

Detection of active compounds in Aloe vera leaf extract and their inhibitory effect on *Pseudomonas aeruginosa*

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Abstract

In the study, two types of Aloe vera leaf extracts were prepared: aqueous and alcoholic extract. The research was conducted at the Department of Life Sciences, Faculty of Science, Tikrit University. From October to January. The active compounds in these alcoholic extracts were detected as tannins, cladosides, saponins, and flavonoids, all of which gave a positive result. The antibacterial activity of the aqueous and alcoholic extracts were estimated at concentrations ranging from (200-12.5) mg/ml, where the alcoholic extract gave a high efficacy of (34.5) mm, while the aqueous extract had a lower efficacy (29.6) mm at the same concentration. Microbial tests were evaluated using different combinations of the extracts on *Pseudomonas aeruginosa* isolates, where the alcoholic extract gave the highest inhibitory efficacy to the bacteria.

Keywords: *Pseudomonas aeruginosa*, tannin, aloe vera, extracts.

Introduction

Aloe vera is a perennial herbaceous plant from the lily family belonging to the cactus family. There are 400 species of cacti, including the Aloe vera cactus, which is characterized by its thick green leaves, and inside it is a sticky white sap, adapted to desert conditions and environments, which is why the proverbial cactus plant tolerates thirst and drought. The plant is called a natural healer because it contains antioxidant and antimicrobial compounds, it is used to inhibit microbes and is a very effective antioxidant, as it helps relieve irritable bowel issues, purifies the brain, and is useful in treating chest pain, stomach diseases and stomach ulcers, liver diseases, and poor digestion, as it is useful in treating rheumatism and joints and acts as a laxative for the large intestine. It treats tumors and pimples, and fresh aqueous aloe vera juice is used to treat inflamed gums, mouth sores, and tumors, and is considered a complementary treatment in lowering blood sugar [1]. Aloe vera leaves consist of three layers: The outer protective layer is composed of carbohydrates and proteins [2], and the outer part is very bitter, its cells are cylindrical and circular and contain compounds that have analgesic and antibacterial activity as antiseptic substances [3].

The middle layer of the leaves is also bitter in taste, and yellowish in color. It consists of anthocyanins and glycosides [2]. The pulp tissue contains proteins, as it contains 20 amino acids, seven of which are essential, in addition to vitamins, mineral salts, enzymes, and lipids, in addition to various carbohydrates, and contains 16 types of polysaccharides, 12 types of polypeptides, and various glycoproteins. Most of these compounds are effective microbial inhibitors and highly effective

antioxidants [4]. The inner layer contains the leaf gel and consists of 99% water with glucomannans, amino acids, lipids, sterols, and vitamins (A, B, C, E), which is one of the few plants that contain vitamin B12, in addition to other active compounds such as minerals, lignin, and saponins. [5]

The trunks of the cactus plant act as a water reservoir, enlarging case of abundant water to be able to store water, and shrinking those trunks in case of water shortage (Pankaj et al., 2013), and most of its species grow in the South Africa, Madagascar, and Saudi Arabia, while the favorite species grows in Southern California. The plant tolerates 44 ° C and freezing temperatures so that its roots do not break, and the main functional compound of cactus leaves is a long chain of acetylated mannose.

[6] in addition to polysaccharides and antioxidant compounds effective against cancer and inflammation [7].

Due to its properties and components, aloe vera is one of the most important plants used in the manufacture of cosmetics and skin creams, as it can be applied to burns, relieves pain in wounds, and moisturizes the skin [8] , treats eczema and psoriasis, and aloe vera works to protect hair from loss, baldness, dryness, and scalp infections [9]. From the above, it is clear that it is important to study the antibacterial activity of aloe leaves, and the extent to which they inhibit the growth of Gram-negative pathogenic bacteria, as the aloe plant contains many antioxidant and antimicrobial compounds.

Materials and Methods: -

1- Preparation of samples:

Aloe vera samples were obtained from nurseries in Salahuddin City, where the leaves were cleaned from the surrounding thorns by the peeler and washed with distilled water several times to clean them from dirt and dust, then left to dry in the air, and then cut into small pieces, dried in the oven for 24 hours and then milled in the electric mill.

2- Preparation of extracts:

2-1: - Alcoholic extract:

The method [10] in the extraction of active substances, the extraction was carried out by placing (20) g of the plant powder of the leaves of the aloe vera plant (Aloe vera) in the Thumble extraction thimble that was placed in the Soxhlet- continuous extraction apparatus. (400) ml of petroleum ether, acetone, ethyl acetate, and IMS (95% ethanol and 5% methanol) were used and the extraction process lasted (7) hours, then the solvent was evaporated using a rotary evaporator under vacuum pressure at a temperature of (45) ° C to obtain the crude alcoholic extract and then dried in the oven at a temperature of (45) ° C to obtain a dry powder and kept in the refrigerator until use [11]

2-2: - Aqueous extract: -

The aqueous extract was prepared as the studied plant parts were dried and ground, then (100) ml of distilled water was added to (20) grams of leaf powder in a glass flask with continuous stirring using a magnetic shaker for (24) hours, then the filtration process was carried out with filter papers (No.1) (The filtrate was concentrated by rotary vacuum evaporator until a very dense extract was obtained by evaporating as much of the solvent as possible, and the final extract was prepared by dissolving 0.4) g in 1 ml of sterile distilled water, placed in opaque bottles and left in the refrigerator until use [12].

3 -Detection of active chemical compounds in Aloe vera leaf extracts.

(Tannins, glycosides, saponins, and flavonoids) were detected according to the Pearson method [13]

1- Tannins Tannine: A small amount of the extract was mixed with the same amount of water and heated in a water bath at 60°C, then the mixture was filtered, and 0.03 g of ferric chloride was added to the filtrate, producing a dark green solution indicating the presence of tannin.

2- Glycosides: Glycosides: 1 g of Aloe vera leaf powder was taken and 10 ml of 10% HCL solution was added, then a few drops of Fahling's solution A and B were added and then neutralized with NaOH solution to form a red precipitate, evidence of the presence of glycosides.

3- Saponine:: Mix 0.2 of the previously prepared Aloe vera leaf extract with 5 ml of distilled water, then heat the mixture until boiling, and the appearance of creamy bubbles on the surface indicates the presence of saponins.

4- Flavonoids: Two solutions were prepared as follows:

The first solution: - 1 ml of the extract was mixed with 10 ml of 95% ethyl alcohol.

The second solution: 50% ethyl alcohol was added to 50% potassium hydroxide solution. A quantity of the first solution containing the extract was mixed with an equal amount of the second solution, and the appearance of yellow color indicates the presence of flavonoids.

4 -Methods of detection of *P. aeruginosa*

Gram-negative *P. aeruginosa* bacteria, obtained from burns and wound patients lying in Salahuddin General Hospital, were selected, and cultures were activated on Nutrient Broth liquid nutrient medium at 37°C for 24 hours, then compared with McFarland's standard solution prepared as mentioned[14] . The agar well diffusion method was used according to [13] as follows:

1- Muller Hinton medium was prepared according to the manufacturer's recommendations, sterilized in an autoclave at 121°C for 15 minutes, poured into 20 ml Petri dishes, and allowed to solidify.

2- Transfer 0.1 ml of the liquid medium containing pathogenic bacteria and spread by L.Shape and incubate the plates at 37°C for two hours, then puncture the plates with a cork drill with a diameter of 5 mm, put 0.1 ml of aqueous and alcoholic extract concentrations and then transferred to the incubator at 37°C for 18 hours and measure the diameter of the inhibition halos with a ruler, including the hole.

5- Results and Discussion:-.

1- Detection of active chemical compounds in Aloe vera leaf extract:

Table (1) shows that the alcoholic and aqueous extract contains the types of active compounds through qualitative and chemical analyses conducted, which gave a positive result for tannins, cladosides, saponins, and flavonoids by the appearance of different colors indicating the detection result, and the result agreed with [8] Aloe Vera methanolic leaf extract, petroleum ether extract, and chlorophyll extract gave a positive result when detecting the presence of tannins, terpenes, clathosides, saponins, and flavonoids and a negative result for phyllopectin and sterols. The result also agreed with [2] who detected the presence of the active compounds of Aloe Vera methanolic leaf extract and acetone extract, which gave a positive result for tannins, saponins, flavonoids, and terpenes and did not contain phyllopectin and sterols.

Table 1: shows the alcoholic and aqueous extracts contain

Na.	Chemical analysis	Alcoholic extract	Aqueous extract	Output
1	Saponins	+	-	Creamy bubbles
2	Flavonoids	+	-	Yellow solution
3	Glycosides	+	-	Red precipitate
4	Tannins	+	-	Dark green

2-The effectiveness of the active compounds of Aloe vera leaf extracts:-

The effectiveness of the compounds is due to the presence of flavonoids, phenols, and flavonoids, and the amount of these compounds is directly related to the antibacterial effectiveness, as well as other secondary compounds such as volatile oils, vitamins, chloroproteins, lectins, carotenoids, organic acids, anthraquinone compounds and polysaccharides such as glucomannan, which is the main compound of the leaves [15].

3 - Inhibitory effect of Aloe vera leaf extract against P. aeruginosa

The table shows that the aqueous and alcoholic extracts of Aloe vera leaves possess antibacterial activity, which varied according to the type of extract and the extraction method, where the highest inhibition rate was found in the alcoholic extract, followed by the aqueous extract.

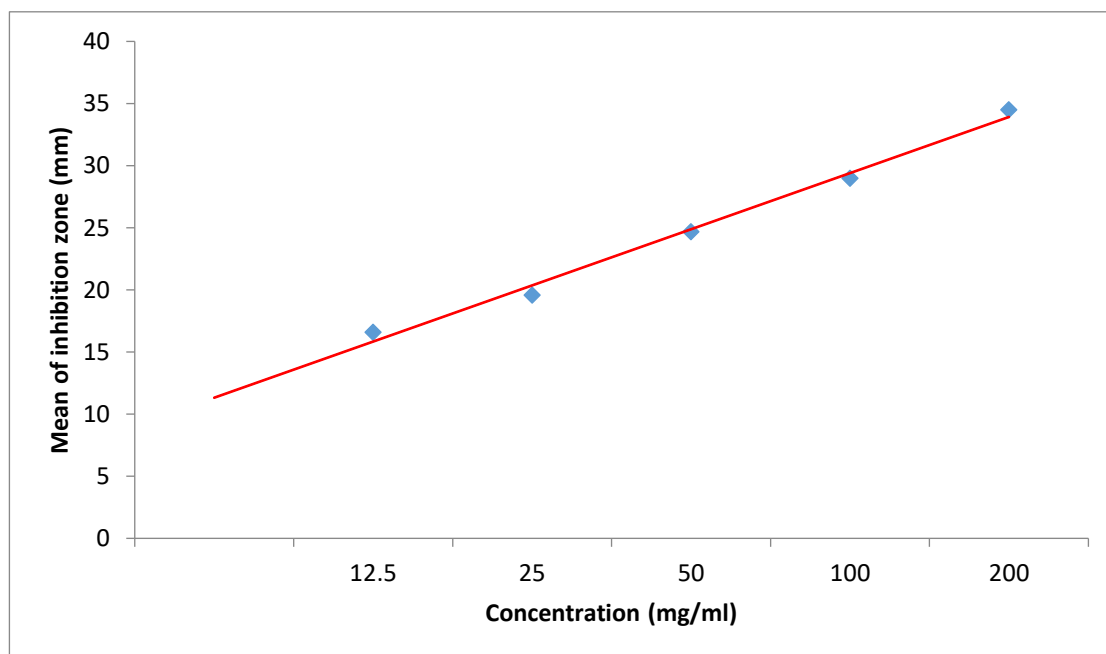


Figure 1: Effect of Aloe vera alcoholic extract on P. aeruginosa.

The inhibitory effect of the alcoholic extract at the concentration of (200) mg/ml, the average inhibition diameter reached (34.5) mm, followed by (100, 50, 25, and 12.5) mg/ml with inhibition diameters (29,

24.7, 19.6, and 16.6) mm. A significant difference ($p < 0.05$) was observed between the different concentrations of the extract Table (1-5) Figure (1-5).

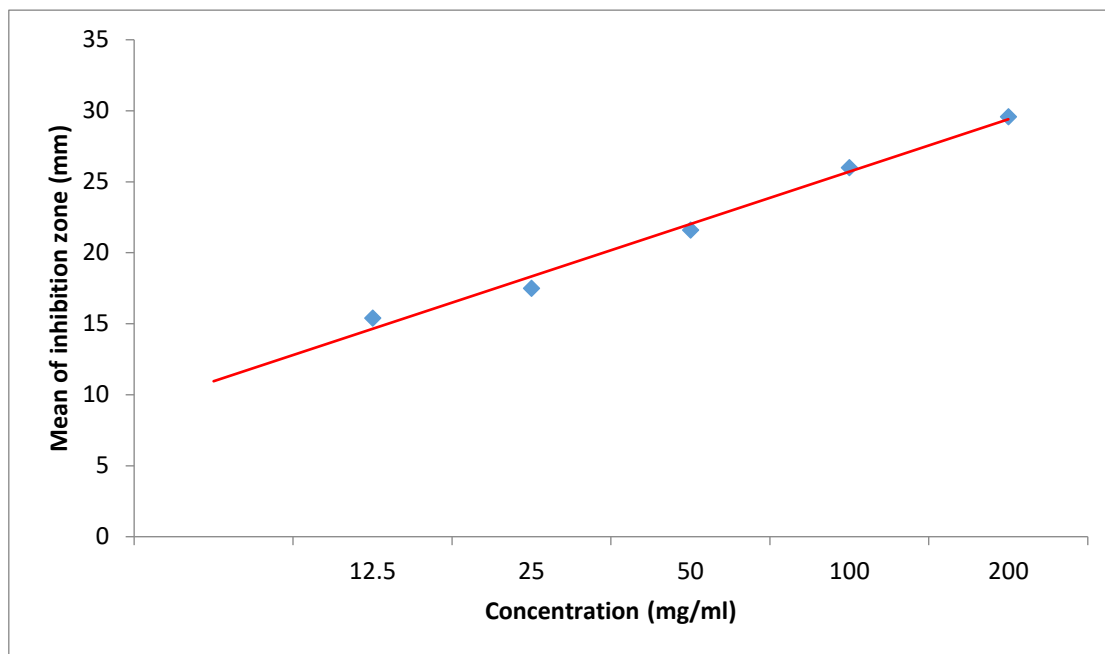


Figure 2: Effect of aloe vera aqueous extract on P. aeruginosa.

Table 2: Effect of alcoholic and aqueous extract of aloe vera on P. aeruginosa

Concentration mg/ml	Aloe vera aqueous extract	Aqueous extract of aloe vera.
	Average diameter of the inhibition zone (mm) ± experimental error	Average diameter of inhibition zone (mm) ± experimental error
200	0.30±29.6*	0.20±34.5
100	0.50±26*	0.50±29*
50	0.30±21.6*	0.30±24.7*
25	0.50±17.5*	0.30±19.6*
12.5	0.30±15.4*	0.20±16.6*

Sign (*) indicates a significant difference.

When the results were statistically compared between the alcoholic and aqueous extract of the Aloe vera plant, it was found that there was a significant difference ($p < 0.05$) in favor of the alcoholic extract as it outperformed the aqueous extract according to its concentration.

The efficacy of alcoholic and aqueous extracts of Aloe vera plant in inhibiting P. aeruginosa was consistent with the results of [12], [16] and converged with [11] who found high effects of aloe vera extracts, as they possess antibacterial activity and vary according to the extraction method. This indicates that the higher the concentration of the extract, the more effective it is, and the effectiveness of aloe vera extracts is attributed to its possession of many active compounds such as phenols and flavonoids, which have an important role in inhibiting the growth of bacteria, as they work to inhibit the enzymes responsible for basic metabolic reactions by their non-specific interference with proteins, leading to Protein denaturation, which causes the death of bacteria, In addition to containing tannins

that have an inhibitory effect on bacteria due to their ability to stimulate phagocytic cells and also have an effectiveness in breaking down proteins and other structures on the bacterial cell wall that bacteria use to adhere to the host's cells, as they can precipitate proteins and other structures on the bacterial cell wall. It can precipitate cell proteins leading to its death. Studies have indicated that aloe vera gel has shown its effectiveness as an antioxidant through its important role in preventing the production and sweeping of free radicals, as well as the aloe vera plant contains antifungal, antibacterial, anti-inflammatory, and antiseptic properties, [10].

Conclusions

We conclude from the study that the alcoholic and aqueous extract of Aloe vera leaves contains active compounds such as tannin, cladosides, saponins, and flavonoids, which can be used to treat burn injuries, wounds, chronic diseases, inflammation, skin ulcers, and others.

Recommendations

It is proposed to conduct another in-depth study and future research on the aloe vera plant to learn more about the properties and proportions of antioxidant compounds that are effective against bacteria and other pathogens and to conduct a biological study of aloe vera plant extracts to determine the proportions of toxic alkaloids contained within them, especially saponins, and their effect on laboratory animals, ending with determining the extent of their safe use in food products.

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