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ISOLATION OF GLYCOSIDES AND TANNINS ACTIVE COMPOUND FROM *EICHHORNIA CRASSIPES* (WATER HYACINTH) AND THE STUDY OF THE ACTIVITY OF THEIR EXTRACTS AND AQUATIC AND ETHANOL EXTRACT AS ANTIMICROBIAL

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ABSTRACT

The current study aimed at isolating and extracting the glycosides and tannins compounds from the Water hyacinth plant *Eichhornia crassipes* (all of plant flowering and non-flowering plant above the soil), preparation of aquatic extracts for plant parts (rhizome, leaves and flowers) flowering plant and (all plant above the soil) non-flowering plant and ethanolic extracts (all plant above the soil) flowering and non-flowering plant and testing the biological activity of their concentrations (25, 50, 100) mg/ml against bacteria (*Staphylococcus aureus*, *Staphylococcus epidermidis* gram positive) and (*Klebsiella pneumonia*, *Shigella* sp gram negative). The plant contains active compounds tannins, glycosides, phenols, saponins and alkaloids, and it our result that tannin extract for the flowering plant with a concentration of 100 mg/ml is most effective in inhibiting the bacterial species studied and the highest inhibition diameter was against *klebsiella pneumonia*, *Shigella* sp gram negative average inhibition (AIZ) 13.3 and 13.3 mm respectively.

KEY WORDS : Isolating, *Eichhornia crassipes,* Active compound .

INTRODUCTION

The Water hyacinth plant *Eichhornia crassipes* is one of the most dangerous invasive aquatic plants found in the form of stable colonies in shallow or mobile waters in deep fresh and non-fresh water (Villamagna, 2009). Its original habitat is the Amazon River basin in Brazil, a perennial plant, currently spread in more than 50 countries between latitudes 39°N and 39 °C (Center *et al*., 2005) growing in the form of a dense green carpet above the surface of the water, a living mass. Large size in a short period as a result of its rapid multiplication, the risk of this plant comes from this characteristic and has a measure of regrowth by various means in the event of exposure to abnormal conditions such as control (Tellez, 2008), rapid response and adaptation to environmental changes and affects Strongly in the ecosystem, the Water hyacinth plant appeared in Iraq in the mid-1980s as an ornamental plant (center and Dray, 2010). Its natural spread occurs by seeds and plant residues, which is a new source of growth and redeployment, and in a short period it became a problem threatening the aquatic environment, which prompted specialists in the field of agriculture to try to stop the spread of this plant (as a jungle) (Wilson *et al.,* 2005), due to the damage caused by the plant by obstructing the move of irrigation water and drainage in the water channels, hindering navigation processes and increasing the loss of water during the process of erosion significantly and providing a suitable climate for the growth and reproduction of harmful insects and threatening fish wealth by reducing the amount of oxygen in the water and blocking light from phytoplankton (Ahmed, 2003). In the study of

Al-Wakaa and Sultan (2012) on the growth and increase of the mass of the Water hyacinth plant in the environment of Iraq, the results showed that the Water hyacinth plant grows rapidly and is a large biomass and occupied the single plant sample that was cultivated an area of 7.5 m^2 within 193 days, and gave 2320 plants and reached a biomass of 48.592 kg/m^2 wet weight and 3.562 kg/m^2 dry weight.

As a result of its rapid spread and significant damage and the lack of an effective, economic and optional method so far to eliminate the Water hyacinth plant, some researchers have been interested in making use of the plant in a number of areas, including the use of animal feed or soil improvement as organic fertilizer or its use in the medical field as a microbial antimicrobial (Ahmed, 2003; Haggag *et al.*, 2014). Due to the plant content of the effective chemical compounds contained in it alkaloid, phthalate derivatives, propanoid and phenyl derivatives) Aboul-Enein *et al.*, 2011), Alkaloids, soaponins and phenols (Baral and Vaidya, 2011), flavonoids, polyphenols, sterols, leucoanthocyanins, terpenoids, tannins (Mahunon *et al.*, 2017); Tridecanoic Acid, 3, 7, 11, 15-Tetramethyl-2-Hexadecen-1-Ol, Cis-10-Nonadecenoic Acid, Hexadecanoic Acid, Methyl Ester, Phytol Acetate, 9-Octadecenoic Acid, 9, 12, 15- Octadecatrienoic Acid (Prabakaran and Mani, 2017). The plant is used as a urine trainer and in increasing sexual arousal and sedative (Greca *et al.*, 1991). It acts as an antioxidant and anti-cancer (Aboul-Enein, 2011).

Used as an active antimicrobial against fungal and bacterial pathogenic species (Aboul-Enein, (2011) and Luis *et al*., 2018). Through a review of available scientific resources, studies that have been interested in studying the anti-microbiology efficacy are very few compared to the wide-spread of the plant and its effective chemical content. The current study was therefore aimed at:

1. Preparation of aquatic and alcoholic extracts for the parts of the flowering Water hyacinth plant (rhizome, leaves, flowers) and the entire non-flowering plant and their use as antibacterial.
2. Separation of glycosides and tannin compounds from the flowering and non-flowering Water hyacinth plant (all plant) and the use of their extracts antibacterial.

MATERIALS AND METHODS

Collection of the plant

Water hyacinth *E. crassipes* vegetable samples were collected in the Tigris River in Salah Al-Din Governorate / Al-Sharqat district. These samples included the entire plant (flowering and non-flowering) as well as each part of the flowering plant separately (rhizome stems, leaves and flowers) and then washed the samples with distilled water. It was dried at room temperature away from light (to prevent photo oxidation).

Glycosidic Extraction

Add 10 g of vegetable powder to 100 ml of ethyl alcohol at 80% concentration and leave the mixture for 24 hours in the refrigerator at a temperature (4-7 m) and filter for the ethanol extract concentrate the extract to a third of its size by the rotary evaporator, and add 50 ml of ether and 5 ml of lead acetate solution 0.3 molari in the suppression of separation with the shake and pulled the water layer. The process was repeated three times and the water layer withdrawn was dried at a temperature of 30°C until the dry ness and then the extracts were placed in bottles with a tight lid and kept frozen until used (Ukida *et al.*, 2006).

Tannic extract

Prepare the water extract by boiling 0.5 g of plant sample powder in 50 ml of distilled water for 30 minutes and filtered the extract and displayed to the centrifuge (2000 cycles/minute) for 20 minutes. Transfer the floating part to a 100 ml volume and add 20 ml of lead acetate solution at a concentrate of 4% and complete the volume with distilled water to (100 ml). Leach and dry in an oven at 60°C until dry and then placed in bottles with a tight lid and kept frozen until used (Ahmad and Nazil, 1989)

Ethanolic Extracts

Grand *et al.* (1988) followed the preparation of ethanol extract This is by dissolving 20 grams of plant powder in 200 ml of ethanol alcohol at a concentrate (80%) with stirring (i.e. at a ratio of 1:10 weight: volume) and then leaving the mixture in the refrigerator for (24) hours for soaking, then filtered Extracted through several layers of gauze and then filtered again using filter papers (Whatman. No. 1) to get rid of the crushed vegetable parts and the remaining fibers, then put in a rotary evaporator at a temperature not exceeding (40 °C) and after the vaporization of the alcohol in the mixture, A dense layer was obtained from the extract, which was dried on air and at laboratory temperature (30-35 °C). The extracts were then placed after being dried

in vials with a tight lid and kept frozen until use.

Aqueous Extraction

The process of extracting with water was done according to what was stated in the Al-Joboory and Al-Rawi (1994) study 40 g of plant powder was mixed with 160 ml of distilled water (i.e. a ratio of 1: 4 weight: volume), add the vegetable powder and stir the mixture, then put it in the refrigerator 24 hours, and subsequently filtered through several layers of medical gauze, and again filtered by Buechner funnel using Whatmann No. 1filter papers. to get rid of the crushed parts and fibers to get the liquid raw vegetable extract, and then put it in a rotary evaporator device with a temperature not exceeding 40 °C. As it works on the basis of evaporation under sieve pressure a dense layer of the extract was obtained which was dried at the laboratory temperature (30- 35 °C). The extracts were then placed after being dried in vials with a tight lid and kept freezing until use.

Chemical disclosures of active compounds

Active compounds in plant species were detected for Glycosides and Tannins based on (Shihata, 1951), Alkaloids (Fa'hmy, 1933) and Saponins, Phenols (Harborne, 1973)

Laboratory micro-organism isolations

In this study, four clinically isolated bacterial species (positive and negative gram) were used: (*Klebsiella pneumonia, Shigella* gram negative), (*Staphylococcus epidermidis, Staphylococcus aureus* gram positive) diagnosed in Wasit University Laboratories/Faculty of Pure Sciences based on Bergey's Manual (2005).

The agar diffusion method was used by drilling (well) according to Egorove (1985) to observe the sensitivity of microbiology to plant extracts studied at concentrations (25, 50 and 100 mg/ml).

RESULTS AND DISCUSSION

Chemical detection of some active groups in whole plant extracts and various parts

The results of the chemical detection of some active substances showed that all studied plant samples (the entire plant and its studied parts) contain tannins, glycosides,phenols, saponins and alkaloids.

Antibacterial active

The results of the current study showed the activity of the extracts of Glycosides, tannin and ethanol and its concentrations (25, 50, 100) in inhibiting the microbiology studied, but there is a marked disparity in their effectiveness.

Table 1 in glycosides and ethanol indicates the treat of tetracycline positive control is the most active in inhibiting *Klebsiella pneumonia* bacteria, with average inhibition zoon (AIZ) 9.33 and 9.33 mm respectively compared to flowering and non-flowering plant concentrations), while in the concentration of tannin extract 100 mg/ml of the flowering plant is more effective against bacteria than (AIZ) 13.3 mm.

Table 2 in glycosides and ethanol indicates the treatment of tetracycline positive control is the most effective in inhibiting *Shigella* bacteria as it (AIZ) reached 7 and 7 mm respectively, furthermore in the concentration tannin extract 100 mg/ml of the flowering plant is the most effective against bacteria compared to control as (AIZ) 13.33 mm .

Table 1. The effects of glycosides, Tannins compounds extracted and ethanol extracted from *Eichhornia crassipes* on *Klebsiella pneumonia*.

Plant	Concentration mg/ml	Extract Glycosides	Tannins	Ethanol
Flower	25	6 b	9.33 b	6 b
	50	6 b	9.66 b	6 b
	100	6 b	13.33 a	6.66 b
Non- Flower	25	6 b	7.33 c	6 b
	50	6 b	6 c	6 b
	100	6 b	10 b	6 b
Tetra		9.33 a	9.33 b	9.33 a
DW		0 c	0 d	0 c

* The numbers represent an average of three iterations

- Similar capital letters in one column mean that there are no significant differences between them at the probability level (0.05).

Table 3 in glycosides and ethanol indicates the treatment of tetracycline positive control is the most effective in inhibiting *Shigella* bacteria as it (AIZ) reached 7 and 7 mm respectively, furthermore in the concentration tannin extract 100 mg/ml of the flowering plant is the most effective against bacteria compared to control as (AIZ) 10 mm .

Table 4 in glycosides and ethanol extracts shows all the concentrations of the flowering and non-flowering plant and the positive control tetracycline there are no moral differences in their activity in inhibiting *S. epidemidis*, furthermore in the concentration tannin extract 100 mg/ml of the flowering plant is the most effective against bacteria compared to control as (AIZ) 11 mm.

Table 5 shows the inactivity of aquatic extracts for different plant parts (rhizome stems, leaves and flowers) and the entire flowering plant and the entire non-flowering plant against *shigella, S. aureus* and *S. epidermidis* compared to the positive control tetracycline, furthermore in *Klebsiella pneumoniae* has shown concentrate of hydroponic extracts of the leg and flowers, the flowering plant and the entire non-flowering plant are active in inhibiting the bacteria tested, that treatof positive control is the most active in inhibition as it is(AIZ) 9.33 mm .

The activity of anti-microbialglycosides is due to the non-sugary part of their composition (Peach & Tracy, 1998) and the non-sugary portion varies from one glycosides to another, which may gain high activity and wider spectrum in effect. The activity of antibacterial tannins is due to their ability to inhibit Adhesins, enzymes and some vector proteins within the cellular membrane (Engels *et al.*, 2011). Tannins are a group of polycyclic phenolic compounds found in almost all parts of the plant with a high susceptibility to inhibition of pyroxides in primitive nucleus organisms (Okuda, 2005). Hussein *et al.* (2010) attributed the antibacterial activity to the fact that tannins can interfere with the function of the

Table 2. The effects of crude Tannins compounds extracted from *Eichhornia crassipes* on *Shigella.*

Extract	Concentration mg/ml	Plant		
		Glycosides	Tannins	Ethanol
Flower	25	6 b	7.66 bc	6 b
	50	6 b	9.33 b	6 b
	100	6 b	13.33 a	6 b
Non- Flower	25	6 b	9.66 b	6 b
	50	6 b	8.33 bc	6 b
	100	6 b	13.33 a	6 b
Tetra		7 a	7 bc	7 a
DW		0 c	0 d	0 c

*The numbers represent an average of three iterations
- Similar capital letters in one column mean that there are no significant differences between them at the probability level (0.05).

Table 3. The effects of glycosides, Tannins compounds extracted and ethanol extracted from *Eichhornia crassipes* on *S. aureus*

Extract	Concentration mg/ml	Plant		
		Glycosides	Tannins	Ethanol
Flower	25	6 b	6.33 c	6 b
	50	6 b	7 c	6 b
	100	6 b	10 a	6 b
Non- Flower	25	6 b	8 b	6 b
	50	6 b	6.66 c	6 b
	100	6 b	8.66 b	6 b
Tetra		7 a	7 c	7 a
DW		0 c	0 d	0 c

* The numbers represent an average of three iterations
- Similar capital letters in one column mean that there are no significant differences between them at the probability level (0.05).

Table 4. The effects of glycosides, Tannins compounds extracted and ethanol extracted from *Eichhornia crassipes* on *S. epidemidis*

Extract	Concentration mg/ml	Plant		
		Glycosides	Tannins	Ethanol
Flower	25	6 a	7 bc	6 a
	50	6 a	8 bc	6 a
	100	6 a	11 a	6 a
Non- Flower	25	6 a	6.66 bc	6 a
	50	6 a	7.66 bc	6 a
	100	6 a	9 bc	6 a
Tetra		6 a	6 bc	6 a
DW		0	0 d	0

* The numbers represent an average of three iterations
- Similar capital letters in one column mean that there are no significant differences between them at the probability level (0.05).

Table 5. The effect of types of plant extracts on laboratory bacteria

Extract plant		Bacteria			
		S.aureus	*S.eidermidis*	*K.pneumonia*	*Shigella*
Glycosides	Flower	6 b	6 b	6 b	6 b
	Non- Flower	6 b	6 b	6 b	6 b
Tannins	Flower	7.77 a	8.66 a	10.88 a	10 a
	Non- Flower	7.77 a	7.77 a	7.77 a	10.44 a
Ethanol	Flower	6 b	6 b	6 b	6 b
	Non- Flower	6 b	6 b	6 b	6 b
Aqueous	Flower	0 c	0 c	3 c	0 c
	Non- Flower	0 c	0 c	3 c	0 c
Average	4.94 A	5.05 A	6 A	5.55 A	

- Similar small letters in a row mean that there are no significant differences between them at the probability level (0.05).
- Similar capital letters in one column mean that there are no significant differences between them at the probability level (0.05).

Table 6. The effects of aqueous extracts from *Eichhornia crassipes* on tested bacteria.

Plant part	Concentration mg/ml	Bacteria			
		S.aureus	*S. epidermidis*	*Klebsiella pneumonia*	*Shigella*
Rhizome	25	0 b	0 b	6 b	0 b
	50	0 b	0 b	6 b	0 b
	100	0 b	0 b	6 b	0 b
Leaves	25	0 b	0 b	0 d	0 b
	50	0 b	0 b	0 d	0 b
	100	0 b	0 b	0 d	0 b
Flowers	25	0 b	0 b	6 b	0 b
	50	0 b	0 b	6 b	0 b
	100	0 b	0 b	6 b	0 b
Whole plant (Flower)	25	0 b	0 b	3 c	0 b
	50	0 b	0 b	3 c	0 b
	100	0 b	0 b	3 c	0 b
Whole plant	25	0 b	0 b	3 c	0 b
(Non-Flower)	50	0 b	0 b	3 c	0 b
	100	0 b	0 b	3 c	0 b
Tetracycline		7 a	6 a	9.33 a	7 a
DW		0 b	0 b	0 d	0 b

* The numbers represent an average of three iterations
- Similar capital letters in one column mean that there are no significant differences between them at the probability level (0.05).

cellular membrane and can affect the active of certain enzymes when they are high in concentrations.These results are consistent with those of the Al-smail (2017) and Al-smail (2020) study which showed the activity of glycosides and tannin extracts for species of plants of the *Erodium* and *Geranium* genus in inhibiting microbiology and Al-smail (2020) study which *Cuscutalehmanniana* showed the activity of glycosides and tannin extractsin inhibiting microbiology. Ethanol extracts are morally different from treatof control, indicating that the microbial efficacy of these extracts is due to their active content (Buazize *et al.,* 2009). The different effect of a single plant extract on the activity of microbiology varies depending on the method of extraction (Nostro *et al.,* 2000).

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