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WATER HYACINTH (*EICHHORNIA CRASSIPES*) : A SOURCE OF BIOREMEDIATION, COMPOST AND MEDICINE

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ABSTRACT

Pollution is an enormous global problem causing multiple harm to the environment including land infertility and health issues. India generates sewage of about 38 billion litres per day, but 78% of it remains untreated and the artificial compost has a major drawback of lacking essential nutrients necessary for soil fertility. The phytochemicals contain widely diverse pharmacological actions assessed by rigorous scientific research to define efficacy and safety. Treatment of all these problems is possible by using one aquatic free floating weed as a resourceful remedy- Eichhorniacrassipes (water hyacinth). Despite being labelled as an ecological threat, the water hyacinth can act as the most efficient absorber and hyper accumulator of all types of effluents including, nutrient pollutants (up to 70-80%), enabling its use for compost preparation. It can be removed from water after phytoremediation, dried and crushed to prepare compost. This plant has also shown anti-microbial, anti-tumor, and wound healing activity from secondary metabolites which can be extracted for pharmacological uses. The drawback like abiotic intolerance and extraction of secondary metabolites creates a problem in its applications which could turn to tolerance by introducing the gene of tolerance and new techniques for extraction, making it the cheapest source of medicine as well as a no wastage principle of treating pollution.

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INTRODUCTION

The potential that lies in different aquatic weeds, like floating, submerged or emergent aquatic macrophytes, are being explored worldwide (Reddy and Smith 1987; Haberl 1997; Trivedy 2001). One of the major aquatic weeds is the common water hyacinth (*Eichhornia crassipes*) which belongs to the order, Commelinales and family Pontederiaceae. It was introduced in many countries as an ornamental specimen for ponds and botanical gardens (Hil and Phiri, 1997). It later became a fast growing water weed, spreading rapidly in high density (over 60 kg/m2) clogging water bodies, leading to hazardous effects on the water ecosystem, environment, and economic activities (Fernandez *et al.*, 1990: Epstein, 1998).

The attractive and noticeable flowers became the reason for the deliberate introduction of this plant into botanical gardens. It was likely established in the 1900s, to Madagascar as an ornamental plant and was firstly accounted in 1920 (Binggeli, 2003). *E. crassipes* may spread rapidly and form thick monotypic mats when introduced to favorable habitats, especially open waters. It has conquered the freshwater systems across 50 countries of five continents.The models of climate change suggest that its distribution may extend to the higher latitudes as rise in the temperatures occurs.

MORPHOLOGY AND GENERAL CHARACTERS OF E. CRASSIPES

E.crassipes is a free-floating macrophyte that grows in colonies as well as single standing plants in both saline and freshwaters (Figure1) (Barrett, 1989). With its reproductive organs on top, it reproduces both sexually and by budding and stolon production (Schmitz *et al.*, 1993). It reproduces sexually through the production of seeds for more rapid spreading.

Shoots consist of axillary buds which also form stolons. The leaves are thick, glossy, broad and ovate. Azotobacter chroococcum is a nitrogen-fixing bacterium which is concentrated around the bases of the petioles of water hyacinth. The fibrous and adventitious roots comprise 50% of biomass of a single water hyacinth. The roots of the water hyacinth shelter macroinvertebrates and small fish that are able to withstand the low dissolved oxygen condition under its mats (Penpound and Earle, 1948). The bacteria fix nitrogen only when the plant is suffering extreme nitrogen-deficiency.

The seeds of water hyacinth can remain viable up to 20 years. Seed germination is favored by high intensity light and alternating low and high temperatures ranging from 5°C to 40°C (Gopal, 1987).

Figure 1: water hyacinth *(Eichhornia crassipes)*

PRODUCTIVITY

Water hyacinths are a major contestant in bio-conversion for energy, producing methane. NASA found that 350 to 411 liters of biogas per kg dry weight (5.7 to 6.6 scf per dry lb) can be obtained in batch studies of anaerobically digested water hyacinths (Wolverton *et al.*, 1975). This biogas consists of approximately 60% methane. Therefore, one hectare of water hyacinths, grown in an enriched and nutrition rich environment in a warm climate for seven months of the year, can produce approximately 58,400 m3 biogas containing 35,100 m3 methane which is more than half of the total (Verma *et al.*, 2007).

GROWING CONDITION FOR WATER HYACINTH

The optimum growing conditions of water hyacinth (Table 1) depends on environmental factors like temperature, salinity and pH of surroundings.

Table 1: Environmental Factors Affecting *E. crassipes*

Environmental Factors	Range of Tolerance	Optimum Growth
Temperature	12 - 35 °C	25–30 °C
Average salinity	0- 15%	0-0.2%
pH	5.0–7.5	7.0

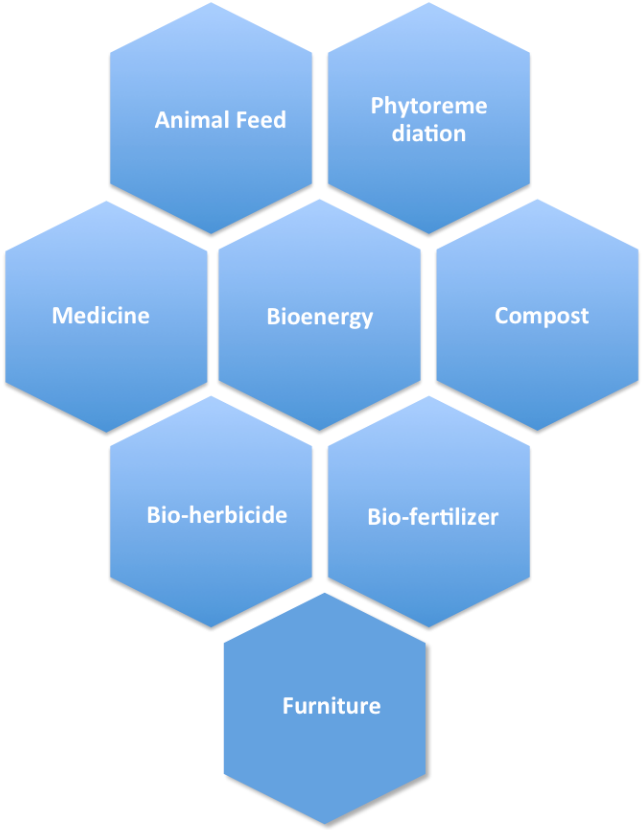


Figure 2: Potential Applications of Water hyacinth

APPLICATIONS OF WATER HYACINTHS

Water hyacinths are used in many different purposes (Figure 2). Wastewater treatment and phytoremediation are some of its important applications. It's application as a medicinal plant and use as fertilizer is significant. Here are a few such applications highlighted in the following section.

APPLICATIONS - WASTE WATER TREATMENT AND PHYTOREMEDIATION

Petrol Refinery Waste Treatment: Large amounts of water are utilized in petrol refineries, producing effluents about 0.4 to 1.6 times the amount of processed crude oil (Kadlecand Knight, 1996; Ishak *et al*. 2012). This untreated wastewater can cause serious problems like environmental and human health issues as it contains toxic and hazardous substances. Over 40% of this wastewater is only partially

treated and discharged as it contains toxic and hazardous substances. Over 40% of this wastewater is only partially treated and discharged into the environment (Igunnuand Chen, 2012; Mustapha et al., 2015; Ishaket al., 2012; Maha *et al.*, 2012). A typical petroleum refinery wastewater contains a wide range of organics, heavy metal oil, ammonia and grease, suspended solids, phenolics, and sulphide (Ahmadun *et al.*, 2009; Kadlec and Knight, 1996)

In a study conducted by Agarry *et al.*, (2018), the effects of *E. crassipes* on refinery waste was found. The DO concentration of the inlet wastewater which was initially 2.10 mg/L increased to 3.20 mg/L. The BOD of the inlet wastewater had a concentration value of 20.40 mg/L, which dropped to 1.96 mg/L. BOD removal efficiency increased positively from 37.3% to 94.6% with increased detention time. The COD concentration of the inlet wastewater was obtained to be 86 mg/L (before treatment) which reduced to 17 mg/L after treatment with -

E. Crassipes. *E. crassipes* successfully removed Cd, Pb, Zn, Cu, Cr, and Fe by 70% to 100% (Table 2).

Table 2: Pollutants in Refineries Initial and Final Concentration after Treatment with *E. Crassipes* (Source: Agarry *et al.*, 2018)

Pollutants	Concentration in Untreated Water (mg L^{-1})	Concentration in *E. crassipes* treated water (mg L^{-1})
Cadmium (Cd)	0.034	0.002
Lead (Pb)	0.12	0.009
Zinc (Zn)	0.31	0.17
Copper (Cu)	0.47	0.080
Chromium (Cr)	1.54	0.033
Nickel (Ni	0.09	0.007
Total Petroleum Hydrocarbon	16.6	1.23
Oil and Grease	18.4	1.77
Nitrate-Nitrogen	4.03	1.10
Sulphate	78.36	6.73
Chloride	1412	597

TEXTILE INDUSTRY WASTE PHYTOREMEDIA-TION

The wastewater from textile industry is one of the primary pollutants (Vilaseca et al., 2010; Awomeso *et al.*, 2010). Textile wastewater is a composite and fluctuant mixture of pollutants such as organic, inorganic, elemental and polymeric products (Brown and Labourer, 1983). Among these, dye wastes are cardinal and are not only poisonous to the biological world but its dark color blocks sunlight leading to ecosystem problems (Choi *et al.*, 2004).

Removal of the dyes could be done by chemical and physical methods but the excessive cost and huge amounts of sludge material production requires another final disposal. There are approximately more than 8000 chemical products which are correlated with the dyeing procedure including several types of dyes like acidic, basic, diazo, disperse, reactive, anthraquinone, azo, and metal-composite dyes (Banat *et al.*, 1996).

The freshwater macrophyte, E. crassipes, has been investigated for the ability to draw heavy metals along with chemical dyes from its surrounding, making it effective for phytoremediation. The mechanisms involved to remove metals and dyes by biosorption can be classified as (Rai *et al.*, 2002)

- extracellular accumulation or precipitation
- cell surface sorption or precipitation
- intracellular accumulation

Dried roots of the *E. crassipes* has the potential to remove basic dyes, Victoria blue and methylene blue, thereby acting as a cheap source of bio-sorbent for basic dyes. In a study conducted by Soni *et al.* (2012), it was found that roots of water hyacinth are efficient absorbers, as in an aqueous solution they removed 95% of the methylene blue (Table 3).

Table 2: Removal Percentage of the dyes by E.crassipes (Nath *et al.*,2013)

TEXTILE DYES	REMOVAL % BY *E. CRASSIPES*
METHYLENE BLUE	90%
CONGO RED	88%
CRYSTAL VIOLET	92%
MALACHITE GREEN	90%

The reduction of pH of textile effluent from alkaline to neutral, was observed in water hyacinth, with the reduction of COD and BOD in the range 40 to 70%, along with highest reduction of total solids by 50.64% (Qaisar *et al.*, 2005).

Figure 3: The industrial sewage discharge in Gomti River, Lucknow.

DAIRY WASTE TREATMENT

One of the most paramount agro-based industries in India is the dairy industry. The range of pH obtained for dairy effluent was between 6.3-6.6. After undergoing aerobic conditions, it was attributed to the decomposition of lactose forming lactic acid that causes corrosion of sewers (Joseph, 1995). Increases in pH cause alkalophilic microorganisms to grow that are a part of indigenous microflora.

A better treatment by *E.crassipes* was possible with lesser retention time where there was 90% reduction in COD and BOD in 2–3 days along with TDS reduction. But the suspended solid reduction was low and water hyacinth is known to reduce suspended solids up to 90% (Reddy and Smith, 1987). Further investigation revealed that, it is required to remove the colloidal solids by some other method before water hyacinth - treatment for dairy waste, which enhances removal of nitrogen than phosphorus (Trivedy and Gudekar, 1985). Removal of sodium, calcium, potassium and magnesium, was not very high and in most cases any reduction in them is not intended (Ingole and Bhole, 2003).

BIOREMEDIATION OF OIL SPILL

Water pollution, caused by regular oil spill accidents, worsen the environmental, ecological and economic problems globally and greatly decreases fresh water resources, threatening the health of people and other living organisms (Aguilera *et al.*, 2010; Beyer *et al.*, 2016).

The suitable material for oil spill sorption should be low-cost and renewable with green raw materials and facile fabrication. Among all, using a cellulose based aerogel from inexpensive biomass fulfills the criteria due to its environmental and economic processing. *E. crassipes* grow swiftly in divergent aquatic ecosystems. Using cellulose aerogel to synthesize it, would have a likelihood of reusing aquatic plants. Cellulose-based aerogels (CBAs) maintained high oil sorption capacity (60.91 g/g for diesel oil) after 16 cycles and so CBA could be a promising super absorbent for spilled oil cleaning (Yin *et al.*, 2017).

Table 4 Various Kinds of Aerogels Manufactured

Type of Aerogels	Examples	References
Organic	Polypropylene And Polyurethane	Aydin and Sonmez, 2015; Diascorn *et al.*, 2015; Wang and Uyama, 2016
Inorganic	SiO_2, Al_2O_3	Gu *et al.*, 2014; Zhao *et al.*, 2015; Zhu *et al.*, 2015
Natural	Cellulose And Proteins	Wan *et al.*, 2015; Wang *et al.*, 2015

Anatomical Changes in *E. crassipes* after Wastewater Treatment:

In the study on *E. crassipes* conducted by Qaisar *et al.* (2005), plant parts like leaves, rhizomes, roots and petioles were harvested after 96 hours, cut and preserved in a toxic preservative-formalin-acetic acid-alcohol (Figure 4). Plant cross sections were observed to understand the changes the plant went through after wastewater treatment.

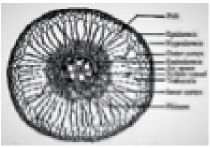
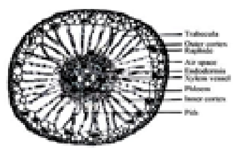
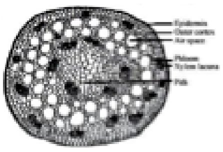
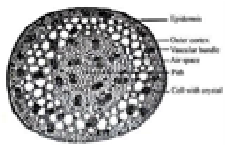
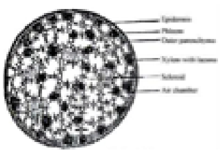
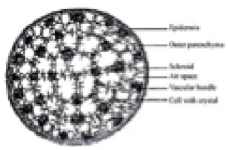
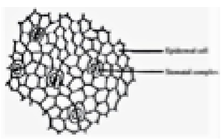
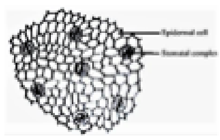
PLANT PARTS	NORMAL ANATOMY	ANATOMY CHANGE AFTER WASTEWATER TREATMENT
ROOTS		
RHIZOME		
PETIOLE		
LEAVES		

Figure 4: The Anatomical Change of *E. crassipes* after Wastewater Treatment (Source: Qaisar M. *et al.* 2005)

COMPOST PREPARATION FROM WATER HYACINTH

Composting is mainly an intense microbial activity which leads to the decomposition of most biodegradable materials such as complex organic materials resulting in stable organic residue (Adani et al., 1997; Weltzien, 1991). Composting is considered to be an ancient treatment to produce an environment safe and agronomic advantageous soil organic alteration at efficient operational cost (Senesi and Brunetti, 1996). Composting requires the continual supply of basic microbial requirements like: moisture, oxygen, temperature control and adequate mixing (Figure 5).

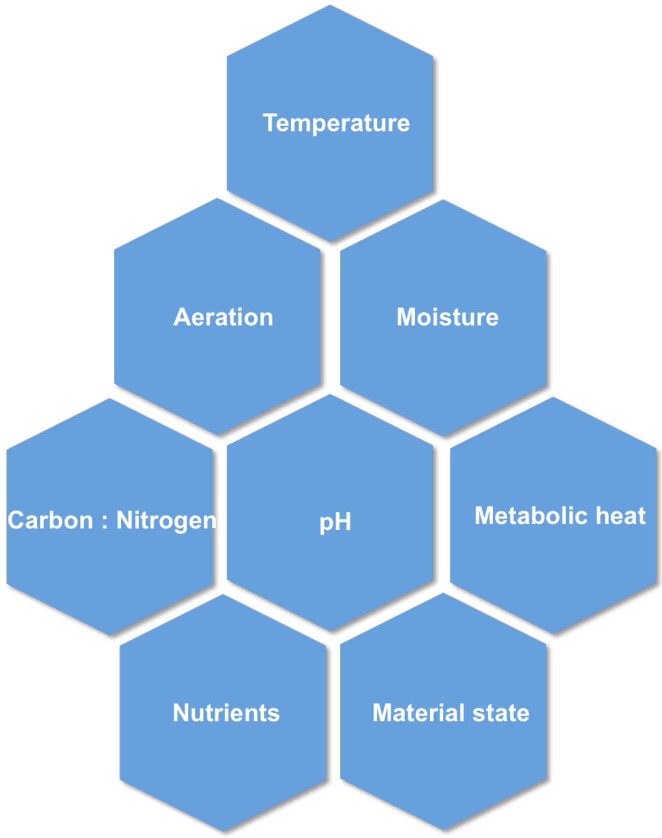


Figure 5: Several inter-related factors at the microbial process involved in the composting process (Adapted from Bea *et al.*, 1996; Sartaj *et al.*, 1995)

The two most important aspects of a compost process are (Boulter et al., 2000)

- the chemical makeup
- the existing population of microorganisms

Water hyacinth can serve to be used as manure, compost, or along with manure (as mulch at the bottom). It facilitates microbial decomposition by breaking down fats, lipids, proteins, sugars and starch. The mixture is kept in piles in tropical countries where warmer climates facilitate the production of rich pathogen free compost that may be transferred to soil. In Sri Lanka, it is mixed with organic municipal waste, ash and soil, composted and sold to native farmers and market gardeners (Dwivedi and Dwivedi, 2018).

The chemistry of plants plays an important role in the quality of compost. Freshwater water hyacinth contains 0.20% K2O, 0.04% N, 0.06% P2O5, 1.0% ash, 3.5% organic and 95.5% moisture. On the basis of zero-moisture, it is 75.8% organic matter, 1.5% Nitrogen, and 24.2% ash. The ash of water hyacinth contains 28.7% potassium oxide, 1.8% sodium oxide, 12.8% Calcium Oxide, 21.0% Chlorine, and 7.0% Phosphorus pentoxide. Water hyacinth roots naturally absorb pollutants, even toxic chemicals such as strontium 90, mercury and lead, and some organic compounds that are believed to be causing cancer (Jafari, 2010).

The nutritional status study of water hyacinth included pH, TOC, EC, TKN, Carbon/Nitrogen ratio, total P and total Ca of vermiwash. Different concentrations of buffalo dung along with gram bran, and *E. crassipes* can be converted into rich organic liquid bio-fertilizer through vermicomposting with the help of *E. fetida* (Shesh and Singh, 2016).

Water hyacinth can be used as a biofertilizer, once incorporated into soil. It inflated the performance of wheat plants when the wheat crop was treated with compost, derived from *E. crassipes*, for fifteen days (Sharda and Girish, 2014). The absorbent material within water hyacinth absorbs Nitrogen, Phosphorus and Potassium from water and may be used as a material for compost; results indicate its potential as organic manure.

Composting by the use of water hyacinth is popular in its indigenous variety (Kondap *et al.*, 1981). Water hyacinth in one hectare comprise of more than two million individual plants with about 300 tons of total wet weight and the sheer biomass of the plant mainly contains lignin, hemicellulose, cellulose and water (Zhao *et al.*, 2014) that marked its use in compost as well as vermicompost. The plant is rich in crude fibre as well as protein which result in large levels of lignin and cellulose transforming into humic materials (Gajalakshmi *et al.*, 2002). In India, water hyacinth compost has been utilized as a fertilizer acting as an organic modification to degraded soils (Balasubramanian *et al.*, 2013).

MEDICINAL APPLICAT-ION OF WATER HYACINTH

Antimicrobial activity:

The antimicrobial and anti-algal property was seen using the bioassay by paper disc diffusion on methanol extract and its fractions against bacteria, fungi, cyanobacteria and green microalgae. The antibacterial activity is exhibited by the crude extract and it's all 5 Total Liquid Chromatography fractions against both the

- Gram-positive bacteria: *Streptococcus faecalis* and *Bacillus subtilis*;
- Gram-negative bacteria: *Staphylococcus aureus* and *Escherichia coli;*
- Candida albicans was impeded by all.
- *Aspergillus niger* and *A. flavus* growth were not stopped by any of these extracts of water hyacinth.

The cyanobacteria *Spirulina platensis* and *Nostoc piscinale* as well as the green microalgae *Chlorella vulgaris* and *Dictyochloropsis splendid* were stopped by the anti-algal activity of the crude extract and its fractions and high anti-algal activity was recorded against Chlorella vulgaris. An alkaloid and 4 phthalate derivatives that had shown the anti-algal and antimicrobial activities were identified by analysis (Shanab *et a* *al*., 2010).

Among the aqueous, ethanol and methanol decoction of the leaves and roots, the aqueous decoction showed better antimicrobial activity, that of the leaves being higher than that of the roots (Fareed et al., 2008).The methanolic extract of water hyacinth showed reduction in the growth of *Aspergillus niger*, but did not show any inhibition of the growth of the *Colletotrichum graminicola* (Bobbarala *et al*., 2009).

In a study, 11 different human pathogenic bacteria and 7 phytopathogenic fungi were taken to observe the antimicrobial activity. reater antibacterial activity was shown by the methanolic fraction, while the part of water obtained by percolation in cold conditions, comparably showed better results against different fungi (Baral *et al*., 2010). The activity against *Aspergillus flavus*, *Fusarium oxysporum*, *Alternaria alternata*, *Rhizoctonia solani*, and *Xanthomonas campestris* was shown by methanolic extract of water hyacinth (Vadlapudi, 2010). Its activity depends on pH, concentration and action time extract showed activity against *Aspergillus niger*, *Escherichia coli*, *Penicillium* and *Staphylococcus aureus* (Zhou *et al.* 2009).

Antioxidant Activity:

The effective antioxidant activity by water hyacinth, the dry hyacinth's leaves had glutathione content about 32 ± 1.6 nmol/gm, as compared to 3.2 nmol/gm of leaves that were fresh (Bodo *et al.*, 2004). The ethanol extract contains polyphenol whose amount in leaves is greater than in stem and also has higher contents of flavonoids. Along with the DPPH radical scavenging capability, the leaf and stem have high reducing power which is 62.7% for stem and 74.6% for leaf (Liu *et al.* 2010).

Hydroponically *Eichhornia crassipes* was exposed for 21 days to a number of concentrations of Cadmium, Chromium, Copper, Mercury, Silver, Nickel, Zinc and Lead which showed incline in the activity of superoxide dismutase, catalase and peroxidase like enzymes. In Eichhornia crassipes, Zinc had the least incitement while Mercury had the highest incitement of the antioxidant enzymes (Odjegba and Fasidi, 2007). In the DPPH assay, greater activity was showed by the extract of ethyl acetate as compared to the methanol and n-hexane extracts, whereas in the ABTS assay, the extract of methanol of crude showed the higher activity than its fractions (Shanab *et al*., 2010).

Wound healing activity:

The leaves yielded methanolic extract in treatment form, at two w/w concentrations of 10% and 15% of leaf extract were investigated for their wound healing potential in rat model. The treatments showed considerably better ability of contraction of wound than of the control (Ali *et al*., 2009).

Antitumor activity:

In water hyacinth, the methanolic leaf extract at different doses from 200 mg per kg of body weight to 500 mg per kg of body weight reported a good response against hybrid mice models like Swiss albino female and C57BL male bearing B16F10, melanoma tumor (Ali *et al.* 2010). The crude extract compared showed greater treating activity against several tumor types. While other fractions showed high anticancer activity in opposition to the hormone dependent type tumor, like cervix and breast cancer against a cancer cell line of the liver. The potential of the crude as compared to its fractions has been ascribed to the auto-coacting effect of these fractions in the same extract (Enien *et al.* 2018).

Larvicidal Activity:

When varying concentrations of crude root extracts of *E. crassipes* was given to *Chironomus ramosus* Chaudhuri larvae and eggs, 100%efficiency was observed (Thorat and Nath 2010). The acetone extract showed toxic activities towards the third stage instar larvae of the tobacco cutworm, *Spodoptera litura* Fab two lepidopteran pests, *Achaea janata* L. and the castor semilooper (Devanand and Usharani, 2008).

In leaves and flowers, the putative cytokinin glucoside-like activity was detected. Pupicidal, Larvicidal as well as repellent activity when carried out on the aqueous extracts, ethyl acetate, light petroleum, methanol and ethanol fractions against *Culex quinquefasciatus* showed good activity in the laboratory.

***E. crassipes* olis extract**:
E. crassipes contains oil comprised of both benzyl alcohol and phenethyl alcohol which are part of many essential oils used in various fields. N1-acetyl-N2-formyl-5-methoxykynuramine along with its precursor melatonin, are potent free radical scavengers and so assisting plant being tolerant to environmental stress (Tan *et al*., 2007).

FUTURE ASPECTS

The only drawback in the cultivation of the *E. crassipes* is the temperature, salinity and pH intolerance as well as the drying and extraction of the secondary metabolites which could be improved and engineered by modern biotechnology approaches by introducing the gene of a temperature tolerant as well as halophytes making it tolerant as well as new techniques for easy extraction of secondary metabolites if deduced then it will make the cheapest source of the medicines.

The temperature tolerance could be induced in the plant by molecular and genetic variation by - molecular and genetic variation by the Quantitative Trait Locus Analysis to identify the loci for heat tolerant gene. The heat shock response is a regulated response involving many elements (Baniwal *et al.*, 2005 and Vierling, 1991) and it was revealed that heat tolerance in two *Potamogeton* species leads to differences in specific species as response and Heat Shock Transcription Factor A2, *HSFA2* and its alleged target gene, Chloroplast-localized Small Heat Shock Protein, *CP-sHSP*, were identified and it was found to be better in heat tolerance,maintaining a higher level of transcription of identical *HSFA2* and *CP-sHSP* genes, to overcome the stress of severe heat (Amano *et al.*, 2012) and so genescan be overexpressed in *Eichhornia* wild-type plants to screen for mutants. The halophytes are the plants which have the potential to grow in the high saline conditions and contain genes which help them in the tolerance of extreme conditions as well as the pH tolerant gene can be introduced in the *E. crassipes* genome for developing tolerance.

There are several other methods that can be utilised for the extraction of the secondary metabolites which will make it the cheapest source of medicines which can treat tumour, microbial infection, algal infection and have a high wound healing ability.

CONCLUSION

India generates 38 billion litres of sewage per day, but 78% of it remains untreated. Due to continuous use of chemicals, lands are becoming barren and artificial compost has a major drawback of lacking few nutrients necessary for soil fertility. Plant produces numerous phytochemicals with pharmacological uses. The free floating plant, *Eichhornia crassipesis* generally regarded as ecological threat, can act as the most efficient absorber -

and hyper accumulator of all types of effluents, among which the nutrient pollutants like Nitrogen and Phosphorous can be absorbed up to 70-80% which enables the use of this plant to prepare compost after drying and crushing, which is rich in every nutrients crucial for soil fertility after the metals from the roots are extracted by different processes thus providing no wastage principle.Water hyacinth plant has shown medicinal properties like anti-oxidant, anti-microbial, anti-tumor, larvicidal and wound healing activity by the secondary metabolites like glycosides, steroids, methanol, phenols and terpenoids.

It cannot survive temperature beyond 35°C and the average salinity greater than 15% making abiotic intolerant, which could be solved by engineering the gene of water hyacinth with the genes of temperature and salinity tolerant plants thus making it suitable to grow at all conditions so that boiled industrial effluent and its salinity and pH not become a barrier in phytoremediation without affecting its activity. The secondary metabolites extraction if simplified and becomes less costly then the plant can treat various diseases by being the cheapest source as no expenditure would be there for providing favourable conditions for its growth due to its invasive properties the plant is cheaper to grow and produce metabolites at a much faster rate. Thus the biggest global problems: pollution, land infertility and health can be solved by just one plant requiring no input cost.

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