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# HYGROPHILA AURICULATA: AS POTENTIAL PHYTOREMEDIENT AND PHYTODISINFECTANT IN WASTE WATER TREATMENT

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**ABSTRACT**

*Hygrophila auriculata* (K. Schum.) Heine (Family: Acanthaceae) is a potent medicinal plant in the Indian systems of medicine. Traditionally the leaves are used in the treatment of diuretic, antibacterial, dropsy, diseases of urinogenital tract, jaundice, hepatic obstruction, rheumatism, inflammation, pain, urinary infection, edema, gout, malaria, and impotence and as an aphrodisiac. The present study was designed to assess findings from a preliminary lab-scale study showed strong potentials of phytodisinfectants as a low-cost, appropriate and ecological alternative technology in purifying water in rural area of Kanyakumari district. It is observed that 95% reduction in bacterial loads of the water sample by *Hygrophila auriculata* after 15 and 30 minutes residence time. The coagulation and the disinfection effect were far better in heavily polluted water than in less polluted water. The phenol coefficient 0.5 indicates that the plant extract is less effective than phenol. Thus *Hygrophila* species proved to be good water purifier even though there are no such related literatures regarding water purification.

**KEYWORDS:** *Hygrophila auriculata,* phytodisinfectants, phenol coefficient, water purification.

## INTRODUCTION

Water pollution is one of the most serious environmental problems due to overpopulation, urbanization, industrialization and illiteracy. (Srivastava and Purnima 1998). It is becoming a huge problem which is faced by all of the human existence and as well as by every wild life species. According to present scales for pollution of water, 10 to 15 billion pounds full of waste materials like garbage is threw in different seas and rivers of the entire world. Not only had this, now, as per the latest records for water pollution in India; had 20 billion gallons of drinking water pollution also dumped in running rivers and seas. This serious problem of water pollution is not only serious

for the present day but, it is also getting worst on a regular day by day basis. As the seas and rivers have a running current of movement; thus, pollution of water does get transported in to various cities and towns on an immense scale. Not only this but, pollution of water also travel to various locations and hence increase water pollution in India. One more reason for the increasing air and water pollution along with drinking water pollution is because of highly growing industrial sector.

These industrial sectors not only results harmfully in increasing drinking water pollution but also increase the air and water pollution on the same time. Another major reason for pollution of water in our country is because of the huge population which is increasing day-by-day. Today, with such huge growing population also the ecosystem is getting effected and giving rise directly to the air and water pollution. Huge population means higher level of water pollution and higher level for pollution of water increases the diseases and death rate for human lives. Thus, rapidly growth in high population is also resulting in increased water pollution in India. Now, if seen clearly and closely on these total water pollution sources, then it can be said that both increasing population and industrialization are the major reason behind the drinking water pollution along with other air and water pollution.

Municipal and industrial wastewaters frequently contain phosphate, nitrate and heavy metals ions. The industrial use of metals increases their concentrations in soil, air and water. The trace metals are widely spread in environment and may enter the food chain from the environment. It is well recognized that the presence of metals ions in the environment can be detrimental to a variety of living species (Benhima *et al.*, 2008). Unlike organic pollutants, metals are non-biodegradable and because of this the removal of heavy metals becomes essential. Also, nitrate and phosphate ions are commonly found in various wastewaters. They can cause serious water pollution and threaten the environment (Barber and Stuckey, 2000).

Phytoremediaton is the process using plants to clean up the environment. The word phytoremediation comes from the Greek word *phyto*, meaning "plant" and the Latin word *remediare*, meaning "to remedy". This word is generally used to describe any system where plants are introduced into an environment to remove contaminants from it. Phytoremediation is done in a variety of ways. The plants can be introduced into an environment and allowed to absorb contaminants into its leaves and roots. These plants can then be harvested and treated as hazardous waste. There have even been studies where these plants have turned the contaminant into a harmless substance and then once harvested can be used for animal feed, paper, etc. In some instances (especially if trees are being used) the plants are left in the environment and allowed to grow and mature as normal (David *et al.*, 1995).

*Hygrophila auriculata* (Schum.) Heine (Family: Acanthaceae) is a wild herb commonly found in moist places on the banks of rivers, ditches and paddy fields throughout India, Sri lanka, Burma, Malaysia, and Nepal. A survey of the ethanobotanical literature shows that the roots, seeds, and aerial parts of the plant are widely used in the traditional system of medicine for the treatment of jaundice, hepatic obstruction, rheumatism, inflammation, pain, urinary infection, edema, gout, malaria, and impotence and as an aphrodisiac (Jain, 1991). In this study, *Hygrophila* species proved to be good water purifier even though there are no such related literatures regarding water purification.

## MATERIALS AND METHODS

### Collection of Plant materials:

Fresh leaves and aerial parts of botanically identified *H. auriculata* at its flowering stage were collected in bulk from waterlogged area of the Paddy fields, Nagercoil, Kanyakumari district during the month of October- November in the year 2015. Cleaned leaves were then dried under shade. The drying process was continued to decrease the moisture content and were ground into a fine powder form using domestic mixer grinder machine. The fine powder of the plant leaves obtained was stored in air tight containers for further processing.

### Preparation of Plant Extract:

A cold methanol extraction was then carried out on 100 g of the plant powder by steeping the powder in 250 ml of methanol for 24 hours. Gravity filtration was carried out using what man filter paper No. 13 and solvent evaporated at room temperature in a fume cupboard.

### Qualitative Phytochemical Screening:

Following different qualitative chemical tests were performed to investigate the chemical composition of *H. auriculata* extract;

Detection of Alkaloids- Mayer's test (Evans, 1997)

Detection of Carbohydrates- Molish's test (Ramakrishnan *et al.,* 1994)

Detection of Phenols- Ferric chloride test (Mace, 1963)

Detection of Flavanoids- Alkaline reagent test (Brain *et al.*, 1975)

Detection of Saponins (Kokate, 1999)

Detection of Tannins (Magadula *et al.*, 2010)

### Identification of Pigments Using Thin Layer Chromatography (Chakraborty *et al.*, 1999)

In thin layer chromatography, separations are carried out within thin layers of almost any material supported on glass plates. A thin layer of adsorbent coated over a glass plate is called chromo plate. By this technique, separations by partition, adsorption and ion exchange can be performed.

In this method, a slurry of the adsorbent, silica gel G was spread uniformly (0.25mm

thick) on a glass plate and allowed to dry. Then the plate was activated by heating in an oven at 100 to120$^0$Cfor 3 hours. This serves to activate the adsorbent. In the pre-activated TLC plates, a base line (3cm above from the bottom) and top line (15cm from the base line was marked). After that the samples (3µ liters) were applied to the plate 2.0-2.5cm from the side edge by means of micropipette or micro syringe over the base line. Then the samples loaded TLC plate was dipped vertically into a glass tank that contains methanol as solvent system. The solvent ascends up the plate and separation of the mixture into components taken place. When the solvent reached the upper line, the plate was taken out and dried for the detection of pigments. In this way, the position of the components was located and their Rf values were determined by the following formula;

$$\text{Rf value} = \frac{\text{Distance travelled by the substance}}{\text{Distance travelled by the solvent}}$$

**Microbial analysis of untreated water sample:**

*Escherichia coli*, coliforms and Total aerobic mesophilic bacterial counts were enumerated on Nutrient agar (Harrigan *et al.,* 1976). A milliliter of the turbid water samples were aseptically diluted serially up to three fold. This was done according to methods of (APHA, 1983), (Cheesbrough, 1984) and (Yongabi, 2004). All these were done before and after coagulation and disinfection.

**Preliminary antibacterial determination of crude methanolic plant extracts from polluted water sample:**

This was carried to confirm if the bacterial reduction previously observed is due to inherent disinfectant ability of the plants. One hundred (100) mg of each of the extract was suspended in 1 ml of distilled water. The extracts were now tested for their *in vitro* antibacterial activity using the agar well-diffusion Method (Mali *et al., 2004*). The bacterial strains used were *Escherchia coli.* The choice of *E. coli* is because *E. coli* are an important indicator of faecal contamination of water. The other bacterial strains include *Bacillus subtilis* and *Pseudomonas aeruginosa*. Nutrient agar media was taken in a pre-sterilized Petri-dish and the microorganisms were grown. The extracts were dissolved separately in distilled water and used in the concentration of 100µg/well in triplicate, placed in petri dishes and incubated at 37$^0$C for 24 hrs. The diameters of zone of inhibition (mm) were recorded and compared with standard drug Ofloxacin (OF) (Walker, 1998).

**Water sample collection:**

It is observed that the pollution of surface and subsurface water has been increased due to domestic and industrial waste water. In this study, we have selected pond of peruvilai village, Nagercoil. The first set of raw waste water sampling has been collected in the second week of December 2011. The appearance/cloudiness of these water samples

were noted by visual observation (Burns and Van, 1974).

The physical, chemical examination of the untreated water sample and experimental plant treated water sample was done (Clarson, 2002). The physical examination include turbidity, total dissolved solids and electrical conductivity whereas the chemical examination includes pH, total hardness, alkalinity, calcium, magnesium, dissolved oxygen, biological oxygen demand and chemical oxygen demand etc.

**Test tube *in vitro* coagulation and disinfection test using methanolic extract of the plant materials in polluted sample and Automobile Service Station Effluent (ASSE):**

This was a novel technique meant to further proof phytodisinfection activity of the plant extract. The preliminary *in vitro* coagulation and disinfection studies was also carried out using the methanolic plant extract.1 ml of the methanolic plant extract was dropped in 5 ml of polluted water and Automobile Service Station Effluent (ASSE) in two seperate test tubes, clearance rate after 15 and 30 minutes residence time was reported visually in both the tubes and a ml of the treated water was cultured for total bacterial counts (Yongabi, 2004).

**Phenol Coefficient Test:**

The phenol coefficient test compares the antimicrobial activity of a chemical compound to that of phenol under standardized experimental conditions. In a test-tube rack, place test tubes of each of the different phenol and methanolic plant extract dilutions.

Rapidly introduce one drop of the *Staphylococcus aureus* culture into each of the test tubes of disinfectant. Note the time when start introducing the microorganism into the disinfectants. Agitate all the test tubes to ensure contact between the disinfectants and the microbes. Using sterile technique, at intervals of 5, 10 and 15 minutes transfer 0.1ml from each of the test tubes containing the disinfectant and microorganisms into the prepared sterile nutrient agar plates using spread plate technique. Label and incubate all nutrient agar plates for 48 hours at $37^0$C and examined for the presence or absence of growth.

The phenol coefficient is determined by dividing the highest dilution of the chemical being tested that destroyed the microorganisms in 10 minutes but not in 5 minutes by the highest dilution of phenol that destroyed the microorganisms in 10 minutes but not in 5 minutes. A phenol coefficient number greater than 1 indicates that this agent is as effective as or less effective than phenol. A phenol coefficient greater than 1 suggests that the chemical is more effective than phenol when employed under test conditions (Cappuccino, 2005).

**RESULT AND DISCUSSION**

The plant materials were collected and the methanolic plant extract were prepared to carry out the phytochemical screening. In the

phytochemical screening, the methanolic plant extract shows the presence of alkaloids, flavanoids and phenol (Table 1). There are also literatures suggesting that *Hygrophila spinosa* contains 1.5% alkaloids corroborated with the present result. Additional to this carbohydrates, phenolic compounds and tannins were also detected in the extract contradict the result of previous worker who reported presence of phytosterol, nitrogen, albuminoids, alkaloids, essential oils (Majumdar and Sengupta, 1978).

**Table 1: Qualitative phytochemical analysis**

| PARAMETERS | RESULTS |
|---|---|
| Carbohydrate | Absent |
| Alkaloids | Present |
| Tannin | Absent |
| Flavanoids | Present |
| Phenol | Present |
| Saponin | Absent |

The thin layer chromatography showed the presence of chlorophyll a, chlorophyll b, lutein, pheophytin, neoxanthin, carotene, violaxanthin. The methanolic plant extract shows high number of pigments such as chlorophyll a, chlorophyll b, lutein, neoxanthin, carotene, violaxanthin whereas the ethanolic plant extract shows the presence of lutein and pheophytin and the acetone plant extract shows the presence of only violaxanthin (Table 2- 4). The Rf value were noted for each pigment and compared with the standard Rf value. In the earlier studies, the compounds identified in *H. spinosa* are mainly phytosterols, fatty acids, minerals, polyphenols, proanthocyanins, mucilage, alkaloids, enzymes, amino acids, carbohydrates, hydrocarbons, flavonoids, terpenoids, vitamins and glycosides. Some of the reported phytoconstituents are lupeol, lupenone, β- carotene, stearic acid, linoleic acid etc. (Arjun Patra *et al.*, 2009).

**Table 2: Detection of pigments using methanolic plant extract**

| Name of the pigments | Rf value for standard pigments | Rf value for methanolic extract | Colour of the pigment |
|---|---|---|---|
| Chlorophyll a | 0.59 | 0.55 | Blue green |
| Lutein | 0.74 | 0.71 | Yellow |
| Carotene | 0.98 | 0.95 | Golden |
| Violaxanthin | 0.65 | 0.65 | Yellow |
| Neoxanthin | 0.32 | 0.37 | Yellow |
| Chlorophyll b | 0.42 | 0.45 | Yellow green |

**Table 3: Detection of pigments using ethanolic plant extract**

| Name of the pigments | Rf value for standard pigments | Rf value for ethanolic extract | Colour of the pigment |
|---|---|---|---|
| Pheophytin | 0.81 | 0.78 | Olive green |
| Lutein | 0.74 | 0.72 | Yellow |

**Table 4: Detection of pigments using acetone plant extract**

| Name of the pigments | Rf value for standard pigments | Rf value for acetone extract | Colour of the pigment |
|---|---|---|---|
| Violaxanthin | 0.65 | 0.67 | Yellow |

The antibacterial activity was performed using methanolic plant extract against the test organisms and it was observed that *Bacillus subtilis* shows the highest zone of inhibition (10 mm) than the other two organisms (Plate 1) (Table 5). The antibiogram using commercial antibiotic Ofloxacin was also performed. It was observed that *Bacillus subtilis* shows the highest zone of inhibition (17 mm) than the other two organisms (Plate 2) (Table 6). Similarly there are also literatures stating that leaf extract of *Hygrophila spinosa* collected during mid-September to October end showed significant antibacterial activity in comparison to the other two extracts, where the activity are almost similar (Arjun patra *et al*., 2009).

**Table 5: Antibacterial activity of methanolic plant extract on test organism**

| SL.NO | TEST ORGANISMS | ZONE OF INHIBITION (mm) |
|---|---|---|
| 1. | *E.coli* | 6 |
| 2. | *Bacillus subtilis* | 10 |
| 3. | *Pseudomonas aeruginosa* | 8 |

**Table 6: Antibiogram of test organisms against the commercial antibiotic ofloxacin**

| SL.NO | NAME OF THE ANTIBIOTIC | CODE | CONCENTRATION/DISC IN mcg | TEST ORGANISMS | ZONE OF INHIBITION (mm) | RESULT |
|---|---|---|---|---|---|---|
| 1. | Ofloxacin | OF | 5 | *E.coli* | 15 | I |
| 2. | Ofloxacin | OF | 5 | *Bacillus subtilis* | 17 | S |
| 3. | Ofloxacin | OF | 5 | *Pseudomonas aeruginosa* | 15 | I |

**Plate 1: Antibacterial activity of methanolic plant extract on test organisms**

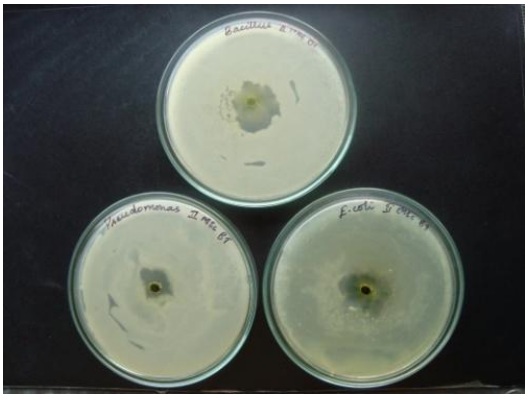


**Plate 2: Antibiogram of test organisms against the commercial antibiotic ofloxacin**

The untreated and the experimental plant treated sample were subjected to physical and chemical analysis. The physical analysis revealed the reduction in turbidity and total dissolved solids in experimental plant treated water sample compared to the untreated water sample (Table 7). The chemical analysis of the experimental plant treated water sample shows the reduction in pH, sodium, magnesium, calcium and increase in potassium, phosphate and iron (Table 8). The average removal efficiency for the plant species that is water hyacinth was 80.26%, for Cr and 71.28%, for Cd. Average removal rates of Cr and Cd were 0.10µg/day and 0.12µg/day. This shows that water logged plants has the ability to remove toxic wastes from the water sample (Satya Narain *et al*., 2011).

**Table 7: physical examination of the water sample**

| SL.NO | PARAMETERS | Untreated water sample mg/l | Experimental plant treated water sample mg/l |
|---|---|---|---|
| 1. | Appearance | Brownish | Brownish |
| 2. | Odour | Algae | Algae |
| 3. | Turbidity NT units | 40 | 27 |
| 4. | Total Dissolved Solids | 203 | 74 |
| 5. | Electrical Conductivity micS/cm | 111 | 308 |

**Table 8: chemical examination of the water sample**

| SL.NO | PARAMETERS | Untreated water sample mg/l | Experimental plant treated water sample mg/l |
|---|---|---|---|
| 1. | pH | 9.04 | 8.28 |
| 2. | Alkalinity-pH as CaCO3 | 16 | 4 |
| 3. | Alkalinity Total as CaCO3 | 16 | 52 |
| 4. | Total Hardness as CaCO3 | 92 | 32 |
| 5. | Calcium as Ca | 26 | 8 |
| 6. | Magnesium as Mg | 7 | 3 |
| 7. | Sodium as Na | 22 | 9 |
| 8. | Potassium as K | 1 | 5 |
| 9. | Iron Total as Fe | 2.71 | 4.47 |
| 10. | Manganese as Mn | 0.33 | 0.33 |
| 11. | Free Ammonia as NH3 | 0.58 | 1.04 |
| 12. | Nitrite as NO2 | 0.07 | 0.39 |
| 13. | Nitrate as NO3 | 2 | 2 |
| 14. | Chlorides Cl | 64 | 20 |
| 15. | Fluoride as F | 0.0 | 0.0 |
| 16. | Sulphate as SO4 | 14 | 4 |
| 17. | Phosphate as PO4 | 1.6 | 2.10 |
| 18. | Dissolved Oxygen | 1.6 | 1.3 |

The BOD content for the untreated water sample was shown to be 25mg/l whereas the plant treated water sample was 6mg/l and the percentage of reduction was shown to be 76. This shows the reduction in the BOD content which is an indication for the purity of the water (Table 9). WHO recommended a BOD limit of 6mg/l for drinking water until 1971, no limit is now recommended. A sample with a 5 day BOD between 1 and 2mg/l indicates a very clean water, 3 to 6mg/l indicates a moderately clean water and >6mg/l indicates a nearby pollution source (WHO, 1996).

**Table 9: Determination of biological oxygen demand**

| SL.NO | BOD ANALYSIS | BOD VALUES(mg/l) | % OF REDUCTION |
|---|---|---|---|
| 1. | Untreated water Sample | 25 | _ |
| 2. | Experimental plant treated water sample | 6 | 76% |

The coagulation and disinfection activity was carried out in both polluted water and Automobile Service Station Effluent using methanolic plant extract. The raw untreated

water sample had an initial total bacterial counts too Numerous to count, which reduced to only 43 colony forming units per ml when treated with the methanolic plant extract after 15 minutes exposure and reduced to 17 colony forming units per ml after 30 minutes exposure in the polluted water (Plate 3) (Table 10). Similarly it was also done for Automobile Service Station Effluent (ASSE) which shows the initial bacterial counts too numerous to count, which shows no growth of organisms when treated with the methanolic plant extract after 15 minutes and 30 minutes exposure (Plate 4) (Table 11). The findings indicated that *Hygrophila auriculata* coagulated well above 90% of the particles in the samples leading to a clear supernatant after 15 and 30 minutes residence time. The coagulation and the disinfection effect were far better in heavily polluted water (ASSE) than in less polluted water. A 95% reduction in bacterial loads of the water samples by *M. oleifera* in fifteen minutes residence time was observed (Amir *et al.*, 2010).

**Table 10: Disinfection effect of methanolic plant extract-total bacterial count of polluted water in cfu/ml**

| SL.NO | INITIAL COUNT OF WATER SAMPLE | TREATMENT | TOTAL BACTERIAL COUNTS AFTER | |
|---|---|---|---|---|
| | | | 15 MINUTES | 30 MINUTES |
| 1. | TNTC | Methanolic plant extract | 43 | 17 |

TNTC- Too Numerous To Count

**Table 11: Disinfection effect of methanolic plant extract- total bacterial count of automobile service station effluent (asse) in cfu/ml**

| SL.NO | INITIAL COUNT OF WATER SAMPLE | TREATMENT | TOTAL BACTERIAL COUNTS AFTER | |
|---|---|---|---|---|
| | | | 15 MINUTES | 30 MINUTES |
| 1. | TNTC | Methanolic plant extract | Nil | Nil |

**Plate 3: Test tube *in vitro* coagulation and disinfection test using methanolic extract of the plant materials in polluted sample**

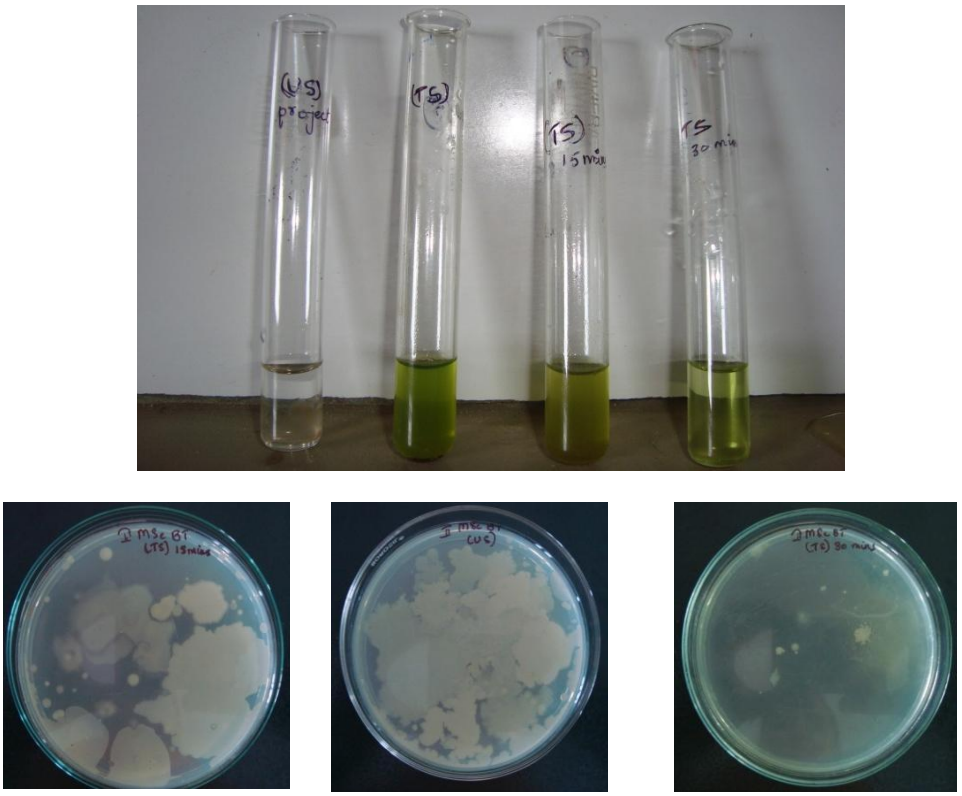
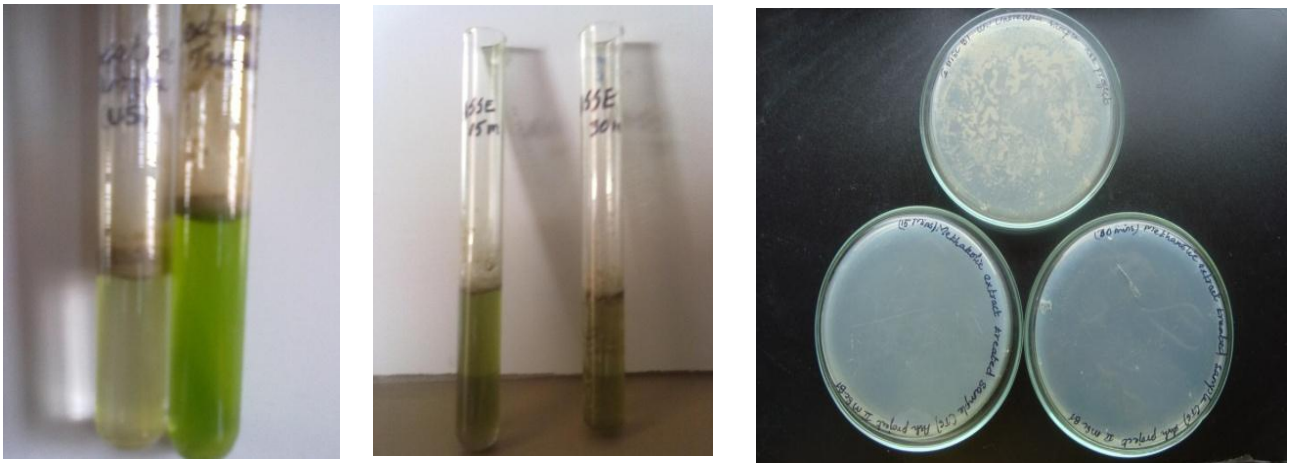


**Plate 4: Test tube *in vitro* coagulation and disinfection test using methanolic extract of the plant materials in automobile service station effluent (ASSE)**



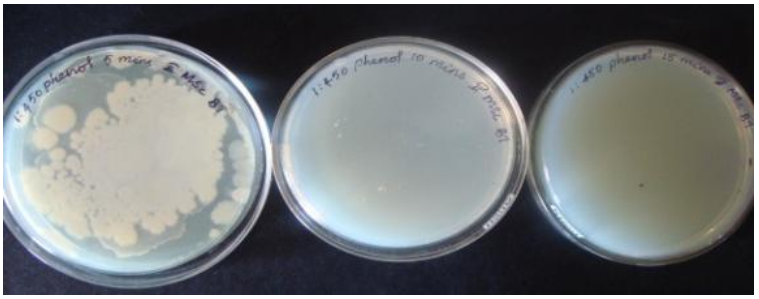
A phenol coefficient of 0.5 indicates that the methanolic plant extract under evaluation was 0.5 times less effective than phenol (Plate 5) (Table 12). The phenol coefficient test is mainly carried out to compare a chemical disinfectant with the phenol. This test compares Lysol with phenol in which the highest dilution of Lysol that destroyed the organism in 10 minutes but not in 5 minutes was 1:450. The highest dilution of phenol that destroyed the organism in 10 minutes but not in 5 minutes was 1:90. Thus the phenol coefficient of test chemical = 450/90 = 5. A phenol coefficient of 5 indicates that the chemical

under evaluation is five times as effective as phenol (Cappuccino, 2005). Thus *Hygrophila* species proved to be good water purifier even though there are no such related literatures regarding water purification.

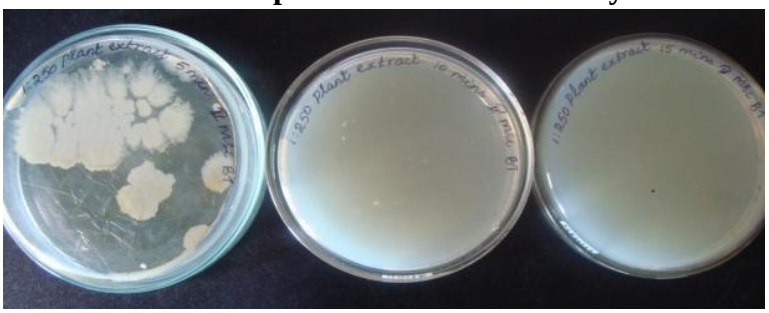
**Table 12: Phenol coefficient determination**

| S.NO | CHEMICAL AGENT AND DILUTION | | PRESENCE OF GROWTH (MINUTES) | | |
|---|---|---|---|---|---|
| | | | 5 | 10 | 15 |
| 1. | TEST DISINFECTANT | **1:200** | - | - | - |
| | | **1:250** | + | - | - |
| | | **1:300** | + | + | - |
| 2. | PHENOL | **1:400** | - | - | - |
| | | **1:450** | + | - | - |
| | | **1:500** | + | + | + |

**Plate 5: Phenol coefficient test highest dilution of phenol that destroys the microorganisms**



**Highest dilution of methanolic plant extract that destroys the microorganisms**



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