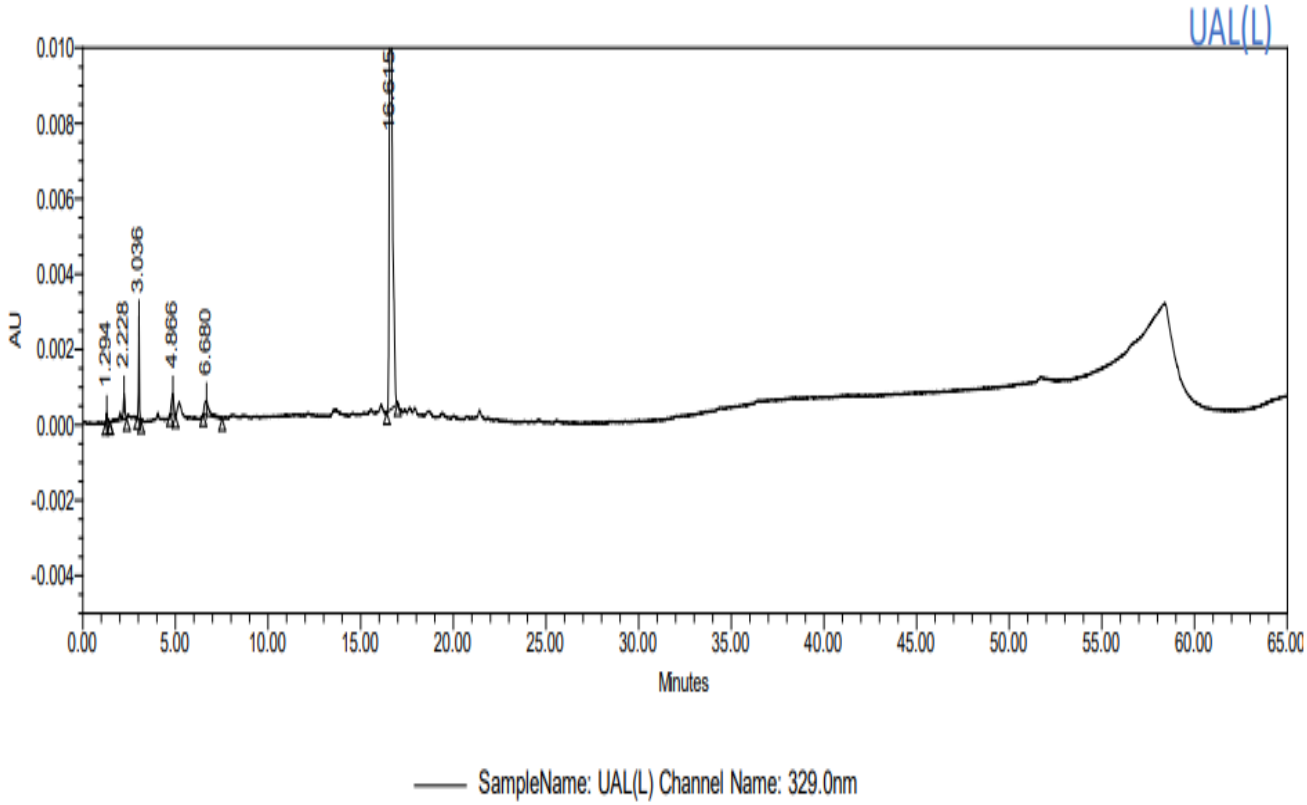

Figure8 (c):UAL(L) at 329 nm.

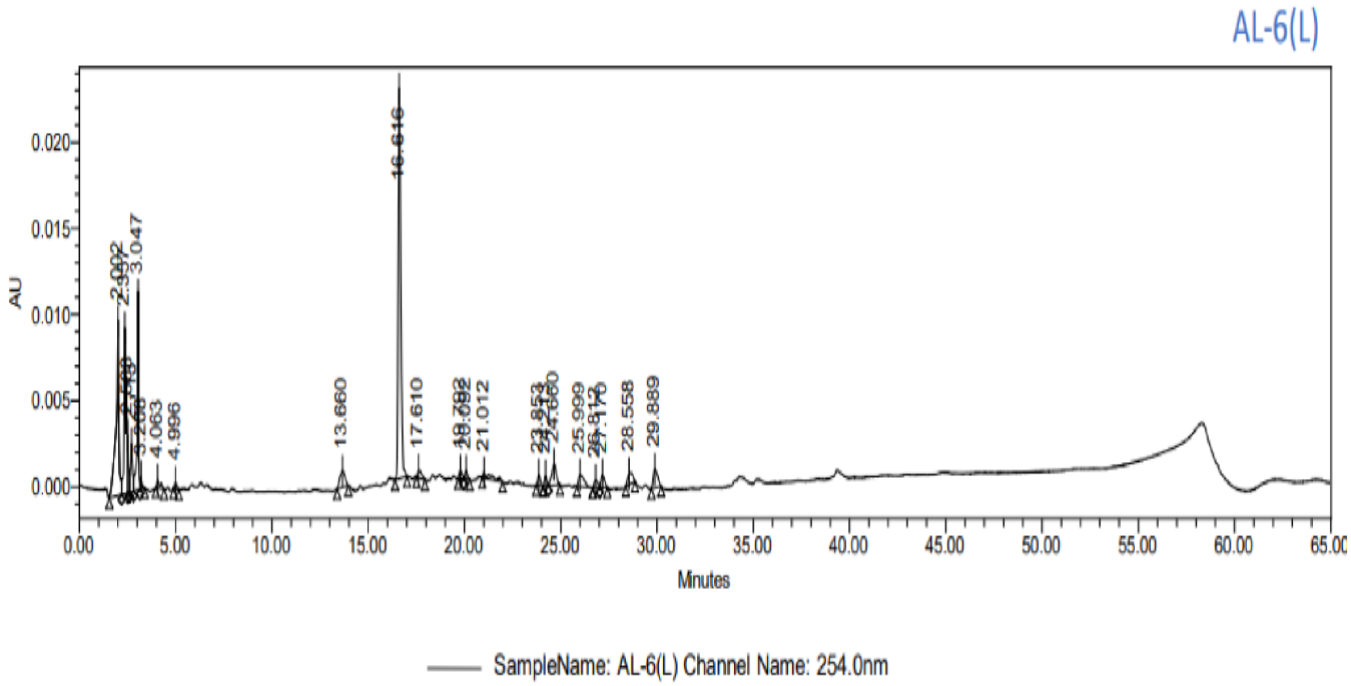

Figure 9 (a): AL-6(L) at 254 nm.

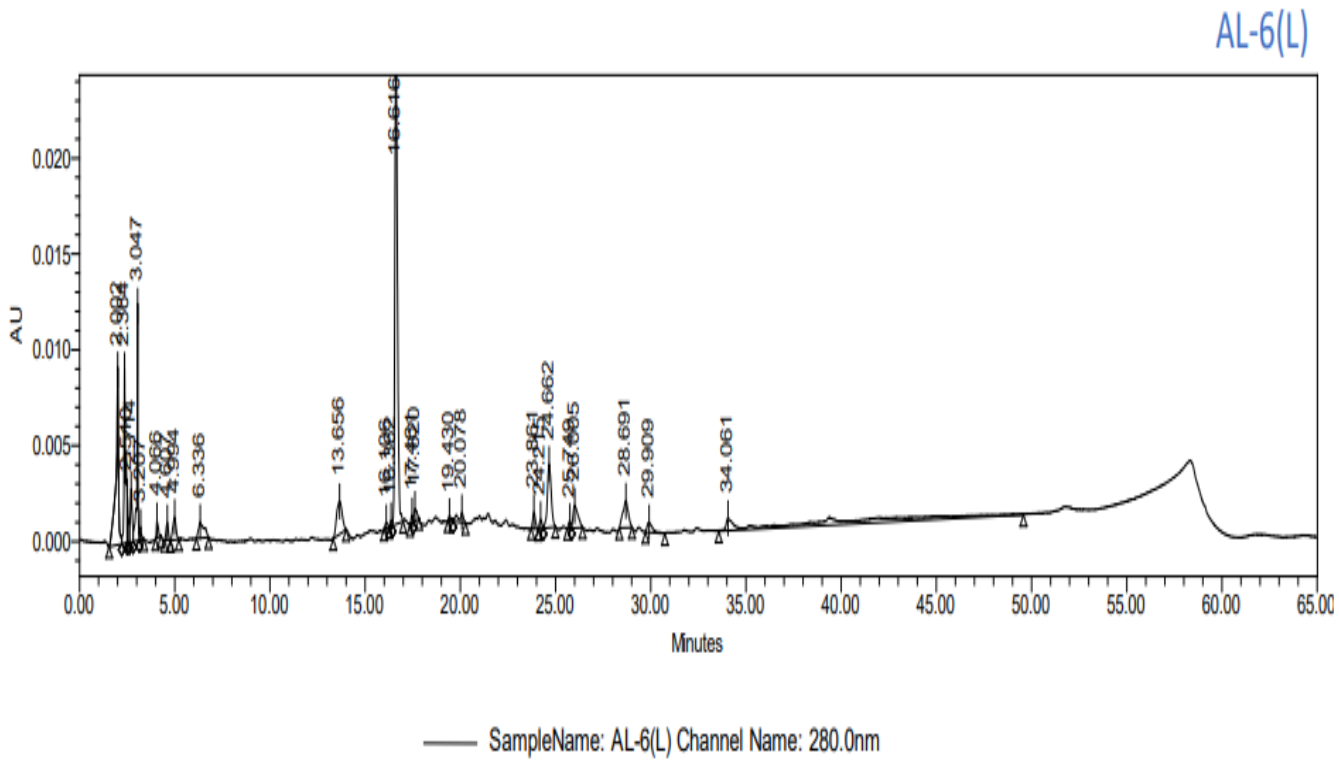

Figure 9 (b): AL-6(L) at 280 nm.

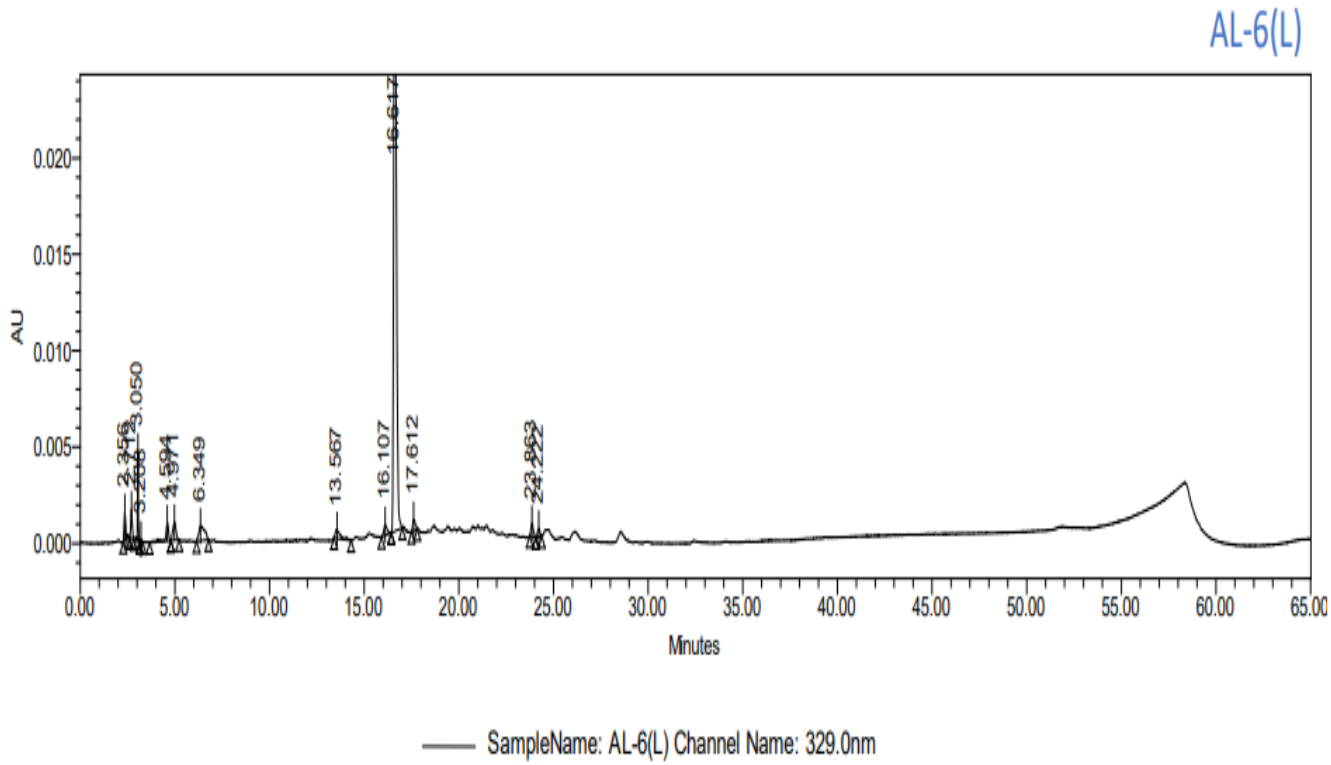

Figure 9(c): AL-8(L) at 329 nm.

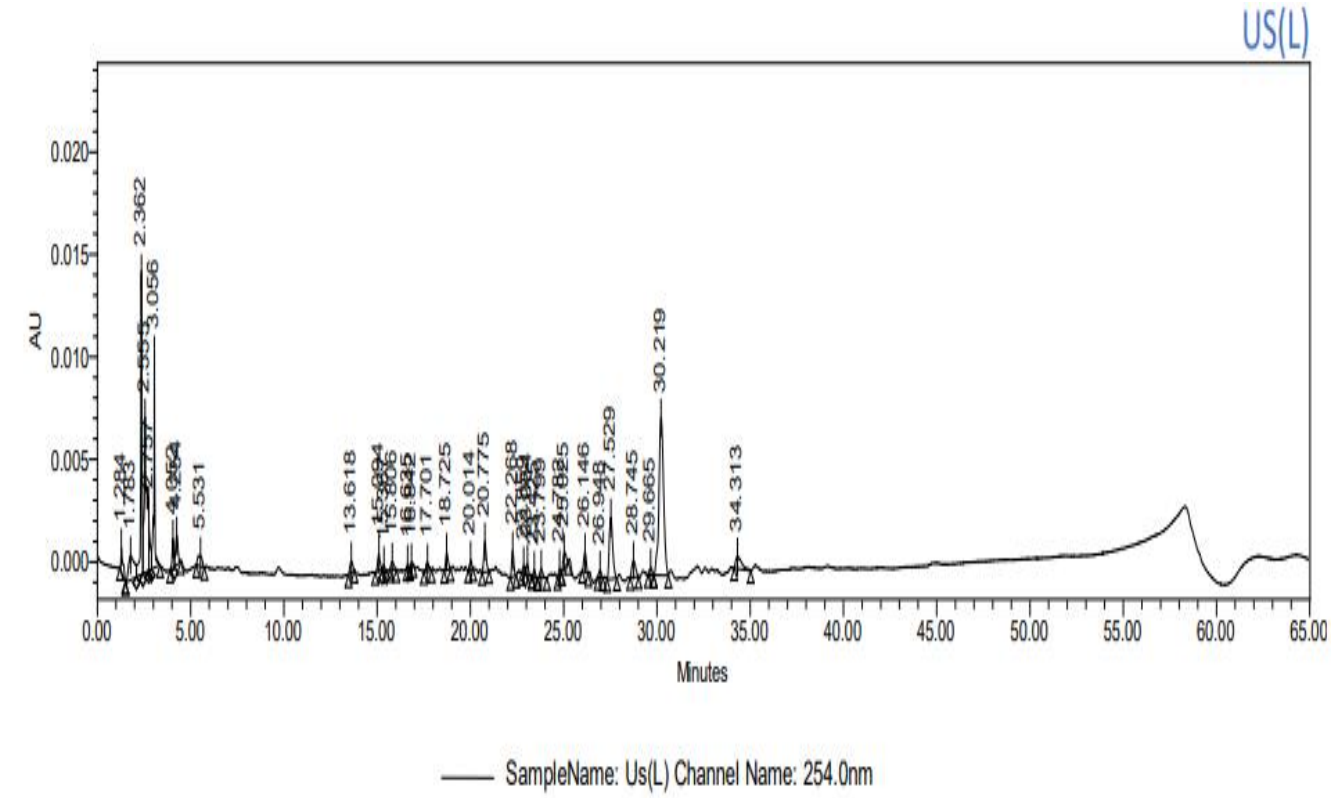

Figure 10 (a):US(L) at 254 nm.

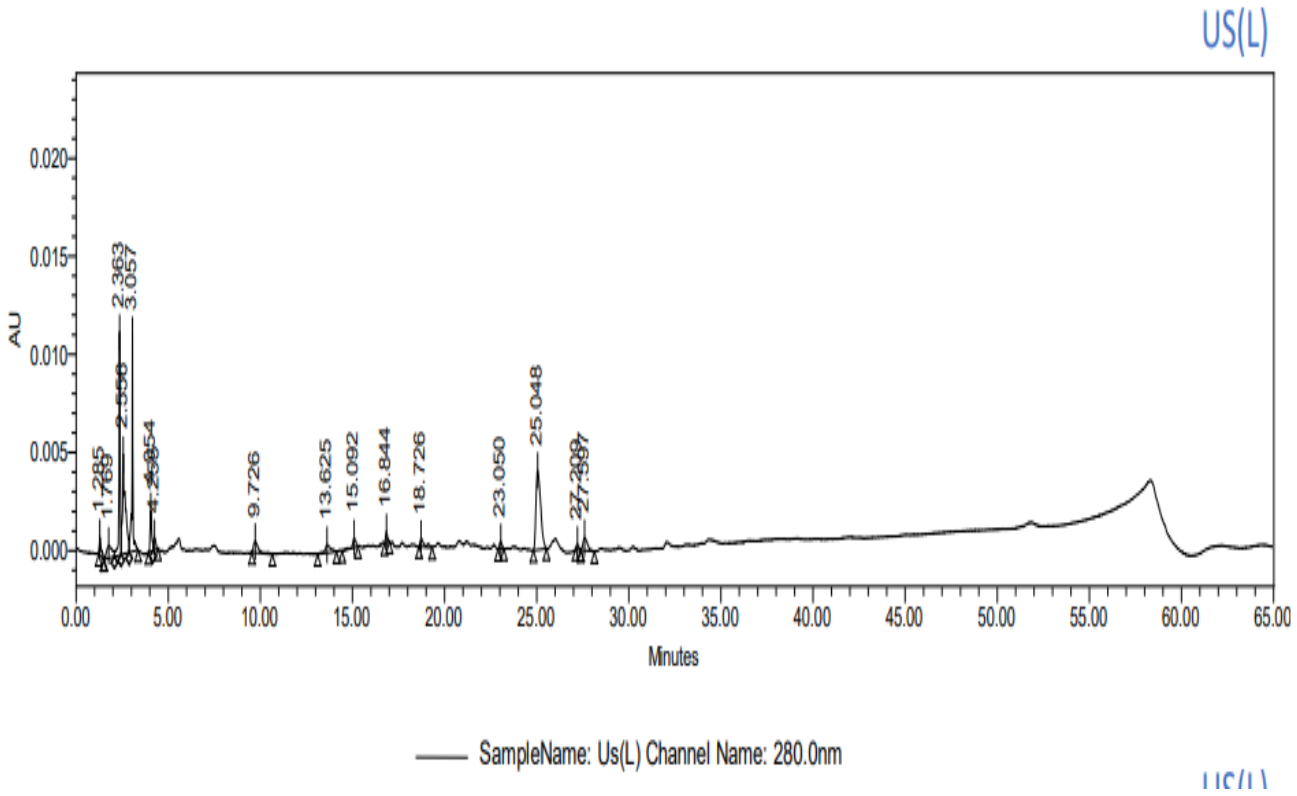

Figure 10 (b):US(L) at 280 nm.

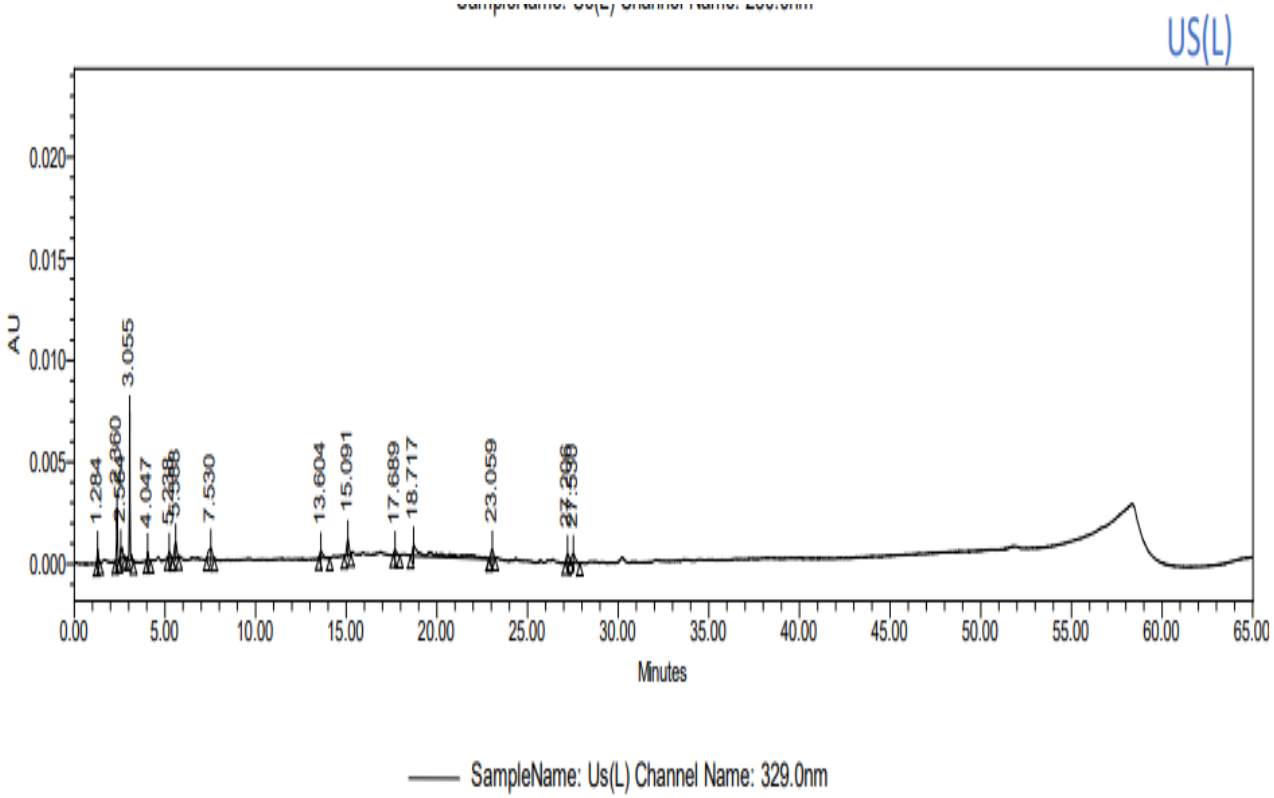

Figure 10 (c):US(L) at 329 nm.

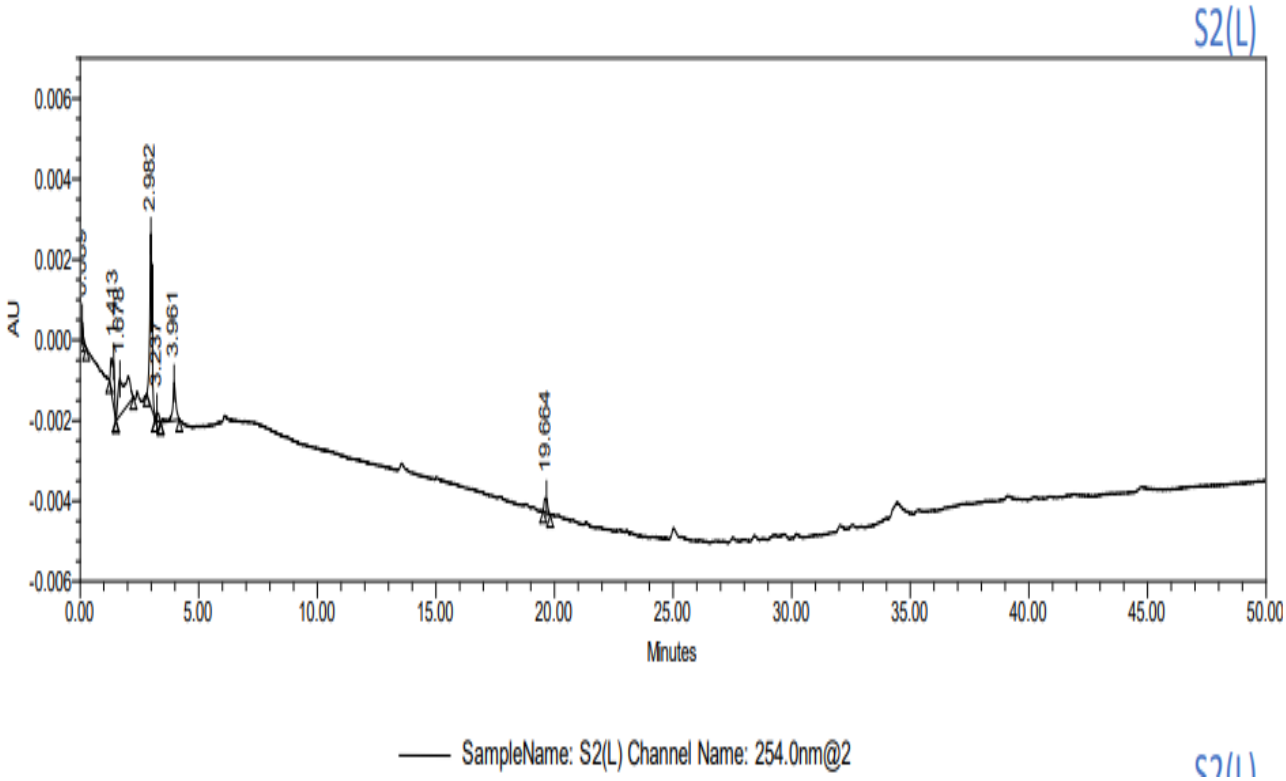

Figure 11 (a):S2(L) at 254 nm.

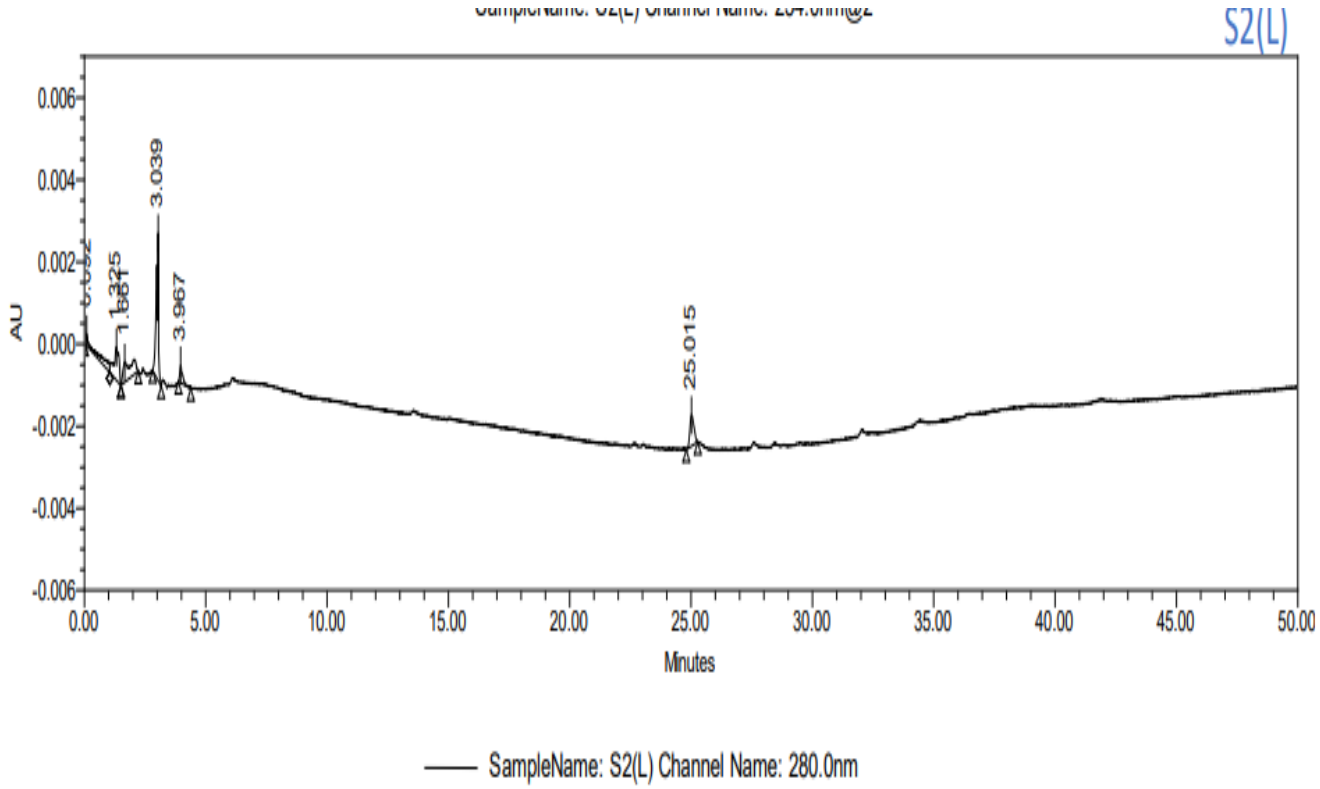

Figure 11 (b):S2(L) at 280 nm.

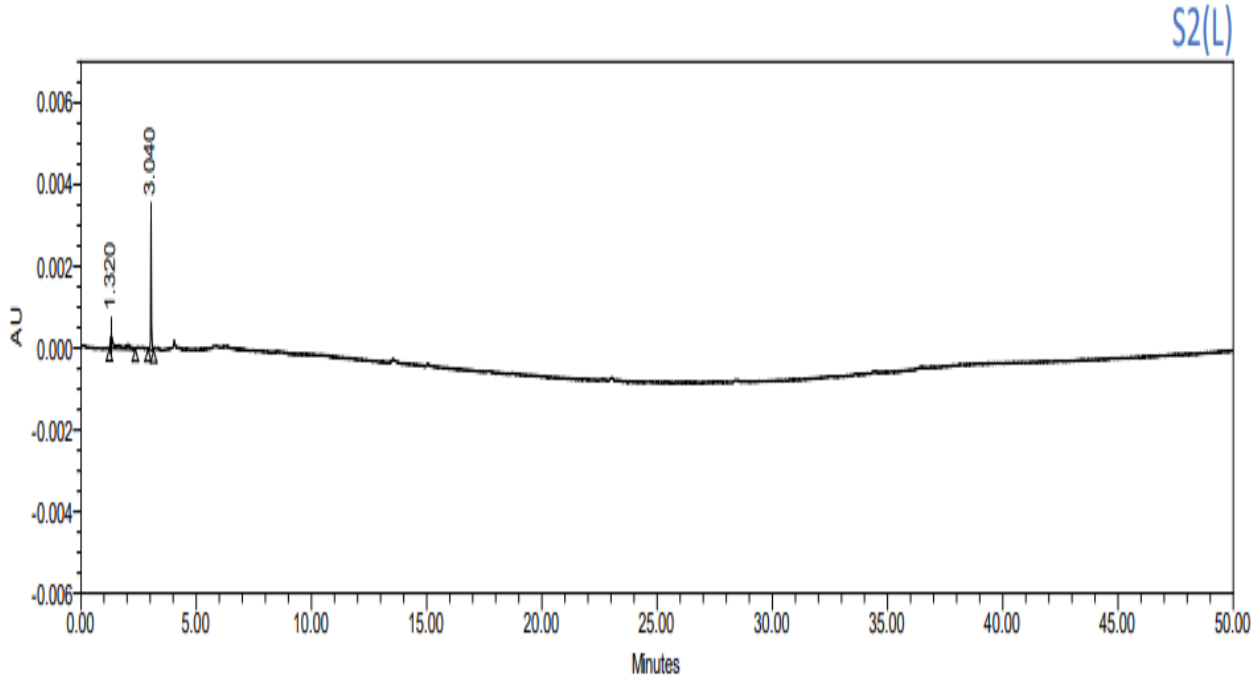

Figure 11 (c): S2(L) at 329 nm.

4. Conclusions

Psudo second order kinetic model is followed in the phytoremediation of As(III) by *Aloe-vera* and *Withaniasomnifera.* Pseudo second order kinetic model is the best fit for phytoremediation but intra- particle diffusion does not take place. Both the plants have the potential to remove arsenic from aqueous medium. Higher accumulation of As(III) by *Withaniasomnifera* and *Aloe-vera*cautions towards the uncontrolled use of medicinal plants as medicine.

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