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A Study on Biodegradation Potential of Various Solvent Extracts of *Sargassum* on Tannery Industry Effluent

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Research Article

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ABSTRACT

The untreated liquid waste generated from tannery industries have managed to take away the calmness and serenity from our hectic and tensed lives by tampering our greatest wealth i.e. health. It has also degraded our environment at a fanatic pace. Moreover, with rapid industrialisation under the guise of automation, modernization and progress have also contaminated our water bodies as the untreated effluent are release directly into them, therefore leading to a catastrophic impact on the quality of water and aquatic life. The present study being first of its kind investigate the impact of different solvent extract of marine algae *Sargassum* on the various physicochemical parameters like TDS, hardness, chloride, nitrate, sulphate and chromium (VI) of tannery industry effluent. Phytochemical analysis of various solvent extract namely SET, SMT, SCT, SBT and SAT were performed and the retentate was used as biofertilizer for growth of *Vigna mungo*. The length and protein content of the plant showed a significant increase in comparison to the control. With the help of Imvic test, the organism isolated from tannery industry effluent was identified as *Pseudomonas* (MIC 1800 mg/l for Cr (VI)). Methanolic extract of *Sargassum* was found to be most effective in improving the quality of water and removal of toxic metals like Cr (VI).

INTRODUCTION

Water is the part and parcel of our existence without which existence of life cannot be even imagined. Water is the worldwide civilization had originated on the banks of large water bodies as water is the most essential commodity for survival. Of late water is polluted by lots of factors among which industrial and domestic effluent play vital role, as they are discharged in the untreated form to the water bodies, canals and drainage ditches, land and water

resources. This method of waste disposal has greatly reduced the amount of potable water. The main constituent in domestic wastewater is human excreta with smaller contributions from food preparations, washings, laundry and surface drainage [1,2]. A large number of enteric bacterial and viral pathogens may be excreted by infected individuals and may therefore be present in untreated domestic wastewater [3]. The limited availability of fresh water is a global crisis. The growing consumption of fresh water by anthropogenic activities has taken its toll on available water resources. Unfortunately, water bodies are still used as sinks for wastewater from domestic and industrial sources. However, in recent times, the need to replenish our water resources has been receiving increasing attention. This has led to the development of strategies to return water to its source in the least toxic form possible, to enable reutilization of water. The untreated liquid wastes generated from tannery are characterized as high-coloured, foul-smelling, acidic and alkaline with high BOD (Biological Oxygen Demand) and COD (Chemical Oxygen Demand) [4,5].

The waste product of electroplating and leather industries are contain huge amount of chromium and it is also major cause for the high influx of chromium to the biosphere [6]. The huge quantity of chromium salts discharge into tannery waste has raised several ecological concerns. The slug generated by chromium based industries is usually damped on the ground which pollutes in surface and subsoil water in the vicinity of industrial units. Hexavalent chromium is formed due to oxidation of Cr (III) compounds which percolates down into the soil during rainy season and polluting underground water [7]. In general, industrial waste contains both hexavalent and trivalent forms of chromium which are most stable and exist in aqueous system [8]. The hexavalent chromium is of particular concern due to its great toxicity. It is known to be carcinogenic and mutagenic to living organisms [9]. Thus it is necessary to remove or recover the chromium before disposal of industrial waste.

Sargassum is an invasive brown seaweed that has recently found its way near the coast of Ireland. Numerous species are distributed throughout the temperate and tropical oceans of the world, where they inhabit shallow water and coral reefs. The species of *Sargassum* containing wide range of bioactive metabolites which has various applications like medicinal importance, biofuel and cosmetic industries [10]. Due to physiochemical and biological activities of *Sargassum* it is used to enhanced the soil quality by nutrients supply and toxicity removal [11,12]. However the genus may be best known for its planktonic species. *Sargassum* is also cultivated and cleaned for use as an herbal remedy. Many Chinese herbalists prescribe powdered *Sargassum* in paper packets, to be dissolve in warm water and drunk as tea. Batch experiment using *Sargassum* biomass indicated that it was possible to attain high removal efficiencies of various parameters including TDS, conductivity and salinity. Hardness and TDS are the major criteria which can be significantly reduced by leaf extract of *Moringa oleifera* and *Murraya koenigii* [13,14]. A similar study using leaf extracts of *Prosopis juliflora*, *Nymphaea ampla*, *Annona squamosa*, *Manilkara zapota* and *Moringa oleifera* were performed to treat the paint industry effluent [14-16].

The present study uniquely utilizes the various solvent extracts of *Sargassum* to improve the physicochemical characteristics and reduce Cr (VI) concentration of tannery industry effluent collected from Nagalkeni village, Pallavaram, Chennai (12°57'51.6"N 80°07'53.8"E). The phytochemical analysis of the solvent extract was performed and the retentate obtained after extraction was used as biofertilizer to grow *Vigna mungo*. Lastly, Cr (VI) resistant bacteria was isolated and was identified using IMVIC test.

MATERIALS AND METHODS

Collection of sample

Leather and paint industry effluents were collected from the surrounding areas of Nagalkeni village, Pallavaram, Chennai (12°57'51.6"N 80°07'53.8"E) and stored in refrigerator for avoiding further contamination in the effluent. *Sargassum* was collected from the coast of Tuticorin (8°45'50.9976"N 78°8'5.4024"E). Leaves were separated manually and dried under shade for 10 days. After complete drying, it was made as a fine powder and was stored.

Preparation of plant extract

The powdered samples were soaked in different solvents such as methanol, ethanol, chloroform, benzene and water for 48 hrs and the extracts were filtered out using whatman No.1 filter paper and were tested by following protocol [14].

Phytochemical analysis of leaf extract

Various qualitative phytochemical test were performed. Briefly for testing terpenoids and triterpenoids salkowski test was done in which few drops of concentrated sulphuric acid was added to 2 ml of extract. Then it was shaken well and left it for some time. Appearance of red color indicates the presence of steroids and yellow color indicates the presence of triterpenoid. For phenol, 2 ml of extracts was taken and few drops of ferric chloride were added. Presence of phenol was confirmed by the appearance of green/blue/bluish green/brown/brownish red color. To test for flavonoids 3 ml of distilled water was added to 2 ml of sample and filtered. Then 10% ferric chloride is added to this filtrate. Appearance of greenish blue/violet color confirms the flavonoids. Neutral ferric chloride test was done for tannins in which few drops of 0.1% ferric chloride was added to 2 ml plant extracts. Appearance of blue/black/bluish green precipitate indicates the presence of tannins. To check the presence of amino acids ninhydrin test was done by adding few drops of ninhydrin into 2 ml of extract. Sodium bicarbonate test was done for checking the presence of carboxylic acid. Presence of glycoside was done by molisch's test in which 2 ml of sample was treated with 2-3 drops of α-napthol and few drops of concentrated sulfuric acid. Keller killani test for cardiac glycoside by adding few drops of glacial acetic acid and 2-3 drops of ferric chloride into 2 ml of extract along with 1 ml of concentrated sulfuric acid. For testing anthraquinone borntrager's test was performed by adding 2 ml of extract was mixed with 10% of 5 ml ammonia. Carbonyl group was tested by treating the 2 ml of plant extract with 2-3 drops of 2,4 diphenyl hydrazine. Saponin was tested by adding 2 ml of plant extract 5 ml distilled water and boiled with vigorous mixing. Coumarin was tested by reacting the plant extract with 1 N NaOH or KOH. For testing phlobatanin, distilled water was added to the extract and then filtered. Filtrate was boiled with 2% HCl.

Treatment of effluent

Tannery effluent was treated with various plant extracts and some important parameters were checked. In which estimation of TDS was done. Briefly the sample was filtered and the sediment leftover on the filter was scrapped off and dried in oven. Then the dry weight of the sediment was measured. Determination of Hardness was done by dissolving an aliquot containing 25 ml of extract in 50 ml of distilled water and 1 or 2 drops of EBT indicator was added to it. The solution was titrated with EDTA solution till the colour changes from reddish to blue tinge. Sulphate concentration was checked by nephlometry method. About 100 ml of sample was treated with 20 ml of buffer

solution A (30 g of $MgCl_2$ was dissolved in 5 g of sodium acetate, 1 g of KNO_3 and 20 ml of CH_3COOH in 500 ml distilled water). A spoonful of $BaCl_2$ was added to it. The turbidity was measured. Using standard graph, the concentration of sulphate was measured. Aliquot containing 50 ml of sample was added to 1 ml of HCl and OD was measured using calorimeter by using phenol disulphonic acid method. The nitrate concentration was measured for the given sample using standard graph.

Hexavalent chromium was measured spectrophotometrically by diphenyl carbizide method which is nearly specific for Cr (VI) adding diphenyl carbized solution to samples develops a pink color which can be measured with a UV-spectrophotometer at 540 nm. Chloride was estimated by adding ten millilitres of effluent samples in a conical flask and 1 ml potassium chromate was added to get light yellow color. It was then titrated with standard silver nitrate solution till color change from yellow to brick red.

Effect of retentate as bio fertilizer on growth of *Vigna mungo*

The soil was tested by soil testing kit (HIMEDIA K054). After the extraction of the phytochemical the plant biomass was mixed with soil in two proportions i.e. 5 g in 50 g soil and 10 g in 50 g soil and the growth and protein content of *Vigna mungo* was checked using lowry method. It was watered with nutrient solution for almost a week.

Microbiological assay

Catalase test: Pure growth of the organism from the agar to a clean slide with a loop or glass rod was transferred. Immediately 3% hydrogen peroxide was added to the growth and the release of bubbles was observed.

Oxidase test: With the help of glass rod, a colony from 24 hrs growth of the test organism was picked up and rubbed on oxidase disc. A change in color from blue or purple within 10 sec was observed.

MRVP test: Organism was inoculated into MR/VP both and incubated at 37°C for atleast 48 hrs. Then the broth was divided into two equal halves and to one tube 0.5 ml of MR reagent was added and other tube 0.2 ml VP reagent A and 0.2 ml VP reagent B was added and was allowed to stand for 15 min.

Citrate utilization test: Citrate agar slant was prepared and the organisms were streaked on it. It was then incubated for 18-24 hrs and the result was read.

Indole test: Tube of tryptone broth was inoculated with organism and was incubated for 24-48 hrs at 37°C. Then 0.2 ml of kovac's reagent was added and it was allowed to stand for few min.

TSI test: A butt and a slant was prepared in the same tube. Organism from top of a single colony was picked and was stabbed at the centre if the agar butt carefully. The needle was withdrawn carefully and then the surface of slant was streaked carefully and was incubated at 37°C for nearly 18-24 hrs.

RESULTS AND DISCUSSION

Phytochemical analysis of various solvent extracts of *Sargassum*

Phytochemical are non-nutritive plant chemicals that have protective or disease preventive properties. It is well known that plant produce these chemicals to protect them but recent research demonstrate that they can also protect humans against diseases. The methanolic extract of *sargassum* contained steroids, terpenoids, amino acid

and carbonyl in medium amount whereas phenol, coumarin, glycoside, cardiac glycoside and saponins were weakly present.

In the ethanolic extract triterpenoids, coumarin, cardiac glycoside and carbonyl were present in medium amount whereas steroid, terpenoids, phenol, flavonoid, tannin, phlobatanin and carboxylic acid were weakly present.

The chloroform extract had steroid, triterpenoids and coumarin in medium amount whereas terpenoids, phenol, flavonoid, tannin, phlobatanin, carboxylic acid and glycoside were weakly present.

However benzene and aqueous extract showed ony triterpenoids in medium amount and aqueous extract showed weak presence of steroid (Table 1).

Table 1. Phytochemical analysis of *Sargassum* in various solvent extract.

S.NO	Phytochemicals	SMT	SET	SCT	SBT	SAT
1	Steroid	++	+	++	-	+
2	Triterpenoids	-	++	++	++	++
3	Terpenoids	++	+	+	-	-
4	Phenol	+	+	+	-	-
5	Flavonoid	-	+	+	-	-
6	Coumarin	+	++	++	-	-
7	Tannin	-	+	+	-	-
8	Phlobatanin	-	+	+	-	-
9	Amino acid	++	-	-	-	-
10	Carboxylic acid	-	+	+	-	-
11	Glycoside	+	-	+	+	+
12	Cardiac glycoside	+	++	++	+	+
13	Carbonyl	++	++	++	++	++
14	Saponins	+	+	+	+	+
15	Anthraquinone	-	++	-	-	-

Note: Highly prominent=+++; Medium amount=++; Fewer amount=+; Absent= -.

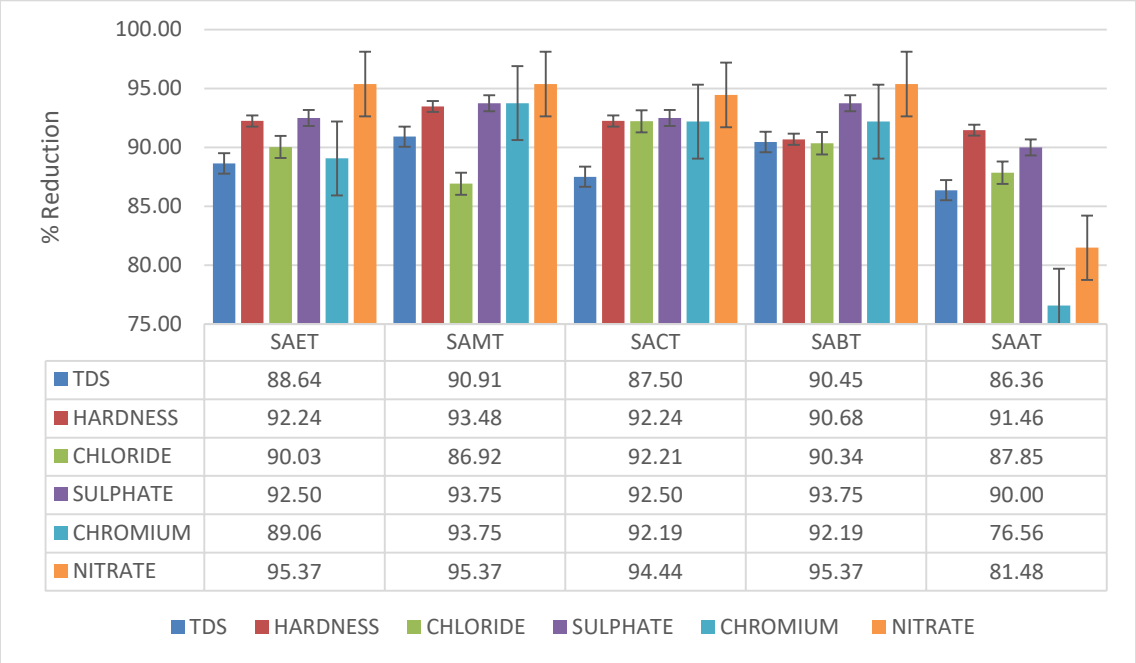
Effect of extracts on physicochemical properties of tannery effluent

Methanolic extract of *sargassum* was most effective in reducing almost all parameters including TDS, Hardness, sulphate, chromium, nitrate except chloride followed by benzene, ethanol, chloroform and aqueous extracts. From the phytochemical analysis, it can be revealed that the methanolic extract has terpenoids and amino acid in maximum amount which may be responsible for bringing maximum reduction in all parameters.

Chloride was reduced maximum in chloroform extract followed by benzene, ethanol, aqueous and methanolic extract. The minimum reduction in chloride in the methanolic extract of *Sargassum* suggested a probability of amino acid hindering with chloride reduction (Figure 1).

Methanolic extract of *Sargassum* was able to remove 93.75% Cr (VI) from the tannery effluent which may be attributed to the presence of phytochemical like steroid, terpenoids, amino acids and carboxyl group.

Figure 1. Percentage reduction of various parameters by solvent extract of *Sargassum*. **Note:** TDS; Hardness; Chloride; Sulphate; Chromium; Nitrate.



	SAET	SAMT	SACT	SABT	SAAT
TDS	88.64	90.91	87.50	90.45	86.36
HARDNESS	92.24	93.48	92.24	90.68	91.46
CHLORIDE	90.03	86.92	92.21	90.34	87.85
SULPHATE	92.50	93.75	92.50	93.75	90.00
CHROMIUM	89.06	93.75	92.19	92.19	76.56
NITRATE	95.37	95.37	94.44	95.37	81.48

Effect of retentate on growth of *Vigna mungo*

A biofertilizer is a substance which contains biological or natural agents which when applied to seed or plant surfaces promotes growth without any ill effect. The retentate after extraction of phytochemical were used as biofertilizer in two different amounts and it showed a positive effect on growth of plant in terms of height and protein content. The seedling without retentate (control) was 13 cm long with 0.38 µg/ml of protein whereas the seedling with 5 g and 10 g of retentate showed a considerable increase in height and protein content (Table 2 and Figure 2).

Table 2. Effect of biofertilizer on growth of green grams.

Amount of biomass	Height of plant (cm)	Protein content (µg/ml)
0 g (control) (CP-1)	13	0.38
5 g (PP-I)	18	0.42
10 g (PP-II)	19	0.46

Identification of isolate

A total of 10 different colonies were isolated and purified. Out of 10 isolates, one colony with MIC 1800 mg/l Cr (VI) was identified using primary and secondary tests and the organism was identified as *Pseudomonas* based on bergey's manual of systematic bacteriology (Tables 3 and 4).

Figure 2. Effect of biofertilizer on plant growth.

Table 3. Morphlogical test result of bacterial strain.

Test	Organism
Colony morphology	
Configuration	Circular
Texture	Moist
Pigment	Blue-green
Opacity	Opaque
Gram's reaction	
Cell shape	Rods
Spore	-
Motility	+
Note: Negative (-); Positive (+).	

Table 4. Biochemical test result of bacterial strain.

Test	Organism
Methyl red test	-
Vogesproskaven test	-
Citrate	-
Indole	+
Catalase	+
Oxidase	+
TSI	
Acid production	+
H_2S production	+
Gas production	+
Note: Negative (-); Positive (+).	

CONCLUSION

Methanolic extract of *Sargassum* has many bioactive compounds like steroids, terpenoids, amino acids and carbonyl. Which are medicinally important as well as they can be used in improving the physicochemical properties of contaminated water and they are also important in remediating many toxic metals like Cr (VI). In future quantitative determination of these phytochemicals can be done and their impact on large scale bioremediation of tannery effluent can be studied. Moreover, the isolated strain can be used for bioreduction of Cr (VI) to Cr (III).

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