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Evaluation of Antibacterial and Antifungal Susceptibility of Water Hyacinth

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Abstract:- Water hyacinth *(Eichhornia crassipes)* is an aquatic weed which proliferates rapidly in lakes and rivers. The rapid growth and spread of water hyacinth has been a great concern worldwide. In Mauritius, it covers an approximate area of 13.67 km² throughout the island. The biodegradability, antibacterial and antifungal activity of water hyacinth's leaves, stalks and roots were studied to explore it's potential as a raw material for product development. The inoculums *Trichoderma viride, Staphylococcus aureus, Klebsiella pneumoniae* and *Aspergillus niger* were used for testing. The biodegradability test was conducted with enzyme cellulase from *Trichoderma viride* using the enzymatic method. The biodegradation levels were determined by computing the percentage weight loss after thirty days. The antibacterial test was performed with the test inoculums *Staphylococcus aureus* and *Klebsiella pneumoniae. Aspergillus niger* derived from ATCC 6275 was used for the antifungal test. The results of the tests provide promising prospect for advancing into the development of ecologically friendly products.

Keywords:- *Water hyacinth, Biodegradability, Antibacterial, Antifungal.*

I. INTRODUCTION

Water hyacinth also known as *Eichhornia crassipes* is a highly invasive aquatic plant that propagates mostly in tropical and sub-tropical regions. Water hyacinth grows into dense mats causing severe obstructions in rivers, lakes and dams which result in floods, blockages to marine transportations and turbines. Water hyacinth propagation also increases eutrophication and cause algal blooms. The weed serves as a habitat for many disease vectors such as malaria. In several developing countries, the spread of water hyacinth is triggering socio-economic problems to communities that depend on freshwater bodies. However, literature and studies also reveals this weed to be a productive plant (Kayathri et al., 2015). Recently, considerable attention has been given to harvest the plant and put to use to partially defray the cost of removing it (Lata and Dubey, 2010). This study focuses on the biodegradability, antibacterial properties and antifungal activity of water hyacinth to investigate its potential for the development of sustainable products. Antimicrobials are substances that neutralize or inhibit the growth of microorganisms such as bacteria, fungi or protozoans through micro biocidal or micro biostatic action. Many plant extracts have a very high antimicrobial potential. The phytochemicals present in the plants have a lot of therapeutic properties that may lead to the development of antimicrobial drugs, cosmetics products including soaps (Cowan, 1999). Several medicinal plants have been studied in the field of pharmaceutical and scientific community (Baral and Vaidya, 2011). Baral and Vaidya, 2011 also state that many researchers are exploring the potential of plants for their antimicrobial usefulness. Since centuries, herbs have been used as the main source of medicinal drugs (Sa'ad et al., 2005). According Shanab et al., 2010, water hyacinth has a high chemical potential for medicinal functions. The leaves of the weed contain phenols, alkaloids, tannins, flavonoids which contribute as antibacterial agents and to antiviral, antifungal, antitumor activities. According to Haggag *et al.* (2017), the leaf extracts and compounds of water hyacinth show anti-microbial and antifungal activities. Phenolics and antioxidants are secondary metabolites that are considered as chemical defence of plants against plant pathogens (Haggag *et al.*, 2017). The weed contains a substantial amount of oxidative enzymes and non-enzymatic antioxidant systems (Thamaraiselvi et al., 2012). Researchers need to show more interest in common weeds which are rich in phytochemicals (Jayanthi and Lalitha, 2013). Antibacterial activity against *Staphylococcus albus* and antifungal activity against *Mucor sp* that has been studied by Jayanthi and Lalitha (2013), demonstrates that water hyacinth can be a potential source for developing antimicrobial agent that can be used in cosmetic products.

II. MATERIALS AND METHODS

A. Sampling:

Water hyacinth leaves, stalks and roots used for testing were harvested from Haut Rive Lake, at Riviere Du Rempart, Mauritius. They were separated and washed thoroughly under running water. They were then dried under the sun. The dried leaves, stalk and leaves were used for the study. These were finely ground and tested for the biodegradability test and antibacterial test. A circular fibre swatch of 38 mm was made with the leaves, stalk and roots, and used for the antifungal test.

B. Biodegradability Test:

Enzymatic method ASTM D 5988-03 standard was used to test the biodegradability of the ground samples (leaves, stalk, roots). 1 gram of the sample was tested using cellulase enzyme *Trichoderma viride for* a period of 30 days. The percentage of weight loss was calculated as follows:

Percentage Weight Loss (%): 100 x ($W_1 – W_2$) / W_1

Where,
W_1: Weight of original sample
W_2: Weight of sample after 30 days

C. Antibacterial Test:

Water hyacinth's leaves, stalks and roots were analysed for their antibacterial activities using ASTM E 2149-13a Method. The samples were ground and 1 gram of each sample was placed into a 100ml of inoculating petri dish. *Staphylococcus aureus* ATCC 6538 (Gram Positive Bacteria) 2.00 x 10^5 and *Klebsiella pneumoniae* ATCC 4352 (Gram Negative Bacteria) 2.00 x 10^5 were used for inoculation. To assess the number of viable bacteria for each petri dish, a colony forming unit (cfu) was used. Sterilization was conducted in an autoclave.

Percentage Reduction of Microorganisms (M): 100 x (Y – X) / Y
Where,
M: Reduction of Microorganisms (%)
X: Number of colonies at 0 hr
Y: Number of colonies at 24 hrs

D. Antifungal Test:

The antifungal test was carried out using the AATCC 30:III-2013 standard method. Each sample was combined to make a circular fibre swatch of 38 mm. The samples were placed in a humidity chamber (>90% RH) at 28°C and incubated for 6 days. *Aspergillus niger* (ATCC 6275) was used as the test inoculation. The number of colonies were counted (X) at '0' hours and (Y) after a period of 24 hours.

III. RESULTS AND DISCUSSION

A. Biodegradability Test:

The biodegradability test was conducted by ASTM D 5988-03 standard test using cellulase enzyme *Trichoderma viride*. The percentage weigh loss of each sample was recorded. The results in Table 1 show the susceptibility of water hyacinth biomass to degradation. It was observed that, for every 1 gram of leaf, stalk and root samples, it takes 30 days to degrade with a weight loss of 99.6%, 99.7% and 99.5% respectively.

Table 1: Biodegradability test

Sample	Weight of sample (W_1)	Weight of sample after 30 day (W_2)	Percentage of weight loss (%)
Water hyacinth leaves	1 gm	0.005 gm	99.6
Water hyacinth stalks	1 gm	0.003 gm	99.7
Water hyacinth roots	1 gm	0.004 gm	99.5

B. Antibacterial Test:

Table 2 shows the results of the antibacterial activity of leaves, stalks and roots with ASTM E 2149 standard test. It records the number of colonies that are seen at '0' hours (X) and the number after '24' hours (Y). The percentage reduction of microorganisms (M) was calculated. The antibacterial activity of the leaves with test cultures *S. aureus* and *K. pneumoniae* was 99.23% and 99.03% respectively. The stalks show an antibacterial activity of 99.87% with *S. aureus* and 99.70% with *K. pneumoniae.* The roots showed 99.88% antibacterial activity with *S. aureus* and 99.85% *K. pneumonia.* From the results it can be inferred that the plant displays good antibacterial properties against S. *aureus* and *K. pneumonia.*

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Table 2: Antibacterial test

Sample	Test Culture	No. of colonies '0' hour (X)	No. of colonies'24' hours (Y)	Reduction of Microorganisms (M) %
 **Leaves**	*S.aureus*	2.08 X 10^5	1.6 X 10^3	99.23%
	K. pneumoniae	2.17 X 10^5	2.1 X 10^3	99.03%
 **Stalks**	*S.aureus*	2.09 X 10^5	3.7 X 10^2	99.87%
	K. pneumoniae	2.11 X 10^5	6.3 X 10^2	99.70%
 **Roots**	*S.aureus*	2.05 X 10^5	2.3 X 10^2	99.88%
	K. pneumoniae	2.06 X 10^5	2.9 X 10^2	99.85%

C. Antifungal Test:

Table 3: Material resistance rating

Growth on specimen	Rating
No growth	0
Trace of Growth (<10%)	1
Light Growth (10 to 30%)	2
Medium Growth (30 to 60%)	3
Heavy Growth (60% to complete coverage)	4

The percentage of surface growth of *Aspergillus niger* was rated on a scale of '0' to '4', as seen in Table 3 for the testing the material resistance rating. 'No growth' is indicated by '0' and a heavy growth of 60% to complete coverage is given a rating of '4'.

Table 4: Antifungal test

Sample	Zone of Inhibition	Rating	Interpretation
Leaves	4.2 mm zone of inhibition	0	A zone of inhibition was identified around the fibre. There was no trace of fungal growth on the sample.
Stalks	4.4 mm zone of inhibition	0	A zone of inhibition was identified around the fibre. No trace of fungal growth was spotted on the sample.
Roots	4.2 mm zone of inhibition	0	A zone of inhibition was identified around the fibre. No growth of fungal was detected.

Large zones of inhibition indicate that the organism is susceptible, small or no zones of inhibition indicate resistance. Table 4 interprets results of the antifungal tests for leaves, stalks and roots. No trace of fungal growth was seen on the samples. All the samples showed antifungal activity. The plant demonstrates a potential for the development of therapeutic or cosmetic products.

IV. CONCLUSION & RECOMMENDATION

The research investigates the biodegradability, antifungal and antibacterial activity of the leaves, stalks and roots of water hyacinth, as a potential raw material for product development. The biodegradability ASTM D 5988-03 test with cellulase enzyme *Trichoderma viride* was carried out for 30 days. The weight loss per gram in the leaves, stalks and roots was 99.5%, 99.7% and 99.6% respectively. The antibacterial activity of water hyacinth leaves tested with the cultures of *S. aureus* and *K. pneumoniae* showed a result of 99.23% and 99.03% respectively. The stalks showed an antibacterial activity of 99.87% with *S. aureus* and 99.70% with *K. pneumoniae*. The antibacterial activity on water hyacinth's root showed 99.88% antibacterial activity with *S. aureus* and 99.85% *K. pneumonia.* There was no fungal growth on the leaves, stalks and roots. A zone of inhibition could be seen when tested for antifungal activity with *Aspergillus niger* derived from ATCC 6275. An inhibition zone of 4.2 mm, 4.4 mm and 4.2 mm were observed in the leaves, stalks and roots respectively. The results show promising prospects for product development. Research is being carried out to develop activated carbon from water hyacinth. Water hyacinth activated carbon can further find a variety of applications, in water purification, food and beverage processing, odour removal, and cosmetics.

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