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Evaluation of some Antioxidants in *Aloe vera* extracts and determination of Antibacterial Activity in Growth of some Pathogenic Bacteria

Hadeel Ahmed Hasan[1], MADEHA H. HUSSAIN[1], ALI S. HASSOON[2] and Emad M. Rashed[1]

[1]Medical Institute Tech. Mansour, Middle Tech. University, Iraq

[2]Musaib Tech. College, Al-Furat Al-AwsatTech, University, Iraq

Corresponding author e-mail: com.hs.ali@atu.edu.iq hadeelahmed@mtu.edu.iq

Abstract

The crude of *Aloe vera* extracts were investigated with aim of determining the anti-bacterial activity in the growth of *Eescherichia coli* and *staphylococcus auerus*. The disc diffusion method was employed to measure the sensitivity of the crude extracts (equeous and methalonic) of aloe vera gel to the tested bacteria. Gentamicin (300 mgml) was utilized as a positive control for comparison with the two extracts activity. The findings of the present investigation were displayed that the tested bacteria were susceptible to both extracts, and the methalonic extracts , showed high antibacterial activity than equeous one and the *staph.anerus* was more sensitive to both extracts than *E.coli* according to the zone of growth inhibition which were measured in (mm) around the disc of two extracts .

Keyword: *Aloe vera* extracts, zone of growth inhibition, *staphylococcus auerus*, *Eescherichia coli*

Introduction

The usage of medicinal plants as a source of ailment alleviation may be dated back over five million years, when they were first discovered.

Anatomically, Neanderthals who lived 60,000 years ago utilized a variety of herbs that are still frequently employed in ethano medicine today.

Untapped potential for many plants as sources of novel medications has yet to be discovered. Only a small percentage has been investigated for phytochemical , pharmacological or biological function (1) .

It is still the most common source of antibacterial chemicals to come from plants. Most people in Asia and across the world rely on them as traditional health remedies, according to surveys; they have few harmful effects if taken correctly (2). A lot of effort and money has recently been spent by pharmaceutical corporations on producing natural medications derived from plants in order to provide more cost-effective cures. Multiple drug resistance in pathogenic bacteria has increased the need of finding novel antibiotic sources. In order to discover if *Aloe vera* possessed antibacterial capabilities, this experiment was carried out due to its extensive use and availability.

Aloe vera linne, also known as Aloe barbadensis Miller, is a succulent plant belonging to the Aloe family (which has 400 distinct species) that originated on the African continent. Its large leaves provide the plant with enough water to let it to endure lengthy periods of drought (3). A broad variety of juices and supplements containing *Aloe vera* have been found to be effective in the treatment of many gastrointestinal ailments.

In addition to being a laxative, dried Aloe leaves are also used to cure hemorrhoids because of their high concentration of extracts. In the United States, aloe vera gel is the most widely used herbal treatment today, and with good reason. It is used to relieve thermal burns, sunburns, and to aid in the healing of wounds (3). Furthermore, research reveals that *Aloe vera* contains antibacterial properties and can assist to activate the body's immune system, among other things (4) As a result, a research was conducted to test the antibacterial properties of *Aloe vera*.

Materials and methods

1 – Plant collection :

From a local market, mature, fresh *Aloe vera* leaves were bought. Cleansing the plant with running water and then rinsing it in distilled water removes any remaining dirt and dust particles. it dissected longitudinally to obtain the *Aloe* gel without fibers .

2 – Plant extraction:

10 grams of *Aloe vera* gel was grinded and soaked in 250 ml of distilled water and hemognized , then left for two weaks in order to prepare the aqueous extract .

Whatmann filter paper No. 1 was used to remove the crude extract. It was necessary to keep the filtrate in a conical flask in the refrigerator at 4 degrees Celsius until it was used.

Preparing methalonic extract of plant was in the same manner of aqueous one except using of methanol 95 % instead of distilled water (5) .

3 – Disc diffusion assay

The conventional dies diffusion susceptibility test on solid medium was used to assess the antibacterial properties of *Aloe vera* extracts (6).

To isolate E.coli and Staph.aureus, 100 microliters of each clinical isolate were prepared and streaked over nutritive solid medium.

A sterile Whatmann filter paper No.5 with a diameter of 6 mm was impregnated with equeous and methalinic extracts separately and put on the aforesaid culture medium, then incubated at 37 C for 24 hours.

The disc's circumference was measured, and the diameter of the growth inhibition zone around it was recorded in millimeters.

Sterile filter disc soaked in a gentamicin solution (300 mg\ml) and used as standard control with to extracts .

Any inhibition zone measuring < 7 mm around the disc of filter paper was considered a bad result.

Analysis of active compound

In order to separate the principal compounds, the researcgers utilized FLC (Fast Liquid Chromatography) with a binary delivery pump model (LC-10A Shimadzu) and a Shimadzu SPD 10A vp to monitor the eluted peaks, and we recorded the results using a Shimadzu C-R8A integrator. (Shimadzu,koyota,Japan).

Table 1: Chromatographic Separation conditions for Some active compound in Aloe Vera using (HPLC)

Column	FLC (Fast Liquid Chromatographic) column , 3 µm particle size, (50 x 2.0 mm I.D) C-8DB column
Mobile Phase	acetonitrile : tetrahydrofuran (THF):,0.1 % asetic acid (6 : 3 : 1, V/V)
Detection	UV set at 254 nm
Flow Rate	1.2 ml/min.
Temp	40 C

Table2: The Retention Time and the area of active compound

Seq	Subjects	Retention time minute	Area	Condensation
1	Aloin	3.54	317910	25ug/ml
2	Aloe-emodin	4.75	300248	25ug/ml
3	Aloetetic acid	5.34	256681	25ug/ml
4	Anthronol	6.80	244268	25ug/ml

The HPLC separation profile of the *Aloe vera* sample extract revealed different chromatographic peaks. The analysis of the separated substances that comprise the principal observed peaks, as well as the summarization of the collected data for each discovered chromatographic peak, are detailed further down. Quantitative measurements of phenols were calculated by comparing the peak areas of the authentic standard and the sample peaks under the same ideal separation conditions, and then using the equation below:

$$\text{Concentration of sample } \mu g/ml = \frac{\text{Area of sample}}{\text{Area of standard}} \times \text{conc. of standard} \times \text{dilution Factor}$$

The results

The antibacterial activity of *Aloe vera* gel extracts was first determined using the disc diffusion method with two E.coli and Staph.aureus isolates obtained from the urine and blood of Al-Yarmook hospital patients.

Table (3) shows the antibacterial efficacy of *Aloe vera* gel methalonic extracts against the growth of two different bacteria . The zone of growth inhibition of *staph.aureus* was more longer than in *E.coli* 18 mm and 15 mm respectively , comparing with Gentamicin as a standard control 24 mm and 20 mm respectively . figure (1) and (2)

Table (4) shows that *Aloe vera* gel aqueous extract inhibited the growth of the examined microorganisms. *staph.aureus* was more sensitive than *E.coli* to the extract , and the methalonic extract was more effective than aqueous one in growth of two tested bacteria figure (3) and (4) .

Table (3) : In comparison to Gentamicin, the antibacterial activity of methalonic extract of *Aloe vera* in the studied bacterium (300 mg\ml)

Aloe vera extarcts	Tested bacteria	Zone of growth inhibition (mm)
Methalonic extract	*Staph.aureus*	18
	E.coli	15
Gentamicin (300 mg\ml)	*Staph.aureus*	24
	E.coli	20

Table (4) : In Comparision to Gentamicin, the antibacterial activity of *Aloe vera* eqeous extract in the studied microorganisms (300 mg\ml)

Aloe vera extarcts	Tested bacteria	Zone of growth inhibition (mm)
Methalonic Extract	*Staph.aureus*	20
	E.coli	13
Gentamicin (300 mg\ml)	*Staph.aureus*	24
	E.coli	20

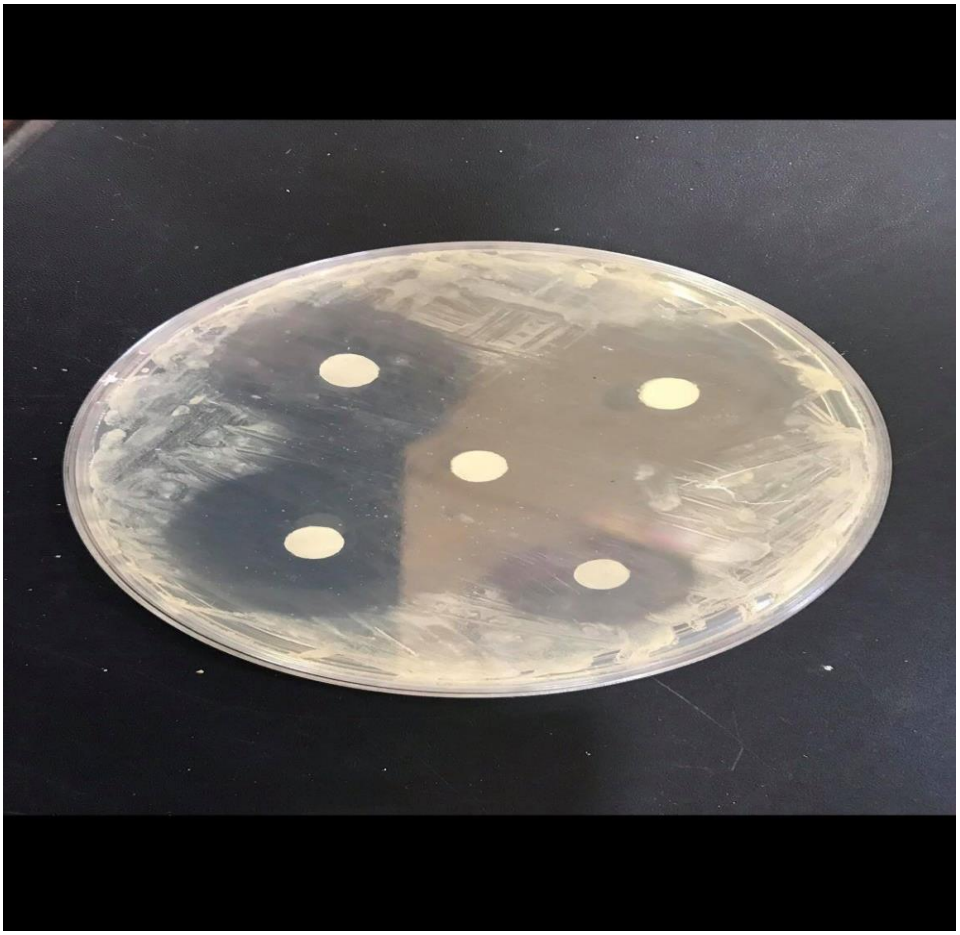

Figure (1) : Staph.aureus growth inhibition zones by *Aloe vera* methalonic extract

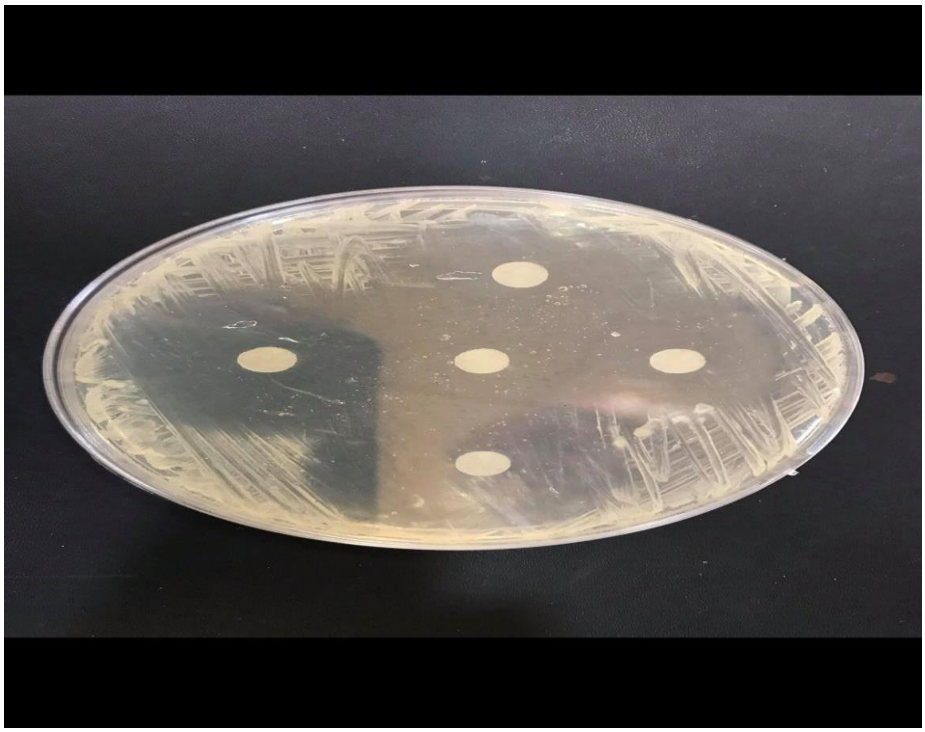

Figure (2) : E.coli growth inhibition zones induced by *Aloe vera* methalonic extract.

Figure (3) : Staph.aureus growth suppression zones by *Aloe vera* equeous extract

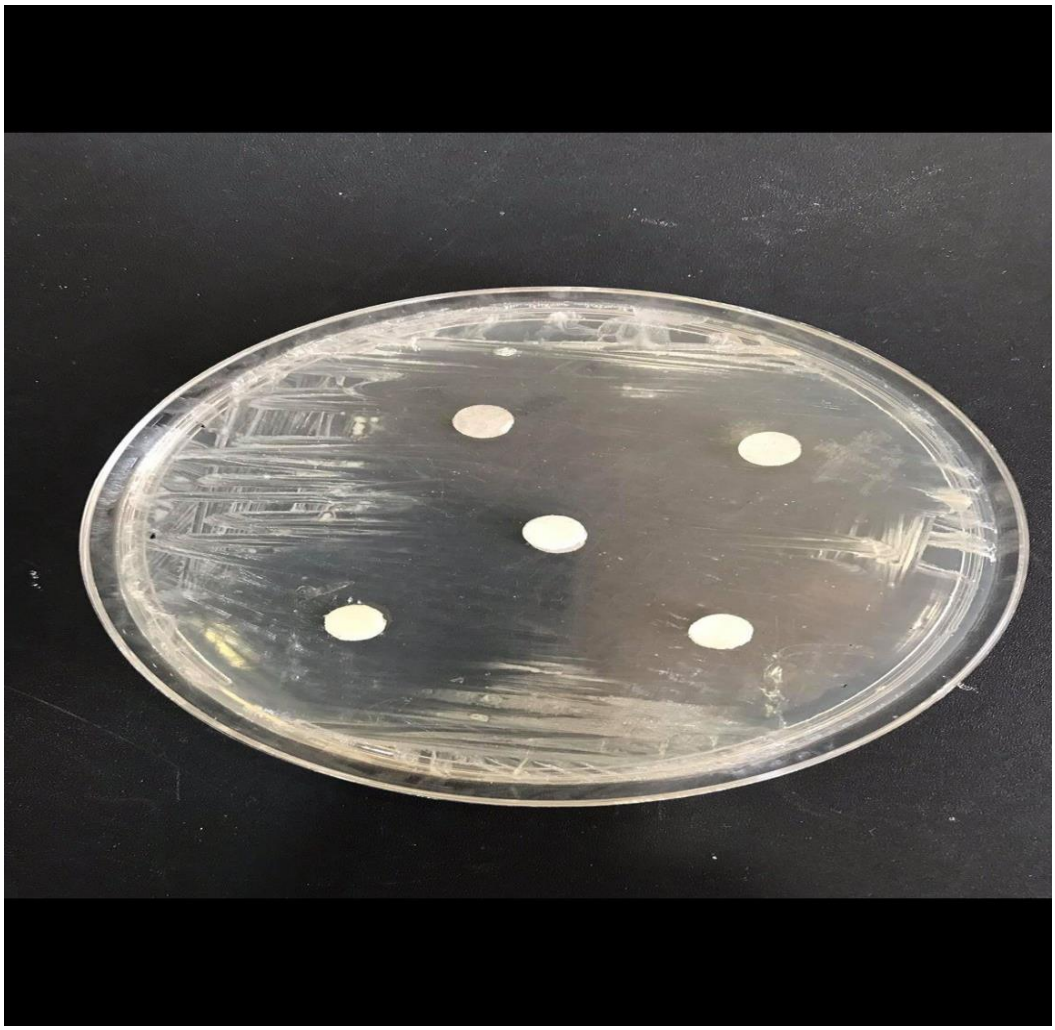

Figure (4) : E.coli growth suppression zones by *Aloe vera* equeous extract.

Table5: Concentration of active compound in the aqueous and alcoholic extract of *Aloe Vera*

Phenols	Concentration ug/ml	
	extract Aqueous	extract Alcoholic
Aloin	432.51	478.34
Aloe-emodin	244.87	265.30
Aloetetic acid	213.18	237.26
Anthronol	187.62	208.89

Discussion

The two extarcts of *Aloe vera* were subjected antibacterial activity against *E.coli* and *staph.aureus* . It was clear from table (1) and (2) that two extracts showed activity against tested bacteria .

The methalonic extract had higher activity than the equous one, which might be attributed to the phytochemical differences between them, as well as the fact that the methalonic extract was more polar than water, which would remove more of the chemicals buried inside the plant cells.

Anthraquinone is made up of phenolic chemicals that are only present in plant sap. Aloes, aloin, aloe-emodin, and barbaloin are all important pain relievers. They also have antibacterial and antiviral properties. It was also said to have antibacterial qualities. Their antibacterial qualities are extremely effective against pathogens including bacteria, fungi, and yeasts.

Other studies have already discovered the presence of pyrocatechol in Aloe vera, and this is not the first time (7). Moreover, the antibacterial activity of pyrocatechol was demonstrated by (8), as previously stated. Pyrocatechol is a hydroxylated phenol that has been shown to be hazardous to microorganisms in the laboratory.

The amount of hydroxyl groups and locations on the phenol group are thought to be related to their relative toxicity to microorganisms, as does the increase in hydroxylation. Among other things, phenolics function by denaturing proteins and disrupting cell membranes.

Their disinfection activities are boosted by organic matter, and they remain active on surfaces for a long time. It is possible that the antibacterial activity of methalonic extract is attributable to the extraction of a phenolic component from it.

The presence of cinnamic acid in *Aloe vera* gel has been found to be comparable to that found in Duke's phytochemical databases (9), and the antibacterial action of cinnamic acid

has been found to be comparable to that found in (10), who suggested that cinnamic acid inhibits glucose uptake and ATP production in bacteria's resting cells.

Coumaric acids have been shown to have antibacterial action according to the research of (11). It has been found that the chemical increases during the lag phase of the microorganism (12).

Likewise, the presence of ascorbic acid in *Aloe vera* gel has been demonstrated utilizing (9). Ascorbic acid inhibits bacterial growth through interfering with cell membranes, enzyme activity, and genetic processes. These findings are consistent with(13),(14), and (15), (16).

As worldwide antibiotic resistance by bacteria is becoming an exciting public health problem and the race to identify a new antibacterial agent is on, it was determined that *Aloe vera* gel, together with its components with potential antibacterial action, might be employed as an alternative herbal medicine.

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