EXTRACTION YIELD, QUALITATIVE PHYTOCHEMICAL ANALYSIS AND ANTI-MICROBIAL ANALYSIS OF EXTRACTS PREPARED FROM *EICHHORNIA CRASSIPES*

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Abstract: *Eichhornia crassipes*, is a floating plant, an invasive plant in much of the world where it often jams rivers and lakes with tons of floating plant matter. *E. crassipes* form mats that clog waterways making fishing impossible and reduces water flow. Water-hyacinth is a rich source of many phytochemicals. In this study, various extracts of water hyacinth have been made using solvents like distilled water, methanol, ethanol and diethyl ether of both shoot and root of the plant and its extraction yield was calculated. The highest yield was found in an extract prepared from the shoot in distilled water. Phytochemical analysis of the fractionates showed the presence of alkaloids, phenols, tannins, saponins, and quinones. The extracts also showed anti-microbial activity against potential human pathogens like *Escherichia coli, Salmonella, Listeria, Pseudomonas and Staphylococcus.*

Index Terms - Eichhornia crassipes, extraction ability, Phytochemicals, bio-assay.

I Introduction

Eichhornia crassipes has become the world's worst invasive aquatic weed due to its rapid proliferation rate, ecological adaptability and detrimental effects caused on the environment, human health and economic development (Njogu et al,2015). On the other hand, water hyacinth has demonstrated abilities to be used as a raw material in various useful applications (Dwivedi et al, 2018). Water hyacinth is a source of chemicals with medicinal function (Ahmad, 2007; Jayanthi and Lalitha, 2011; Thamaraiselvi et al., 2012; Joshi and Kaur, 2013). The leaf extract of this plant contains flavonoids, alkaloids, tannins, phenols, which have biological activities such as antiviral, antifungal antitumor and antibacterial agents (Ali et al., 2009; Shanab et al., 2010; Jayanthi and Lalitha, 2011; Aravind et al., 2013). Various studies show the potential of the plant in the production of bio-fuel and manures for sustainable agricultural practices. In this study, the plant has been proved to be a brilliant source of various phytocompounds like tannins, saponins, and alkaloids. This study also shows the anti-microbial nature of various extracts prepared from *E. crassipes*.

II Research methodology

Collection of plant sample

Disease-free plant material was collected from Dabhoi (22.1323° N, 73.4121° E), Vadodara, Gujarat, India. The entire plant was washed thoroughly with running water three times and then once with distilled water. The root and the shoot of the plant were separated from one

another and were allowed to shade-dry and finally powdered and stored in an air-tight bag until further use.

Preparation of extracts

Plant materials (root and shoot) extracts were prepared using a Soxhlet extraction unit, a quantity of 50gm plant materials (root and shoot) were weighed and suspended with 500 ml of solvent. The extraction for each plant material is carried out by using methanol, ethanol, diethyl ether, and distilled water. The extracts were dried by using a water bath and desiccator until they were fully dried and stored in a refrigerator at 4ºC for further analysis.

Determination of extraction yield

After the extracts were prepared, the extraction yield was determined using the following formula:

$$\text{Extraction yield (\%)} = \frac{Weight\ to\ the\ dried\ sample}{Weight\ of\ the\ original\ sample} \times 100$$

Phytochemical analysis

Phytochemical screening of the shoot and root extracts of *E. crassipes* was carried out as per the standard procedure (Harborne, 2005).

(1) Test for Alkaloids:

Wagner's test: A fraction of extract was treated with Wagner's reagent (1.27 g of iodine and 2 g of potassium iodide in 100 ml water) and observed for the formation of a reddish-brown color precipitate.

(2) Test for Carbohydrates:

Benedict's test: The extract(100mg) is dissolved in 5ml water and filtered. To 0.5ml filtrate, 0.5ml of Benedict's reagent is added. The mixture is heated in a boiling water bath for 2 minutes. A characteristic colored precipitate indicates the presence of sugar.

(3) Test for glycosides:

Borntrager's test: 50 mg plant extract was added to concentrated HCl and kept on a Boiling water bath for 2 hours and filtered. 2 ml of this filtrate was taken and 3ml chloroform was added and shaken. Separate the chloroform layer and add 10% ammonia solution and observe for the formation of pink color.

(4) Test for saponins:

The extract(50 mg) is diluted with distilled water and made up to 20 ml. The suspension is shaken in a test tube for 15 mins. A 2 cm layer foam indicates the presence of saponins.

(5) Test for proteins and amino acids:

The extract(100 mg) is dissolved in 10 ml of distilled water and filtered through Whatman No. 1 filter paper and the filtrate is subjected to tests for proteins and amino acids.

Ninhydrin test: Two drops of ninhydrin solution(10 mg of ninhydrin in 200 ml of acetone) is added to 2 ml of aqueous filtrate. A characteristic purple color indicates the presence of amino acids.

(6) Test for sterols :

Libermann- Burchard's test: The extract(50 mg) is dissolved in 2 ml acetic anhydride. To this, one or two drops of concentrated sulphuric acid are added slowly along the sides of the test tubes. An array of color changes show the presence of phytosterols.

(7) Test for phenol:

Ferric chloride test (Mace, 1963): The extract (50 mg) is dissolved in 5 ml of distilled water. To this, a few drops of neutral 5 % ferric chloride solution are added. Dark green color indicates the presence of phenolic compounds.

(8) Test for flavonoids:

Alkaline reagent test: An aqueous solution of the extract is treated with 10% ammonium hydroxide solution. Yellow fluorescence indicates the presence of flavonoids.

(9) Test for tannins:

Lead acetate test: The extract (50 mg) is dissolved in distilled water and to this, 3 ml of 10% lead acetate solution is added. A bulky white precipitate indicates the presence of tannins.

(10) Test for Quinones:

A small amount of extract was treated with concentrated HCl and observed for the formation of a yellow color precipitate.

(11) Test for terpenoids (Ayoola et al, 2008):

Salkowski test: To 0.5 g each of the extracts was added 2 ml of chloroform. Concentrated H2S04 (3 ml) was carefully added to form a layer. A reddish-brown coloration of the interface indicates the presence of terpenoids.

(12) Test for Anthraquinone:

Borntrager's test: About 50 mg of powdered extract was heated with 10% ferric chloride solution and 1ml concentrated HCl. The extract was cooled, filtered and the filtrate was shaken with diethyl ether. The ether extract was further extracted with strong ammonia; pink or deep red colorations of aqueous layer indicate the presence of anthraquinone.

(13) Test for anthocyanin:

NaOH test: A small amount of extract was treated with 2M NaOH and observed for the formation of a blue-green color.

Determination of the anti-microbial activity of the fractionates

The potential of the crude extracts and fractionates of fresh Eichhornia crassipes to inhibit the growth of bacteria was determined by antibacterial activity [N. Kannan, Laboratory manual in general microbiology, Palani paramount publications, Palani, Tamilnadu]. The aqueous, methanol, ethanol and diethyl ether extracts were used for the study. The test microorganism used in this study (E.Coli, Staphylococcus aureus, Pseudomonas aeruginosa, Listeria spp and Salmonella typhi) were obtained from Uka Tarsadia University, Bardoli.

The inoculums were prepared in a fresh N-agar medium from the given slant culture and standardized. 1 ml of this culture was added to sterile 5ml hot top agar and poured onto N-agar plates. After the solidification cups were bored onto the plates using sterile cup-borer. The extracts were added into the cups aseptically with the help of a micropipette and allowed to diffuse. The plates were incubated at 37° C for 24 hours. The zones of inhibition were measured.

III Result and discussion:

Extraction yield:

The extraction yield for all the extracts was calculated by using the formula given earlier.

It was observed that the aqueous extract of the shoot of water hyacinth showed maximum extraction yield and the diethyl ether extract of the root of water hyacinth showed minimum yield among all the eight extracts.

table 1. extraction yield of various extracts of water hyacinth

Part of the plant	Extraction yield (%)	
Shoot	Distilled water	18.1
	Ethanol	16.2
	Methanol	9.0
	Diethyl ether	2.0
Root	Distilled water	13.8
	Ethanol	2.2
	Methanol	5.0
	Diethyl ether	1.7

Phytochemical analysis:

Methanolic extract of water hyacinth shoot suggests the presence of compounds like alkaloids, carbohydrates, proteins, amino acids, tannins, and saponins. The most effective solvent found was ethanol.

table 2: phytochemical analysis of shoot extract of *eichhornia crassipes*

Phytocompounds	**Distilled Water**	**Diethyl Ether**	**Ethanol**	**Methanol**
Alkaloids	+	-	+	+
Flavonoids	-	-	-	-
Phenols	+	-	-	-
Carbohydrates	+	+	+	+
Proteins	+	-	+	-
Amino Acids	+	-	+	-
Tannins	+	-	+	+
Terpenoids	+	+	+	+
Sterols	+	-	+	+
Anthraquinones	-	-	+	-
Anthocyanin	-	-	+	+
Quinone	+	-	+	+
Saponins	+	-	+	+

+ indicates presence ; - indicates absence

Methanolic extract of water hyacinth root suggests the presence of compounds like alkaloids, carbohydrates, proteins, amino acids, tannins, saponins, terpenoids, and quinones. The most effective solvents found were distilled water and ethanol.

table 3: phytochemical analysis of root extract of *eichhornia crassipes*

Phytocompounds	**Distilled Water**	**Diethyl Ether**	**Ethanol**	**Methanol**
Alkaloids	+	-	+	+
Flavonoids	-	-	-	-
Phenols	-	-	+	+
Carbohydrates	+	+	+	+
Proteins	+	-	+	-
Amino Acids	+	-	+	-
Phytocompounds	**Distilled Water**	**Diethyl Ether**	**Ethanol**	**Methanol**
Tannins	+	-	+	+
Terpenoids	+	+	+	+
Sterols	-	+	+	-
Anthraquinones	+	-	+	+
Anthocyanin	-	-	-	-
Quinone	+	-	+	+
Saponins	+	-	+	+

Determination of the anti-microbial activity of plant extracts:

table 4: antibacterial activity of *eichhornia crassipes* against pathogenic bacteria

Name of the pathogen	Zone of inhibition (mm)								
	Distilled water extract		Ethanolic extract		Methanolic extract		Diethyl ether extract		Streptomycin
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	
Escherichia coli	2.0	1.5	-	-	-	-	-	-	4.3
Salmonella	-	-	7.0	4.0	2.0	2.0	-	-	5.0

typhi									
Staphylococcus aureus	8.0	6.0	-	1.0	-	1.0	-	-	3.6
Listeria spp	3.0	3.0	1.0	-	-	1	2.0	3.0	3.3
Pseudomonas aeruginosa	1.0	-	-	0.5	-	1.0	-	1.0	-

Note: All experiments were performed in triplicate

- no zone

The results showed the considerable anti-microbial activity of aqueous extracts of root and shoot water hyacinth against pathogens like *E. coli*, *Staphylococcus* and *Listeria spp*. Ethanolic extract of root of the plant showed notable activity against *Salmonella typhi*. Diethyl ether exacts showed minimum activity against these pathogens.

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