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RESEARCH ARTICLE

FUNCTIONAL GROUPS AND COMPOUNDS ANALYSIS USING CHROMATOGRAPHIC AND SPECTROSCOPIC TECHNIQUES OF *SESBANIA GRANDIFLORA* GROWN IN UNTREATED AND BIO REMEDIATED SILK DYEING EFFLUENT

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ABSTRACT

The unique low cost effective technology for the slender entrepreneurs of Silk dyeing effluent has been planned and executed. Environmental outlets like Silk dyeing effluent are harmful and needs a high cost Common Effluent Treatment Plant (CETP) to achieve Zero effluent discharge limits which is not affordable for low investers. In remediating this effluent using the Biofertilizers with multifunctioning activities with Biodegradation, Biocontrol and Plant growth promoting properties were selected. TheGreen leafy vegetable *Sesbania grandiflora* sowed seeds was treated withraw silk dyeing effluent, *Pseudomonas fluorescens* and *Azospirillum sp.* Biofertilizers separately. After 45th days the GLV's extracts *Sesbania grandiflora* were grown in fresh water (SGN), *Sesbania grandiflora* were grown in crude effluent (SGE) and *Sesbania grandiflora* were grown in biotreated effluent (SGT) were subjected to UV, FTIR and HPLC analysis. Thus from the functional group studies by FT-IR, the alcohol, alkane, alkyl halide and amine groups were found in all the GLV treatments, even in crude effluent, the plants managed to synthesize these organic compounds. The amide group was completely absent in the treatments. The isocyanide group was found only in *S.grandiflora* grown in fresh water, which was unable to synthesize isocyanide group in plants grown in crude effluent and biotreated effluent. Irrespective of the treatments, the aromatic groups were synthesized by *S.grandiflora* in biotreated effluent. The HPLC analysis of *S.grandiflora* grown in fresh water revealed 6 peaks of retention time 2.6, 4.8, 6.4, 15.1, 15.3 and 17.4 minutes of which only 3 peaks were present in *S.grandiflora* grown in effluent water. In the biotreated plant 4 peaks with the retention time of 2.5, 4.4, 6.6 and 11.2 minutes were found.

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INTRODUCTION

Water detoxification has become an alarming trend worldwide caused by industrial effluent discharges with heightened concentration of nutrients, sediments and toxic substances. It has become a question of extensive public and scientific concern in the light of evidence of their acute toxicity to human health and to biological ecosystems (Hassan *et al.*, 2013). The textile industry interprets on behalf two-third of the total dyestuff market. More than 10,000 diversified textile dyes with a probable annual production of $7x10^5$ metric tons are commercially accessible worldwide (Aksu, 2005). Wastewater outlets in printing and dyeing units is frequently rich in color, containing residues of dyes and chemicals and needs proper treatment before being released into the environment.

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Without adequate treatment, these dyes can remain in the environment for a longer period of time (Allegre *et al*., 2006). The silk industry comprises water intensive processes that need large volume of good quality of water. The silk industry customs a lot of dyes generating about 3773 metric tons per annum (MTA) of hazardous waste of which only 3.25% is recyclable. Process of dyeing emits volatile organic compounds (VOCs). Some of the small scale dyeing units are devouring storage facilities for hazardous waste but apt disposal facilities are not available, which pollute water sources as they are also released into drainage system and cause crucialmenace to the health of indigenous communities, in addition poisoning the drinking water (Nupur, 2009). At contemporaneous, the Common Effluent Treatment Plant (CETP) established in major places involves some physico-chemical methods such as filtration, specific coagulation, absorption by means of activated carbon, chemical flocculation, precipitation, ozone ionizing radiation, ultra-

filtration etc. The wastewater disposal going on agricultural land is the potentially phytotoxic nature of organic wastes, mainly as an consequence of combination of factors such as high salinity or excess of ammonium ions and organic compounds (Lazarova and Bahri, 2005). The terrible need for the small scale industries is a low-cost, competent method for biotoxification of toxic compounds which should also be environmentally approachable. The ever-present nature of bacteria makes invaluable tools in the effluent biotreatment. Bacteria are very essential to the breakdown of the toxic component of the effluent. Microbial decolorization and degradation is an environmental friendly and cost reasonable alternative to chemical decomposition process (Watson and Cichra, 2006). Biological systems proficiently reduce silk dyeing wastewaters because they are cheaper and produce lesser amounts of sludge, as sludge treatment and disposal is a vital component in the global cost of treatment. However, the advanced biological processes have established increasing attention due to low cost, effectivity, less sludge generation and ecofriendly nature (Lotito*et al.,* 2012). The plant *Sesbania grandiflora* (Fabaceae) is commonly known as "Sesbania" and "Agathi" in ayurvedic system of medicine, selected as an experimental plant for the analysis. It is an open branching tree of fast growing nature (Sreelatha *et al.,* 2011).

MATERIALS AND METHODS

Collection of Seeds: Seeds of Agati (*Sesbania grandiflora*) were collected from Superseeds Nursery, Coimbatore.

Soil preparation for the study: The red soil and the sand were mixed at the ratio of 3:1. Each pot was filled with 7 kg of soil. In Phase 1 and Phase 3, *Sesbania grandiflora* GLV were grown with four replicates. In phase 2, three pots for each of the four different concentrations (25%, 50%, 75% and 100%) were used. The biofertilizer, *Pseudomonas fluorescens* was mixed at the rate of 5 tonnes ha^{-1} with crude effluent and used in Phase 3. The bacterial concentration of the biofertilizer was 10^8 Colony forming units (CFU) ml^{-1}.

Seed sowing and maintenance of plants: About 20 seeds were sown in each pot and were allowed to germinate. Neem cake was mixed with water and poured around the pots as pest control. Fresh water, silk dyeing effluent of different concentrations (25%, 50%, 75% and 100%) and crude silk dyeing effluent treated with *Pseudomonas fluorescens* have been used in Phase 1, Phase 2 and Phase 3 respectively. After germination, 100% moisture condition was maintained throughout the study.

Harvest methodology: The GLV was harvested on the 45th day without any damage. The adhering soil particles were removed by washing gently with water and the water droplets were removed by blotting with the filter paper. Then these GLVs were separately dried with a division of different phases and subjected to various analysis as follows.

Identification of functional groups and compounds in selected GLV of different treatments using spectroscopic and chromatographic techniques

- UV visible analysis was carried out on the methanolic extract of the selected GLV Extract grown in Fresh Water, effluent and Biotreated water.
- FT-IR analysis of the selected GLV grown in fresh water, crude effluent and biotreated effluent and selected dyes in silk dyeing effluent was performed.
- HPLC analysis was carried out in the selected GLV grown in fresh water, crude effluent and biotreated effluent and untreated silk dyeing effluent and biotreated effluent.

The detailed experimental design of the four phases of the study are as follows:

UV visible analysis of selected GLVs: The *Sesbania grandiflora* plants were air dried and 20gms of finely powdered material of each were taken in a thimble and extracted using 200ml of HPLC grade methanol in Soxhlet apparatus. The methanolic extracts of selected GLVs were subjected to Bio-nano UV visible spectrophotometer at different wavelength to find its maximum absorbance peak.

FT-IR analysis of selected GLVs plants and selected dyes: FT-IR (Fourier Transform Infrared) analysis provides spectral information that is essentially a molecular fingerprint for organic, polymeric and in some case inorganic materials. This technique is extremely useful for identifying base polymer compositions and organic contaminants. The FT-IR spectrum of the unknown material can be compared for "best matches" with libraries of spectra that have been cataloged for known materials. The FT-IR analysis was carried out for the selected plants of the GLVs grown in fresh water, crude effluent and biotreated effluent to identify the organic compounds.

1. **In Phase 1,** the selected GLVAgati (*Sesbania grandiflora*) treated with the fresh water were removed on the 45th day, air dried, powdered and subjected to FT-IR.

2. **In Phase 2,**

a) The selected plants were grown in crude silk dyeing effluent till 45 days. The effluent exposed plant were air dried, powdered and subjected to FT-IR.
b) From the technical information obtained from the small scale industry, the dyes (Direct 2y2, Direct yellow 5gll, Procell pineapple) used were subjected to FT-IR.

3. **In Phase 3,** the selected GLV was treated with the biotreated silk dyeing effluent. The effluent biotreated plants were air dried, powdered which was subjected to FT-IR.

HPLC analysis of selected GLV plants

Sample preparation for HPLC analysis

1. **In Phase 1,** the selected *Sesbania grandiflora* treated with the fresh water, on the 45th day were removed, air dried and ground into powder. The HPLC grade methanol was purchased from Fischer Scientific & Co. 20 grams of each dried powder sample was weighed and then packed in Whatmann filter paper placed in thimble of soxhlet apparatus. The HPLC grade methanol of 200 ml each was taken in round bottom flask and the extract was prepared, then stored in the dark at 4°C which was subjected to HPLC analysis at 450 nm.

2. **In Phase 2,** the selected plant was treated with the crude silk dyeing effluent. At the end of the 45th day, the effluent exposed plants were removed, air dried and powdered. The methanolic extracts of the plants were obtained from soxhlet apparatus as in phase 1 and subjected to HPLC analysis at 450 nm.

   i. The collected crude silk industrial effluent and degraded effluent by *Pseudomonas fluorescens* were filtered and subjected to HPLC analysis at 510 nm.

3. **In Phase 3,** the selected plants were treated with the biotreated silk dyeing effluent. The plants grown in biotreated effluent were uprooted on the 45th day and air dried and powdered. The methanolic extracts of selected plants were obtained from soxhlet apparatus as in phase 1 and subjected to HPLC analysis at 450 nm. All the peaks were analysed between the phases and were compared with the standards of pigments (chlorophyll C2, chlorophyll C3, chlorophyll B, carotene), alkaloid (caffeine) and monosaccharides (glucose, fructose, mannose and galactose) which was subjected to HPLC analysis at 450 nm.

Chromatographic conditions: The chromatographic system was equipped with column C18 with 3µl particle size (50×4.6 mm I.D) and detector UV- VIS model SPD 20A at specific nanometer at a flow rate of 1ml/min. The solvent HPLC methanol was used with the stream of liquid N2 until it reached nearly 0.5 ml and then some mobile phase was added to reach 1ml. Then 20µl of the methanolic extract of the sample were injected into HPLC column. The presence of each compound was determined by comparison of peak area of the samples with that of the standard.

Mobile phase and solutions

1. The mobile phase prepared with a mixture of methanol: water (70:30) was used for the HPLC analysis of methanolic extracts of the untreated, crude effluent and biotreated plants.
2. The mobile phase with a binary mixture of acetonitrile: water (60:40) was used for crude effluent and biotreated silk dyeing industrial effluent.
3. The mobile phase for standards such as pigments, alkaloid caffeine and monosaccharides were prepared with 20% methanol and 0.2% phosphoric acid, methanol: water (80:20) and acetonitrile: water (90:10) respectively.

RESULTS AND DISCUSSION

Analysis of the methanolic extracts of selected GLVs grown in fresh water in bionano UV visible spectrophotometer: Figure 1 depicts the spectra of methanolic extracts of selected GLV grown in fresh water subjected to bionano UV visible spectrophotometer. The methanolic extracts of the GLV *S.grandiflora* were subjected to UV visible spectrophotometer at different wavelength from 250-800nm. The visible range of 400-700nm was taken into consideration. The GLV extracts in common have peaks in between 650-700nm. The GLVs have an elevated peak at 400nm.

FT-IR spectrum of *S.grandiflora* grown in fresh water: Figure 2 reveals the spectrum of *S.grandiflora* grown in fresh water subjected to FT-IR. The FT-IR spectrum of *S.grandiflora* grown in fresh water has considerable absorption bands. The band of strong broad intensity at 3456 wavenumber cm^{-1} belongs to alcohol group and 2924 and 2847 wavenumber cm^{-1} with C-H stretch belongs to alkane group. Similarly in addition, there are intense absorptions between the 3600 and 2750 cm−1 regions that are assignable to the O–H and C–H stretching in the FTIR spectral lines of Cotton fibers (Yongliang Liu *et al.,* 2017). The isocyanide group indicated at 2052 and 1975 wavenumber cm^{-1} peak. The N-H bend exemplifies at 1635 wavenumber cm^{-1} peak reveals the amide group. The absorption bands at 1381 and 1319 wavenumber cm^{-1} peak with N-O symmetric stretch correspond to nitro groups. The wavenumber 1134 and 1064 cm^{-1} peak of absorbance has resemblance to aliphatic amine and 1435, 895 wavenumber cm^{-1} correlates to aromatics groups. In correlation to the above studies the band at 895 cm^{-1} has been assigned to the β-glycosidic linkage in cellulose of cotton fibers (Yongliang Liu *et al.,* 2017).All the other peaks such as 1257, 663 and 532 wavenumber cm^{-1} with small bands corresponds to alkyl halide.

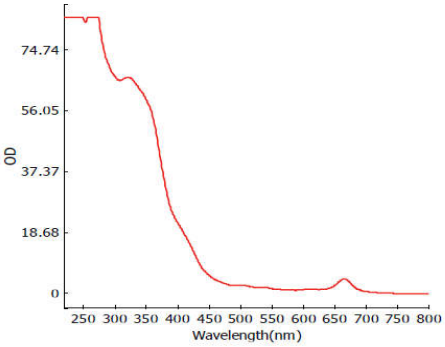


Figure 1. UV visible spectra of the selected GLV

FT-IR spectrum of *S.grandiflora* grown in crude effluent: Figure 3 shows the spectrum of *S.grandiflora* grown in crude silk dyeing effluent subjected to FT-IR. The FT-IR spectrum represents the characteristic absorption bands at 2924 and 2854 wavenumber cm^{-1} as previously discussed to be alkane and bands such as 2376 and 2337 wavenumbercm^{-1} were not detected. Also wavenumber 1381 and 532 cm^{-1} of spectral bands were similar to the *S.grandiflora* grown in fresh water. The wavenumber 1381, 1249 and 918 cm^{-1} confirms the amine groups of various categories.

FT-IR spectrum of *S.grandiflora* grown in biotreated effluent: Figure 4 represents the spectrum of *S.grandiflora* grown in biotreated effluent subjected to FT-IR. The FT-IR spectrum of *S.grandiflora* grown in biotreated effluent of spectral bands detected at 3402, 2924 and 2854 cm^{-1} indicates the alcohol and alkane respectively. Similarly FTIR spectra of new cellulose paper show absorbance peaks located close to 3329 cm^{-1} that represents an O–H functional group, located close to 2922 and 2854 cm^{-1} that represent a C–H functional group, and located close to 1050 cm^{-1} that represents a C–O functional group investigated of Transformer Paper in Mineral Oil-Paper Composite Insulation under Accelerated Thermal Aging (Abi Munajad *et al.,* 2018). These bands were repeated throughout the spectral study in other GLVs. The nitro groups were observed with the N-O stretch intensity at 1550 and 1319 cm^{-1} and amine group detected at 1273 and 1041 wavenumber cm^{-1} with C-N stretch. The alkyl halide groups were also revealed at few bands absorbed at wavenumber 671, 609 and 532 cm^{-1}.

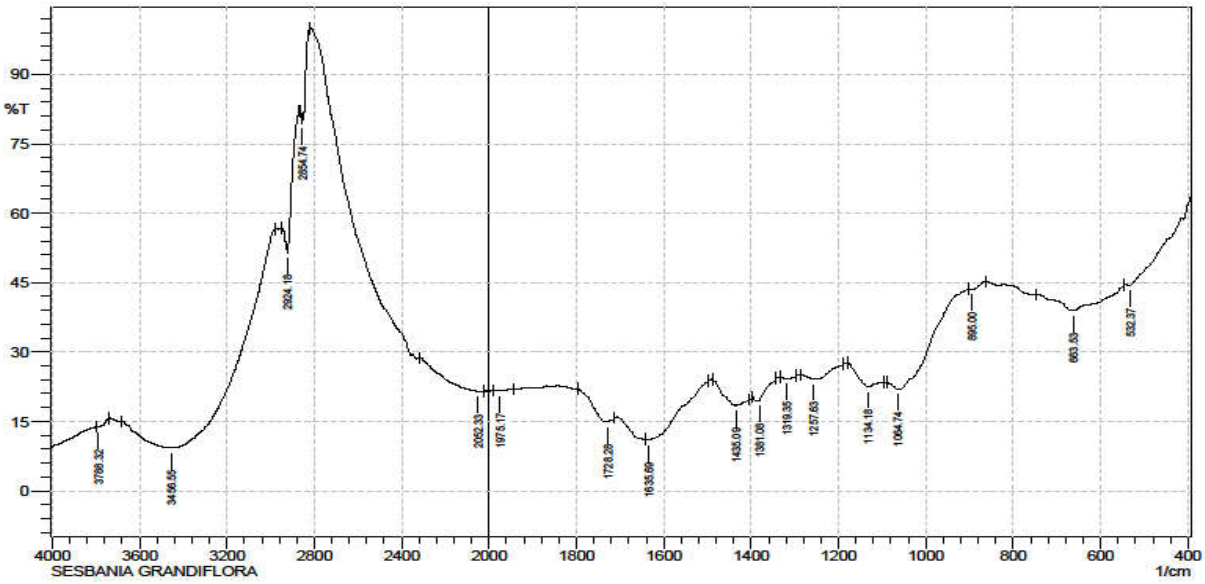


Figure 2. FT-IR spectrum of *S.grandiflora* grown in fresh water

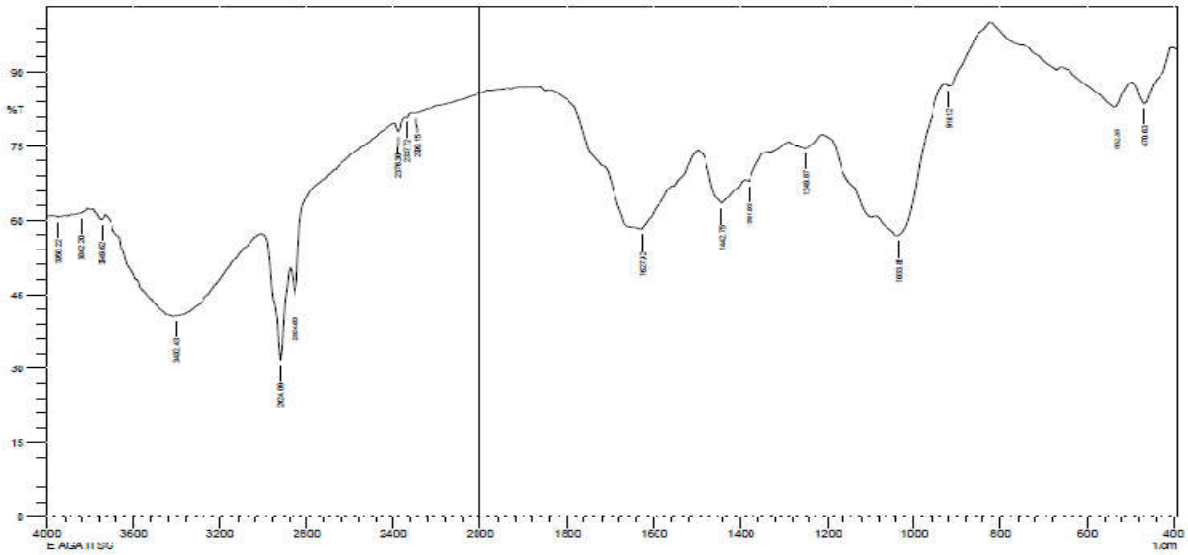


Figure 3. FT-IR spectrum of *S.grandiflora* grown in crude effluent

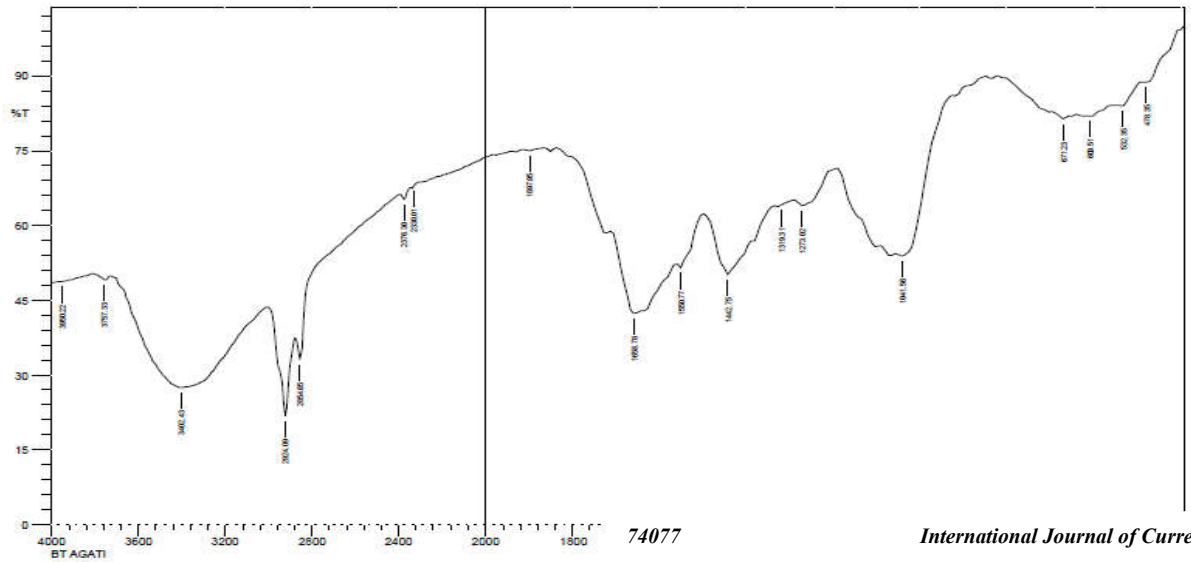


Figure 4. FT-IR spectrum of *S.grandiflora* grown in biotreated effluent

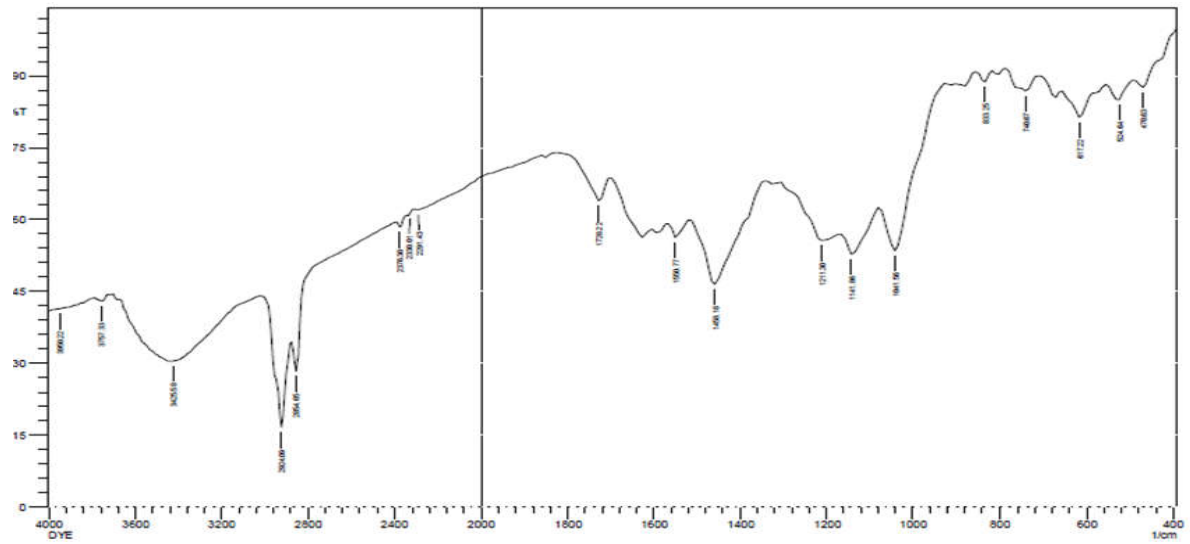
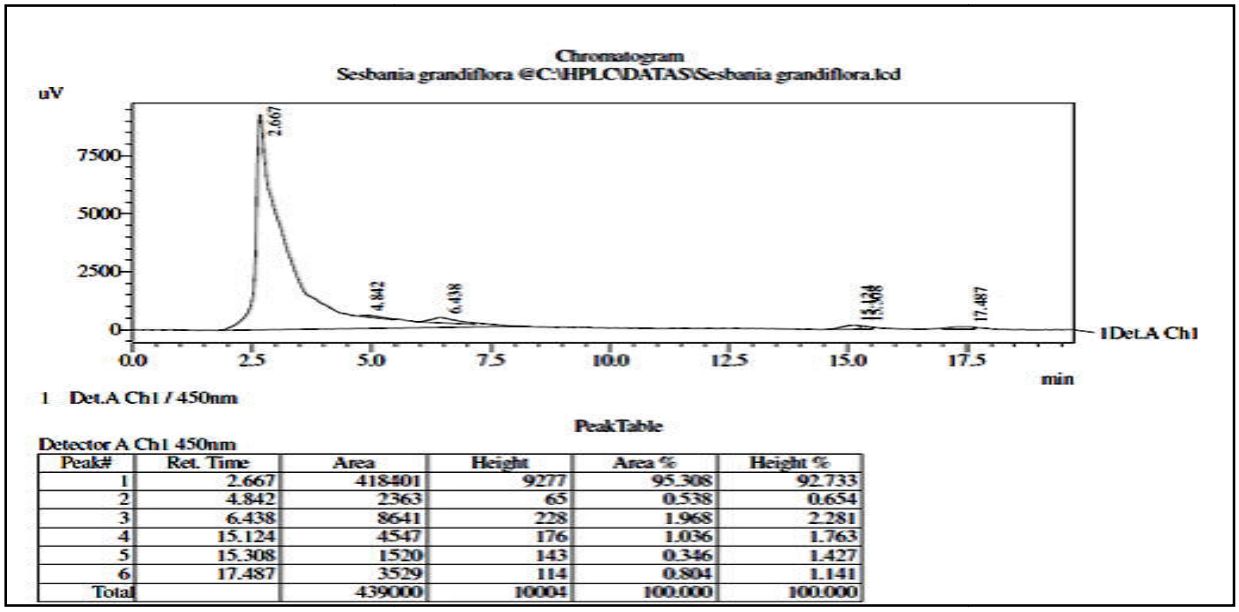


Figure 5. FT-IR spectrum of the selected dyes of silk dyeing effluent

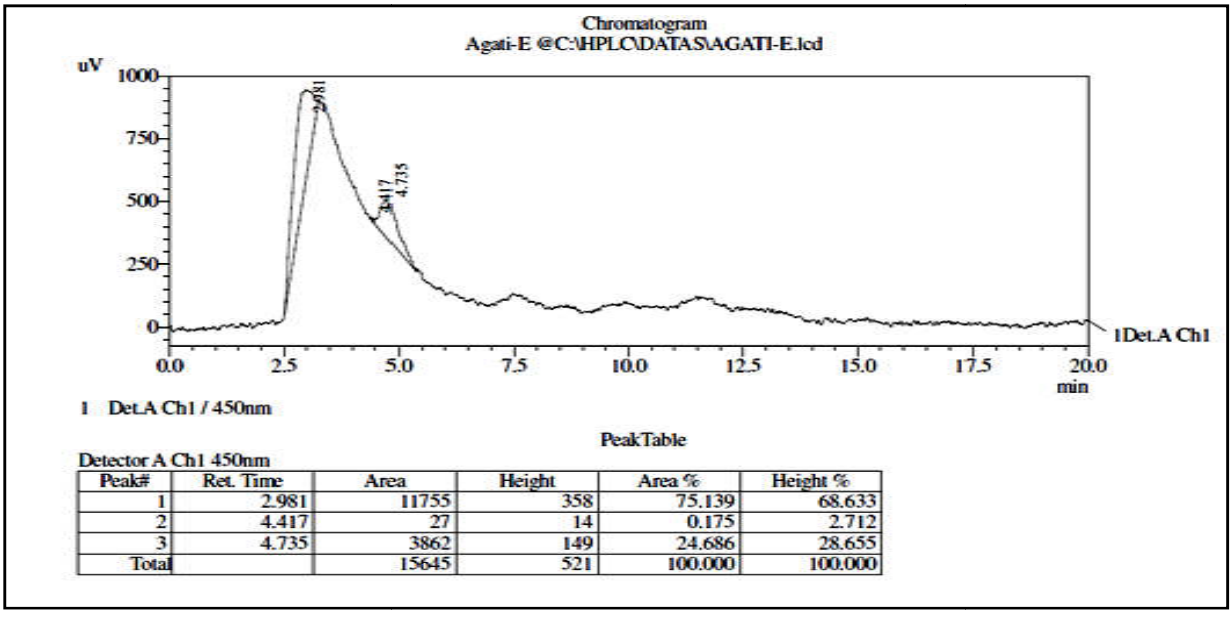


PeakTable

Detector A Ch1 450nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.667	418401	9277	95.308	92.733
2	4.842	2363	65	0.538	0.654
3	6.438	8641	228	1.968	2.281
4	15.124	4547	176	1.036	1.763
5	15.308	1520	143	0.346	1.427
6	17.487	3529	114	0.804	1.141
Total		439000	10004	100.000	100.000

Figure 6. HPLC of *S.grandiflora* grown in fresh water

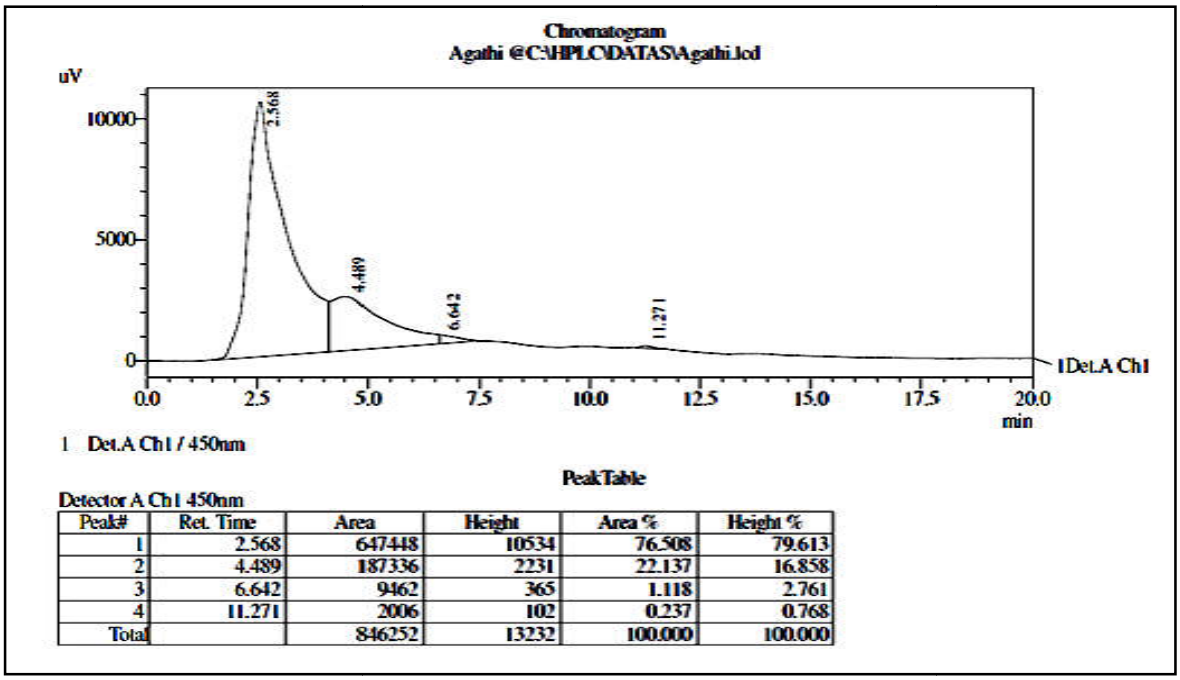


PeakTable

Detector A Ch1 450nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.981	11755	358	75.139	68.633
2	4.417	27	14	0.175	2.712
3	4.735	3862	149	24.686	28.655
Total		15645	521	100.000	100.000

Figure 7. HPLC of *S.grandiflora* grown in crude effluent



Peak Table

Detector A Ch1 450nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.568	647448	10534	76.508	79.613
2	4.489	187336	2231	22.137	16.858
3	6.642	9462	365	1.118	2.761
4	11.271	2006	102	0.237	0.768
Total		846252	13232	100.000	100.000

Figure 8. HPLC of *S.grandiflora* grown in biotreated effluent

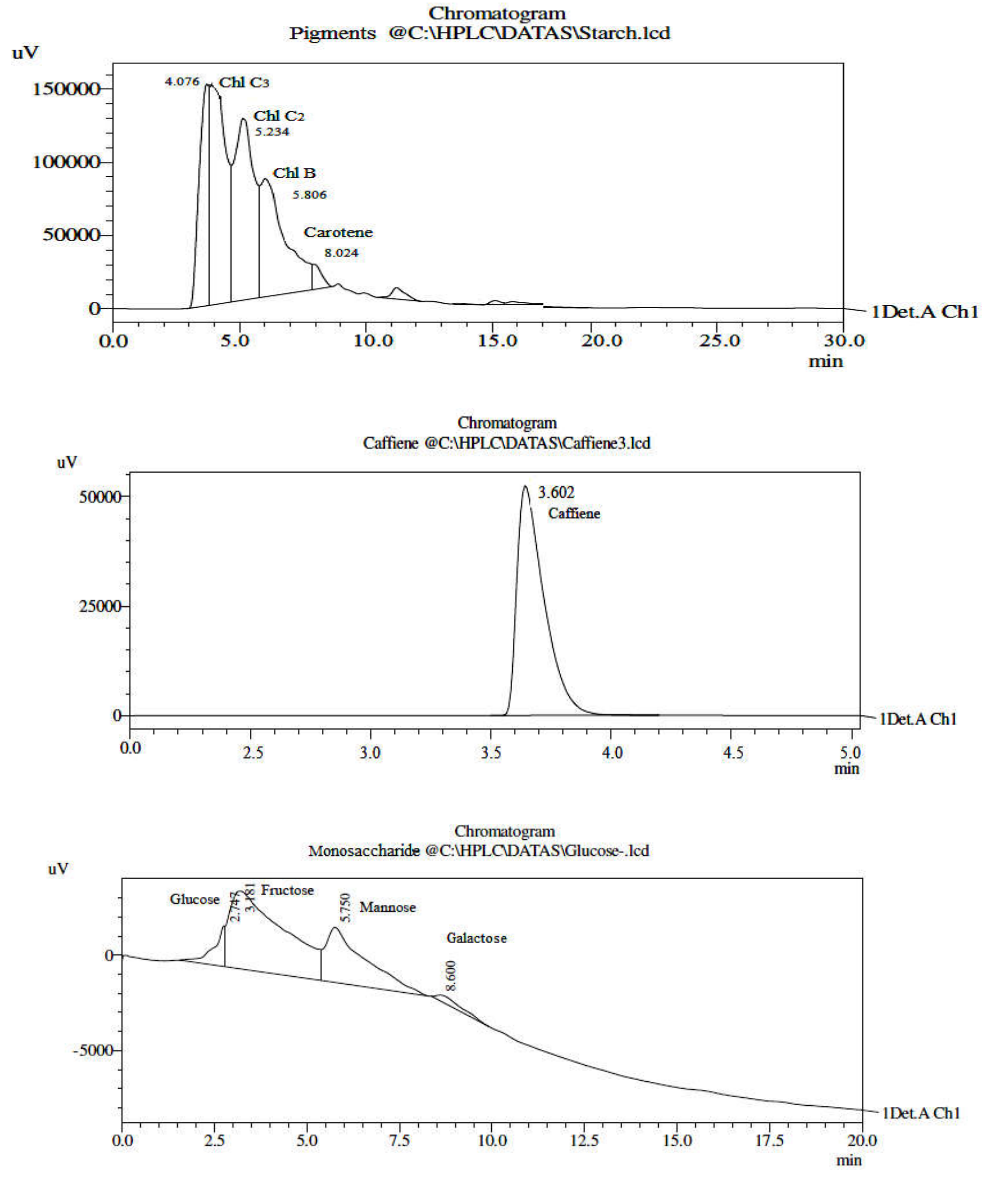


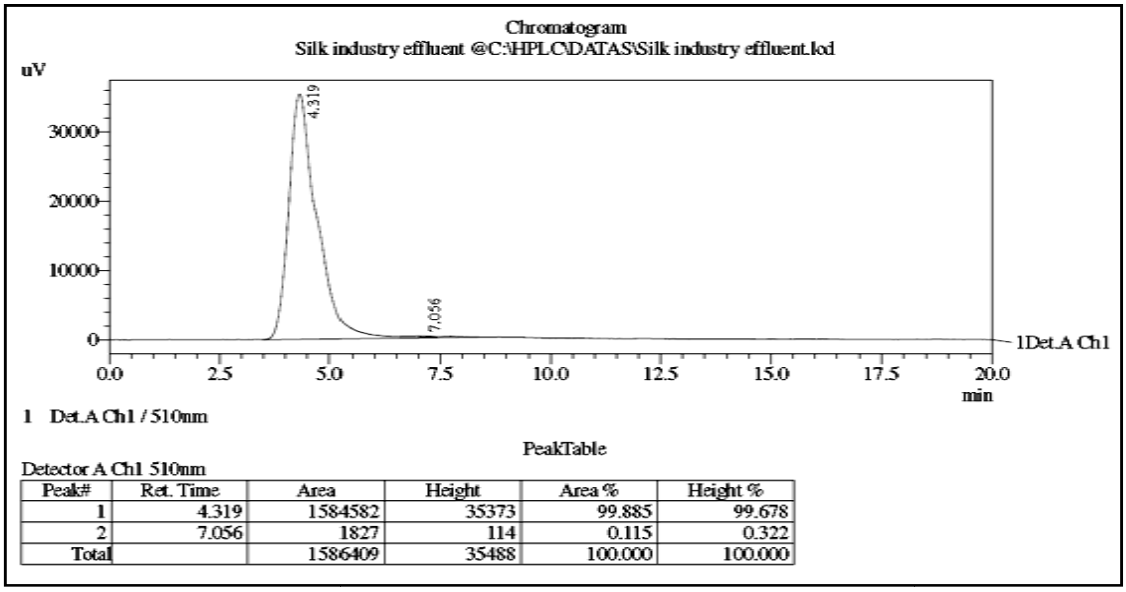
Figure 9. Standard HPLC of pigments, alkaloid and monosaccharides

Table 1. Functional groups detected in the FT-IR spectra of different GLVs in different treatments

Functional group	*S.grandiflora*		
	SGN	SGE	SGT
Alcohol	+	+	+
Alkane	+	+	+
Alkene	+	+	+
Alkynes	-	-	-
Carboxylic acid	-	+	-
Esters	-	+	-
Ethers	-	+	-
Isocyanide	+	-	-
Phosphine	-	-	-
Aromatic	+	+	+
Nitro groups	+	+	+
Amide	-	-	-
Aldehyde	+	-	-
Amine	+	+	+
Alkyl halide	+	+	+

+ Presence - Absence

SGN:Sesbania grandiflora were grown in fresh water.SGE:Sesbania grandiflora were grown in crude effluent:SGT:Sesbania grandiflora were grown in biotreated effluent.

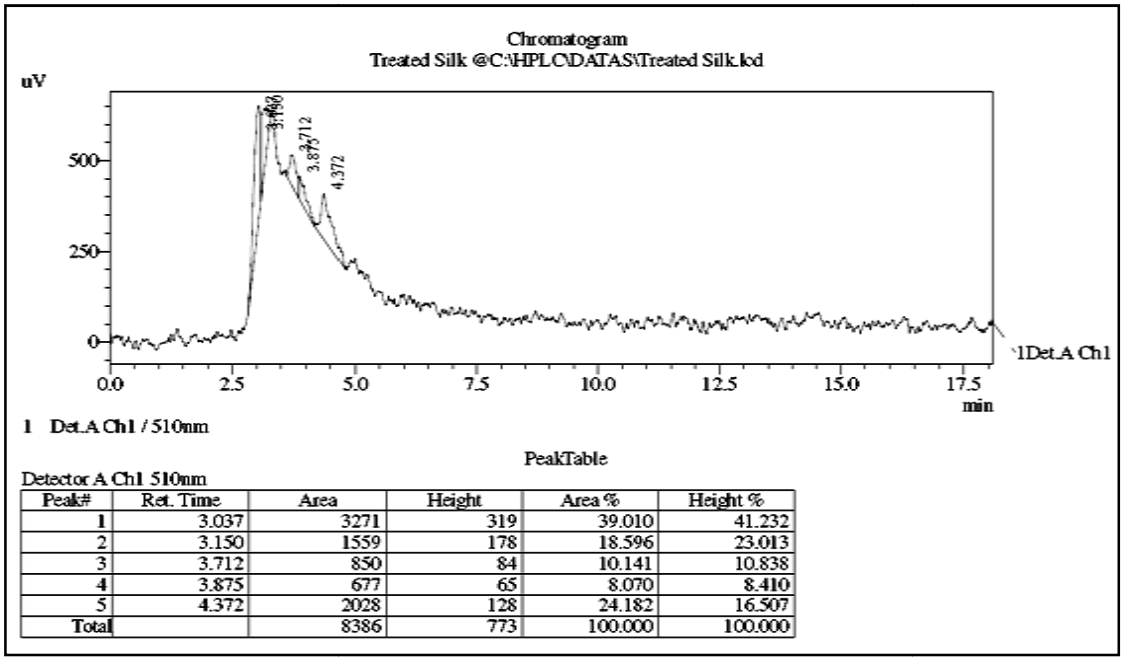


PeakTable

Detector A Ch1 510nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.319	1584582	35373	99.885	99.678
2	7.056	1827	114	0.115	0.322
Total		1586409	35488	100.000	100.000

Figure 10. HPLC of silk dyeing industrial effluent



PeakTable

Detector A Ch1 510nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.037	3271	319	39.010	41.232
2	3.150	1559	178	18.596	23.013
3	3.712	850	84	10.141	10.838
4	3.875	677	65	8.070	8.410
5	4.372	2028	128	24.182	16.507
Total		8386	773	100.000	100.000

Figure 11. HPLC of biotreated effluent

(Direct 2y2, Direct yellow 5gll, Procell pineapple) subjected to FT-IR. The dye powder subjected to FT-IR analysis confirms the presence of alcohol, alkane, alkynes, aldehyde, nitro groups, aliphatic amines, primary and secondary amine, aromatic group and alkyl halide groups.

Functional groups detection from FT-IR spectrum of selected GLVs: Table 1 depicts the analysis of functional groups in the selected GLV of different treatments. Thus from the functional group studies by FT-IR, the alcohol, alkane, alkyl halide and amine groups were found in all the GLVs irrespective of the treatments, even in crude effluent, the plants managed to synthesize these organic compounds. The amide group was completely absent in all the treatments. The isocyanide group was found only in *S.grandiflora* grown in fresh water, which was unable to synthesize isocyanide group in plants grown in crude effluent and biotreated effluent. Irrespective of the treatments, the aromatic groups were synthesized by *S.grandiflora* grown in both crude effluent and biotreated effluent. The alkene group was detected in different treatments of *S.grandiflora* grown in fresh water.

HPLC analysis of the methanolic extracts of the selected GLV grown in different treatments, untreated and biotreated silk dyeing effluent

HPLC of *S.grandiflora* grown in fresh water: Figure 6 depicts the chromatogram of methanolic extract of *S.grandiflora* grown in fresh water.

HPLC of *S.grandiflora* grown in crude effluent: Figure 7 views the chromatogram of methanolic extract of *S.grandiflora* grown in crude silk dyeing effluent.

HPLC of *S.grandiflora* grown in biotreated effluent: Figure 8 reveals the chromatogram of methanolic extract of *S.grandiflora* grown in biotreated effluent. The HPLC analysis of *S.grandiflora* grown in fresh water revealed 6 peaks of retention time 2.6, 4.8, 6.4, 15.1, 15.3 and 17.4 minutes of which only 3 peaks were present in *S.grandiflora* grown in effluent water. The smaller peaks of least percentage area 1.036, 0.346, 0.804 of retention time 15.1, 5.3, 17.4 minutes has been vanished from the chromatogram of effluent exposed plants. In the biotreated plant 4 peaks with the retention time of 2.5, 4.4, 6.6 and 11.2 minutes were found.

Standard HPLC of pigments, alkaloid and monosaccharides: Figure 9 shows the chromatogram of the standard pigments such as (Chl C_3, Chl C_2, Chl B and carotene), alkaloid (caffeine) and monosaccharides (glucose, fructose, mannose and galactose). Figure 9 depicts the standard such as pigments of Chl C_3, Chl C_2, Chl B, carotene, alkaloid caffeine and monosaccharides such as glucose, fructose, mannose and galactose had peaks with the retention time (tR) of 4.0, 5.2, 5.8, 8.0, 3.6, 2.74, 3.18, 5.7 and 8.6 minutes respectively. Thus the chromatographic studies, the compounds were identified on comparison of these standards with the HPLC of methanolic extract of the selected GLVs of different treatments with the difference of ±0.5 retention time (tR). Comparison of the retention time (tR) has revealed the presence of the pigments chlorophyll C_3, chlorophyll C_2, chlorophyll B, carotene grown in fresh water and biotreated effluent).The alkaloid caffeine was absent in all extracts of different treatment. The monosaccharide glucose was present in all the extracts of the GLV.

HPLC of silk dyeing industrial effluent: Figure 10 indicates the chromatogram of silk dyeing effluent.

HPLC of biotreated effluent: Figure 11 depicts the chromatogram of biotreated effluent by *Pseudomonas fluorescens.* Thus from the present study, the HPLC chromatogram of silk dyeing industrial effluent at the wavelength of 510nm has shown two peaks of retention time (tR) 4.3, 7.0 minutes with 99.88 % and 0.115 % area.

Conclusion

The chromatogram of biotreated effluent has shown five peaks with retention time (tR) 3.0, 3.1, 3.7, 3.8 and 4.3 minutes with reduced percentage area of 39%, 18.5%, 10.1%, 8% and 24.18% which clearly indicates that the dye in the effluent has been degraded by *Pseudomonas fluorescens*.Thus the chromatographic studies, the compounds were identified on comparison of these standards with the HPLC of methanolic extract of the selected GLVs of biotreatments with the difference of ±0.5 retention time (tR) and Comparison of the retention time (tR) has revealed the presence of the pigments chlorophyll C_3, chlorophyll C_2, chlorophyll B, carotene grown in fresh water and biotreated effluent. Thus to attain the zero discharge of silk dyeing effluent, the *Pseudomonas fluorescens* can be used to biodegrad the dye molecules. Hence it can be concluded that the null hypothesis (H_0) "*Pseudomonas fluorescens*donot degrade the silk dyeing effluent" is rejected.

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