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# Identification of Bioactive Compounds and Short Term Cytotoxicity Analysis of the Aquatic Weed, *Eichhornia crassipes*

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**Abstract:** The aquatic weed, *Eichhornia crassipes* commonly called as water hyancinth has numerous bioactive compounds. By gas chromatographic-mass spectroscopic (GC-MS) analysis, the active compounds and the cytotoxicity efficiency (short term) of the ethanolic leaf extracts of *E. crassipes* were analysed. Totally 17 active compounds and their pharmacological values were studied. The bioactive compounds showed anti-cancerous, anti-oxidant, anti-inflammatory, antibacterial, antifungal, anti-analgesic etc. *In vitro* cytotoxicity (short term) against MCF-7 (human breast cancer cell line) were studied by Trypan blue dye method. MCF-7 cancer cell line mortality was observed as 11.90±0.27, 18.18±0.57, 27.36±0.52, 43.33±0.38, and 63.59±0.41% for doses of 100, 250, 500, 750, and 1000 µg/ml, respectively whereas the $IC_{50}$ values were observed at the dose of 853 µg/ml. The results concluded that the ethanolic leaf extract of *E. crassipes* has numerous bioactive compound with wide pharmacognostical values.

**Keywords:** *E. crassipes*, Cytotoxicity, GC-MS, Bioactive compounds, MCF-7

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## Introduction

A wild freshwater fern, the water hyacinth (*Eichhornia crassipes*) is a member of the Pontederiaceae family. Although it originated in South America, this plant has spread to many tropical and subtropical areas worldwide. The water hyacinth, which is also a source of biomass, is thought to be the aquatic plant that grows and spreads the fastest in the world. Despite being the most significant aquatic weed in the world, water hyacinth is also a species with high ornamental value that is employed in gardening due to the beauty of its foliage and flowers (Lancar and

Krake, 2002; Rufchaei *et al.*, 2022).

Water hyacinth has a number of potential secondary metabolites that have not yet been employed medicinally. Plants may contain medicinal chemicals, and several countries use them for medical purposes (Dev, 2010). According to Haroon (2006), the bulk of plant drugs work through secondary metabolites. Natural plant-based products are crucial to the healing process at this time. Natural materials are the source of up to 50% of currently prescribed drugs, including cancer treatments. Plant secondary metabolites are mostly responsible for the drug's bioactivity (Mtewa *et al.*, 2020).

Numerous phytochemicals contained in homeopathic and ayurvedic treatments have also been proved to be potent anticancer drugs. One of the most crucial markers for biological assessment in *in vitro* research is cytotoxicity. Chemicals like medications and pesticides have a variety of lethal effects *in vitro*, including irreversible binding to receptors, degradation of cell membranes, and inhibition of protein synthesis. There is a need for inexpensive, dependable, and repeatable short-term cytotoxicity and cell viability assays to measure the cell death brought on by these effects. Assays for cell viability and cytotoxicity are predicated on different cell functions. The disciplines of toxicology and pharmacology today employ a wide range of cytotoxicity tests. This study was planned to identify the bioactive compounds present in the leaf extracts of the water hyacinth and their short term cytotoxicity analysis by Trypan blue assay on MCF-7 cells.

## Materials and Methods

The experimental plant *Eichhronia crassipes* commonly called as water hyacinth were taxonomically identified and their physico-chemical properties were studied (Priya and Jeyanthi, 2023).

### *Plant extract preparation*

The powdered samples were soaked in ethanol solvent to extract the crude chemical from the plant components of *Eichhornia crassipes*. The resulting extract was collected, concentrated, and stored at room temperature to be used for further purification of the bioactive fractions. By eluting 100 ml of each ethyl acetate:hexane mixture in the ratios of 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9, the concentrated ethanolic leaf extracts of *E. crassipes* were further fractionated by (100-200 mesh) column chromatography (Merck, Germany) for the compound structure identification.

### *GC-MS analysis*

Gas Chromatography (GC-7890A/MS-5975C) manufactured by Agilent Technologies, USA, was used to identify the bioactive chemicals from the bioactive fraction of *E. crassipes* leaves. The column size was 30 m in length, 250 µm in diameter, and 0.25 µm in film thickness. A high energy of 70 eV is employed for electron ionisation in mass spectroscopy detection. It is made of 99.95% pure helium gas, starting temperature range of 50 to 150 °C, and end temperature range of 300 °C, increasing at 3 °C per minute to 10 °C per minute, respectively (Anuradha *et al.*, 2023).

In splitless mode, 1 µl of leaf extract diluted with the appropriate solvent (E) was injected. The percentage peaks in the GC chromatogram represent the relative amounts of the bioactive chemicals found in each crude extract. Retention time was used to identify the bioactive components based on NIST libraries. The components of the GC-MS instrument were identified by comparison with those found in the computer library (Willey and NIST), and the outcomes were tabulated.

### *Cytotoxicity analysis*

Human breast cancer cell line (MCF-7) are squamous in origin and are in charge of material distribution throughout the tissue. They develop naturally as a monolayer *in vitro*. Using Minimum Essential Medium supplemented with 3% L-Glutamine, 10% Fetal Bovine Serum, Pencillin (100 IU/ml), Streptomycin (100 µg/ml), and Amphotericin B, the cells were cultivated in a culture flask with 7.5% sodium bicarbonate at

37°C in a humidified atmosphere with 5% $CO_2$. In order to obtain the necessary number of flasks for additional research, the cells underwent passaging.

The short term *in vitro* cytotoxicity was evaluated by incubating various doses of *E. crassipes* leaf extracts at 37°C for 3 h, utilising the breast cancer cell line (MCF-7). Each six-well plate received 0.5 ml of the cell suspension, which was then cultured for 24 h at a $CO_2$ incubator. Subsequently, 0.5 ml of varying leaf extract concentrations (100, 250, 500, 750, and 1000 µg/ml) were introduced into every well and allowed to incubate for three hours in an incubator. Add 0.5 ml of trypsin into every well and allow to settle for 5 min. Then add 20 µl of the isotonic solution's 0.2% Trypan blue dye (Unnikrishnan and Ramadasan, 1998). Control group without *E. crassipes* leaf extracts was maintained. Viable (unstained) and non-viable (stained) cell counts were performed utilising following formula:

$$Dead\ cells\ (\%) = \left(\frac{Total\ cells\ counted - Total\ viable\ cells}{Total\ cells\ counted}\right) \times 100$$

## Results and Discussion

### *GC-MS analysis*

Seventeen bioactive chemicals were detected by GCMS analysis of crude extracts of *E. crassipes* leaves. The chemical compounds, such as acetic acid, had antimicrobial (bacterial and fungal) properties, propiolic acid had anti-analgesic properties, hexadecanoic acid and methyl ester had hypocholesterolemic, antioxidant, pesticidal, antiandrogenic, nematicidal, hemolytic, and reductase inhibitor properties, The compounds dibutyl phthalate and octadecatrienoic acid had anti-cancer, anti-analgesic, anti-acne, hepato-protective, nematicidal, insect repellent, anti-allergens, anti-arthritic, 5-reductase inhibitor, and antiandrogenic properties; phytol (Mol. Wt. 296) had anti-cancer, anti-analgesic, and diuretic properties.

Mol. wt. 306 included ethyl 9, 12, 15-octadecatrienoate, which has anti-aging properties. The compounds ethanol, 2(9octadecenyloxy), and Z (Mol. wt. 312) had anti-abortive, anti-acne, antiseptic, sexually dysfunctional, antibiotic, anti-cancerous, anti-helminthetic, and antineoplastic properties. The anti-inflammatory and anti-aging effects of octadecanal (Mol. wt. 347) were obseved; Octadecane, 3-ethyl-5-(2-ethylbutyl) (Mol. wt. 366) which has anti-tumor, anti-corrosive, antioxidant, and anti-microbial properties; Hexasiloxane (Mol. wt. 412) has antimicrobial, antitumor, diuretic, anti-analgesic, and antioxidant properties; Cyclohexasiloxane and dodecamethyl (Mol. wt. 444) has defoaming properties, lubricant, and emollient properties; Hexatriacontane (Mol. wt. 506) has anti-tubercular properties; Oleic acid (Mol. wt. 563) has insect-repelling, anti-cancerous, anti-analgesic, and hypocholesterolemic properties; Tetracosamethyl and cyclododecasiloxane (Mol. wt. 889) has hepatoprotective activity, anti-rheumatic, anti-spasmodic, insect-repellent, and appetizer properties; octasiloxane (Mol. wt. 577) had antioxidant, antibacterial, antifungal, and anti-helminthic properties (Table 1).

Das and Sudhakar Swamy (2016) employed GC-MS to determine the bioactive ingredients in fruit methanol extracts and performed a comparative study of three Atalantia species. Twenty-seven compounds were identified using the mass spectra that were gathered. 1, 3, 4, 5-tetrahydroxycyclohexanecarboxylic acid was the primary chemical discovered. The most prevalent of the 27 compounds identified in *Atalantia racemosa* was n-hexadecanoic acid. Uraku (2016) looked into methanol extracts of *Spilanthes uliginosa* leaves. Docosane aldehyde (41.72%), 5-hydroxylmethyl heptadecane (14.02%), hexadecanoic acid, hepta-9, 10, 11-trienoic acid octadecenoic acid, and 1-ethoxyoctadecane (8.08%) are the primary phytocompounds present in the leaf extracts.

### *In vitro anticancerous studies: Trypan blue dye exclusion method*

The short-term *in vitro* anticancer technique

Table 1: *Eichhornia crassipes* leaf extracts of GC-MS spectral analysis and their pharmacological properties

| S. No. | Chemical name | Pharmacological property |
|---|---|---|
| 1 | Acetic acid | Antimicrobial (bacterial and fungal) agent |
| 2 | Propiolic acid | Anti-analgesic agent |
| 3 | Hexadecanoic acid, methyl ester | Hypocholesterolemic, Antioxidant, Pesticidal, Antiandrogenicagent, Nematicidal, Hemolysis, α-reductase inhibitor |
| 4 | Dibutyl phthalate | Anticancerous agent |
| 5 | Octadecatrienoic Acid | Anti-analegsic, Hypocholesterolemic agent, Anticancerous, Antiacne,Hepatoprotective,Nematicidal, Insect repellent, Anti-allergens, Antiarthritic, 5-αreductase inhibitor Antiandrogenic agent |
| 6 | Phytol | Anti-cancerous, anti-analgesic, anti-analgesic, diuretic agent |
| 7 | Ethyl 9,12,15-octadecatrienoate | Anti-ageing |
| 8 | Ethanol, 2(9octadecenyloxy),(Z) | Anti-abortive agent, Anti-acne agents, Antiseptics, Sexual disorders, antibiotics, anti-cancerous, anti-helminthetic agent, antineoplastic agent |
| 9 | Octadecanal | Anti-inflammatory, anti-ageingproperties |
| 10 | Octadecane, 3-ethyl-5-(2-ethylbutyl)- | Anti-tumour, anti-corrosive agent, antioxidant, anti-microbial |
| 11 | Stigmasterol | Antimicrobial, Antitumour, Diuretic, Anti - analgesic, Antioxidant. |
| 12 | Hexasiloxane | Antimicrobial, Antiseptic, Hair and skin conditioning Agent, Emollient, lubricant |
| 13 | Cyclohexasiloxane, dodecamethyl | Defoaming property, lubricant, emollient |
| 14 | Hexatriacontane | Anti-tubercular agent, |
| 15 | Oleic acid | Insect repellent, anti-cancerous, anti-analgesic, hypocholesterolemic agent |
| 16 | Octasiloxane | Antioxidant, antibacterial, antifungal and anti-helminthic agents |
| 17 | Tetracosamethylcyclododecasiloxane | Hepatoprotective activity, anti-rheumatic agent, anti-spasmodic agent, insect-repellent, appetizer agent |

known as Trypan blue dye exclusion aids in determining whether the examined leaf extracts of *E. crassipes* have anticancer properties. Breast cancer cell lines (MCF-7) were treated with varying concentrations (100, 250, 500, 750, and 1000 µg/ml) of *E. crassipes* leaf crude extracts. The percentage of cancer cells that died rose with higher leaf extract concentrations. Table 2 shows the MCF-7 cancer cell line death percentage as 11.90±0.27, 18.18±0.57, 27.36±0.52, 43.33±0.38, and 63.59±0.41% for doses of 100, 250, 500, 750, and 1000 µg/ml, respectively. From the results, Inhibitory concentration (50% of the mortality) i.e., $IC_{50}$ value were measured as 853 µg/ml.

MCF-7 was significantly inhibited by the aqueous extracts of *Cornusmas* leaves, *C. alba,* and *C. officinalis*. Total tannin content is known to be high in these three species. Condensed tannins with a significant cytotoxic activity against MCF-7 cells ($IC_{50}$ value 38.33 ± 2.08 µg/ml) were successfully extracted from *Leucaena leucocephala* leaves (Zarin *et al.*, 2016). When compared to the inhibition of other cancer cell types, such as human liver carcinoma (HepG2), human cervical

Table 2: *In vitro* cytotoxic potential of *E. crassipes* leaf extract against MCF-7 cell lines by Trypan blue dye exclusion method

| Concentration (µg/ml) | Mortality rate (%) |
|---|---|
| 100 | 11.90±0.27 |
| 250 | 18.18±0.57 |
| 500 | 27.36±0.52 |
| 750 | 43.33±0.38 |
| 1000 | 63.59±0.41 |

carcinoma (HeLa), and human colon carcinoma (HT29), the inhibitory value of MCF-7 cancer cells is more significant (Zarin *et al.*, 2016). The entire extract of water hyacinth has been shown to cause cytotoxicity in MCF-7 cells with an $IC_{50}$ value of 1.2±0.2 µg/ml, whereas leaf extract at a concentration of 100 µg/ml caused the death of almost 80% of cells. Maceration with methanol and 80% ethanol, respectively, was the extraction technique employed in the two outcomes. According to these investigations, the cytotoxic activity of the total extract, which ranged from 11.1 to 69.1 ppm, was higher than that of nine pure fractions (fractions A–I) that were isolated (Aboul-Enein *et al.*, 2014; Taqi *et al.*, 2019).

## Conclusion

Leaf extract of *E. crassipes* confirms the presence of active compound by GC-MS analysis. Seventeen active compounds were observed and their pharmacological activities were identified. For *in vitro* anticancerous activities, Trypan blue dye exclusion method was followed whereas the $IC_{50}$ values observed at 853 µg/ml. Our study concluded that the aquatic weed *E. crassipes* has numerous active phytocompounds which showed a significant results.

## Ethical Statement

No animal has been used or sacrificed for this study, hence Ethical Approval not required.

## Author Contributions

Each author has contributed equally to the study's planning, analysis, writing, and editing. All authors have read and agreed to the published version of the manuscript.


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## Conflict of Interest

The authors declare no conflicts of interest.

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