Biomedical applications of *Eichhornia crassipes*

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Abstract
Water hyacinth Eichhornia crassipes is an oceanic macrophyte and one of the worst sea-going weeds in the world and Iraq. The grouping of substantial metals in water hyacinth was higher in roots than in shoots. Nutrient content e.g. ammonia, nitrate, nitrite and phosphate were higher in the shoots of water hyacinth. Water hyacinth is a wellspring of phytochemicals having a medicinal function.

In this study, the anti-bacterial and anti-cancer activities of E. crassipes were detected in leafs extracts. The ethanolic leaves extracts were screened for antimicrobial activities. The leaf extracts of E. crassipes exhibited antimicrobial activity against some bacterial strains such as Escherichia coli and Staphylococcus aureus by using the well diffusion method. The anti-cancer activity of leaf extract was tested against breast cancer cell line (MCF-7). The results indicated that the leaf extracts of E. crassipes have potential activity as bacteriocidial agent and anti-cancer agent and could be used in future for biomedical applications studies.

Keywords: *Eichhornia crassipes*, Anti-bacterial activity, Cytotoxicity, Apoptosis.

Introduction

E. crassipeso is known as water hyacinth, is a warm water aquatic plant and the world worst aquatic weed in the world; the plant was recorded in Egypt, India and Australia. It can grow quickly, thereby completely closing water bodies[1] and negatively impacting fisheries and related commercial activities. Moreover, it affects the effectiveness of irrigation canals, navigation, transport and hydroelectric programs[11] and increases water flow and flood levels. Water hyacinth could be a source of chemicals materials with medicinal applications and benefit[8]. The leaf extract of this plant contains flavonoids, alkaloids, tannins, phenols and others which have biological activities such as antiviral, antifungal, antitumor and antibacterial agents[3]. In addition, water hyacinth has rich oxidative enzymes and non-enzymatic antioxidant systems[12].

Secondary metabolites such as phenolic and anti-oxidant components, have been demonstrated to be involved as very important chemical defenses of plants against invaded microorganisms[6]. An alternative approach for detection and development of new and innovative products is increasing the interest of researchers in antimicrobial and antifungal activities of medicinal plants rather than in common weeds which are also the source of several antimicrobial and antifungal compounds[4]. There have been studies on the use of plant products as disease control agents, with less toxicity and fewer environmental effects. Thus, the aim of this study is to evaluate metal and nutrient accumulation, phytochemical compounds, antifungal and antimicrobial activities of different leaf extracts.

Material and Methods

Collection of plant: Fresh, leaves of *E. crassipes* were collected from Tigris river, Al-Suwairh, Iraq. The leaves were washed thoroughly twice with tap water and once with DW; leaves were then air-dried in room temperature.

Ethanolic extraction: Ethanolic extraction of *E. crassipes* leaves was prepared by dissolving of (50 gm) of leaves in (250 ml) of 80% ethanol. These extracts were concentrated to dryness in a controlled temperature (25-37ºC) and they were stored in a refrigerator.

Antibacterial activities of *E. crassipes*: The antibacterial activities of ethanolic extracts were tested against the selected bacterial strains using concentration of 500 mg m^{-1}. The sterilized muller hinton agar medium was poured into each sterile Petri plates and allowed to solidify. By using sterile cotton swabs, fresh bacterial strains were spread over the plates by following the spread plate technique. 6 mm wells were made into the agar plates with the help of a sterile cork borer. The wells were loaded with 100µL of ethanolic extract of *E. crassipes* in to separated wells. The plates were incubated for 24 hours at 37ºC. Each experiment was repeated 2 times and the mean of the diameter of the inhibition zones was measured around the walls.

Maintenance of cell line: MCF-7 cell line was obtained from the Iraq Biotech Cell Bank Unit and maintained in RPMI-1640 supplemented with 10% Fetal bovine, 100 units/mL penicillin and 100 µg/mL streptomycin. Cells were passaged using Trypsin-EDTA and re-seeded at 50% confluence twice a week and incubated at 37°C.

MTT assay: This assay was done according to Majid et al[10]. Briefly, MCF-7 cell line was seeded at 1×10^5 cells/mL in 96 well microtiter plates in RPMI medium. The cells were incubated overnight for attachment. cells treated with leaf extract of *E. crassipes* at different concentrations (6.25, 12.5,

25, 50, 100 µg/ml) in triplicate and incubated for 72 hrs. Thereafter, the cells were stained with MTT at concentration 2µg/ml. The samples were incubated at 37ºC, after 3 hr DMSO (Dimethyl Sulfoxide) was added to each well; the absorbance was measured at 492 nm using microplate reader. The inhibition rate of cell growth (the percentage of cytotoxicity) was calculated as the following equation:

Inhibition rate = A-B/A *100

where A and B are the optical density of control and the optical density of test.

To visualize the shape of cells under inverted microscope, 200 µL of cell suspensions were seed in 96-well micro-titration plates at density $1x10^4$ cells mL^{-1} and incubated for 48 hrs at 37ºC. Then the medium was removed and add leaf extract of *E. crassipes* at 26.7µg/ml (IC50). After exposure time, the plates were stained with 50 µL with Crystal violet and incubated at 37ºC for 15 min; the stain was washed gently with tap water until the dye was removed. The cell observed under inverted microscope at 100x magnification microscope was filed and photographed with digital camera. (MAJID NEW).

Acridine orange –Ethidium bromide (AO/EtBr) dual staining: The induction of apoptosis in MCF-7 cell line was performed using (AO/EtBr) dual staining method. Briefly, the cells in 96- well plates were treated with *E crassipes* leaves extract and incubated for 24 hrs. The cells were washed twice using PBS. Dual fluorescent dyes (10 µL) were added into the cells at equal volumes. Finally, the cells were visualized under fluorescence microscopy [9].

Statistical analysis: The obtained data were statically performed using unpaired t.test with GraphPad Prism 6. Values were presented as the mean ± S.D of the three replicate of each experiments [10].

Results and Discussion

Antibacterial activity of *E. crassipes*: In the present study, two standard strains; *S.aureus*, Escherichia coli were used. Antimicrobial activity of *E crassipes* had been studied. The results of antibacterial activity of alcoholic leaves extract against *S.aureus* showed that extract exhibited good antibacterial activity better than activity against *E.coli*. Figure 1 showed the antibacterial activity of leaves extract against *S.aureus* and *E.coli* .The results could be attributed to the presence of the outer membrane in gram-negative microorganisms.

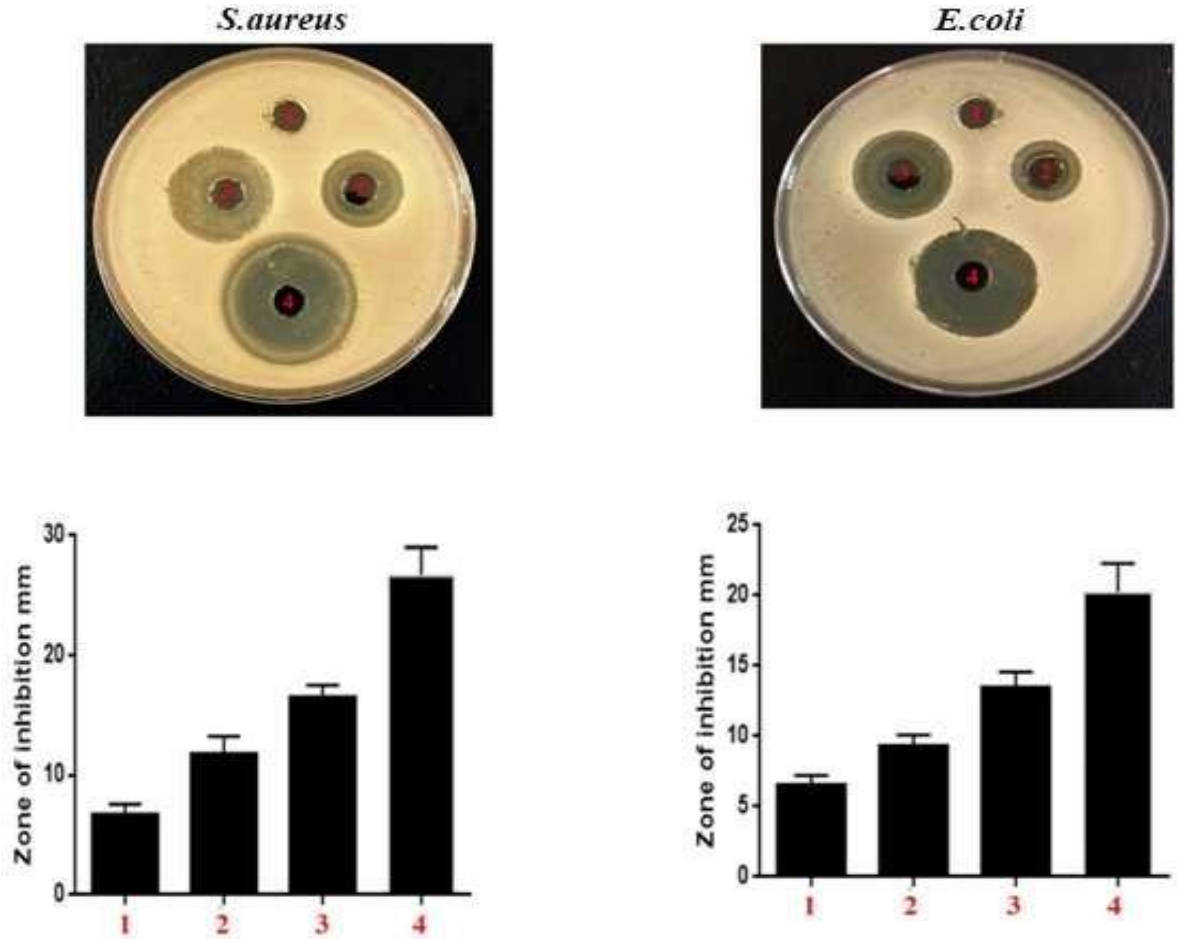

Fig. 1: Antibacterial activity of E. crassipes against bacterial strain as indicated. 1 negative control (DW), 2 concentration 250 µg/ml, 3 concentration 500µg/ml, 4 concentration 1000µg/ml.

Anti- cancer activity of *E crassipes* leaves extract: The cytotoxic effects of leaves extract on the viability of breast cancer cell lines MCF-7 after 72 hrs of exposure were examined as shown in figure 2. The results illustrated that treatment with leaves extract inhibited the growth of cells significantly. Leaves extract at concentration 100µg/ml exhibited more than 80% of cells were dead. The results indicate that the leaves extract are considered to be particularly valuable source of effective antiproliferative and cytotoxic agents. The apoptogenic property was investigated through morphological changes in MCF-7cell line using inverted phase contrast microscope.

As seen in figure 3, the control (untreated) cells showed that the cells maintained their original morphology form, that most of the control cells were adherent to the tissue culture flask. In contrast, MCF-7 cells treated with *E. crassipes* leaves extract at concentration 26.22 µg/mL exhibited high efficiency on the proliferation and morphology.

Acridine orange –Ethidium bromide (AO/EtBr) dual staining: This analysis was employed to examine the changes in nuclear morphology of MCF-7 treated cells. The apoptotic cells were evaluated based on DNA damage. In this study, the efficiency of Eichhornia crassipes at concentrations 26.22 µg/mL was studied. The using of AO-EB dual staining was specific to different parts of the cell and determined distinct apoptotic signs characteristics to nucleate alternations. Viable and nonapoptotic cells were shown green and apoptotic cells were shown orange or red as they stained with AO – EtBr as shown in figure 4.

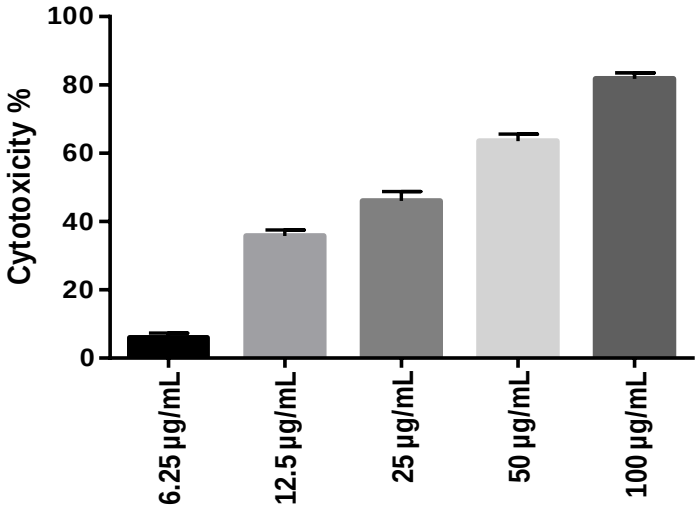

Fig. 2: Anti-cancer activity of *E. crassipes* against breast cancer cell line (MCF-7)

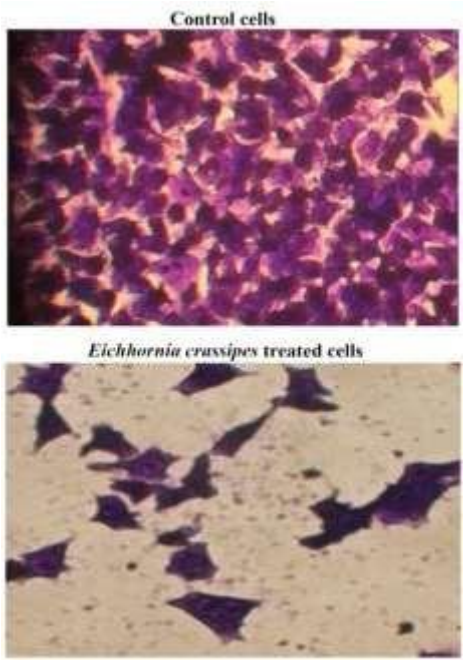

Fig. 3: Anti-proliferative activity of *E. crassipes* against MCF-7 cells

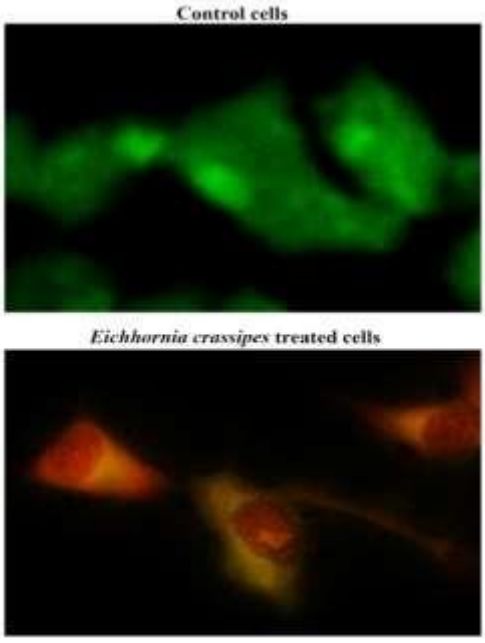

Fig. 4: *E. crassipes* induces apoptosis in MCF-7

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