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IN VITRO ANTI-BACTERIAL EFFECTS OF *ALOE BARBADENSIS* (ALOE VERA) EXTRACTS ON *STAPHYLOCOCCUS AUREUS*

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ABSTRACT

Objective: This study aimed to investigate the antibacterial activity of aqueous and alcoholic extracts of *Aloe vera* (*Aloe barbadensis*) on the growth of gram-positive bacteria *Staphylococcus aureus*. **Methods:** The cultured medium for bacteria was obtained in a dehydrated form and contained beef extract, yeast extract, peptone, sodium chloride, and agar. The antibacterial activity of different concentrations of the aqueous and ethanolic extracts of *A. barbadensis* was tested against Gram-positive bacteria (*S. aureus*). The methods used to determine the antibacterial activity of the plant extracts were agar well diffusion assay and gentamicin was used as a positive control and distilled water as a negative control. All the tests were performed in triplets. **Results:** All concentrations of the aqueous and alcoholic extracts of *A. barbadensis* exhibited a significantly high inhibitory effect against the linear growth of *S. aureus* compared to gentamycin. The highest concentration of the aqueous and alcoholic extracts of *A. barbadensis* at (25, 50 and 100%), gave significantly higher inhibition zones (16, 14 and 10%) respectively compared to the untreated control in day four after inoculation. The aqueous extract was the most effective in suppressing the bacteria growth than its alcoholic extracts and gentamycin. **Conclusion:** As the concentration of the extracts increased, the radius of the zones of inhibition also increased which implies good antibacterial activity.

KEYWORDS

A. barbadensis, *S. Aureus*, Agar well diffusion assay, Zones of inhibition and Antibacterial activity.

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INTRODUCTION

Nowadays, continuous and incorrect utilization of antimicrobial drugs has developed the resistant bacteria and difficulty in the management of infected wounds, so consideration to new antibacterial agents and the least adverse effects seem to be a necessity¹. *Staphylococcus aureus* has posed an important problem in hospitals, nursing homes, and other health care settings. Serious

infections due to these organisms currently necessitate the use of non- β -lactam antibacterial therapy². Many hospitals - acquired MRSA strains are only susceptible to vancomycin³, thus, there is a strong concern about the possible development and spread of vancomycin resistance in MRSA. Some vancomycin-resistant MRSA strains have been reported since 1996⁴. There are several plants with known therapeutic values, a point in case is *Aloe barbadensis* which has been identified to have quite several studied pharmacological activities such as anti-oxidant, wound healing potential, anti-inflammatory, antimicrobial activities among others, in addition to this, a lot of studies on antibacterial activity of *Aloe barbadensis* has been conducted on many species⁵. The main constituents of *A. barbadensis* gel are mucopolysaccharides (glucomannans, polymannoses, about 10% of total solids), enzymes, anthranoids, lignin, saponins, vitamins, amino acids (almost 50% of the total amount consisting of 8 of the 10 essential amino acids) and minerals (quantities not given), total solids are in the range of 1.3 to 2%, the rest being water⁶. Therefore, this study aimed at investigating the *in-vitro* antibacterial effect of *A. barbadensis* against *S. Aureus*.

MATERIAL AND METHODS

Preparation of extracts

A. barbadensis leaves were obtained from University of Gezira, Horticulture Department, Faculty of Agriculture, washed with distilled water, and then chopped into small pieces, air-dried and ground into powder. The powder was then extracted with 80% ethanol. The mixture was then centrifuged at 3000rpm for 10 minutes and the supernatant collected was allowed to evaporate over a dry oven. The gelatinous extract thus prepared was weighed and using distilled water, serial dilutions of 25mg/75ml, 50mg/50ml, and 100mg (w/v) were made to obtain 25, 50, and 100% concentrations, respectively.

Preparation of nutrient agar

This was prepared according to the protocol followed by⁷. The medium was then allowed to boil until it was completely dissolved. The pH of the medium was adjusted to pH 7.4 \pm 0.2 and then

sterilized in an autoclave at 121°C (115b\in²) for 15 min.

Preparation of test organism

The normal agar was mixed well and poured on the sterile Petri plates. The agar media on Petri plates were allowed to settle for a few minutes. Normal agar plates were inoculated with respective bacteria, *S. aureus* which was obtained from University of Gezira, Plant Pathology Center, and then incubated at 37°C for overnight. Each time, a fresh bacterial culture was prepared.

Preparation of the antimicrobial agent

The antibacterial agent gentamicin was dissolved in distilled water. Further dilutions were made using the same solvent according to CLSI document M100-S18. Gentamicin was used in concentrations 25, 50, and 100%.

Antibacterial activity

This was measured by using the paper disc diffusion method. The normal agar was mixed well and poured on the sterile Petri plates. The agar media on Petri plates were allowed to set harden for a few minutes. Normal agar plates were inoculated with respective bacteria. The small autoclaved discs of Whatman filter paper were used. The test organism was spread on the Petri plates by using sterilized glass spreader. During the paper-disc diffusion method, the sterile discs were dipped in the different crude extracts of medicinal plants and antibiotic drugs with the help of sterilized forceps and placed on the Petri plates. Distilled water was used as a control to check the comparison of antibacterial activity with different crude extracts of medicinal plants. The Petri plates were sealed with the parafilm. Then, the Petri plates were left at room temperature for 30 minutes, to allow the diffusion of the test sample and then incubated at 37°C overnight. The diameter of the zones of inhibition was measured in mm⁸.

Statistical analysis

The obtained data were statistically analyzed by computer software MSTATC according to the analysis of variance (ANOVA); Duncan's Multiple Range Test was used for mean separation.

RESULTS AND DISCUSSION

The results depicted in (Table No.1 and Figure No.1) indicate that the high concentrations of *Aloe vera* aqueous extract (100%) used in this study had the highest inhibitory effects (18.2mm) against the tested microorganisms. However, this extract showed inhibition action of 12mm even at a minimal concentration (25%) used in this study. The other concentrations of the aqueous phase (50%) gave inhibition zones of 15mm. Ethanol extract of *Aloe vera* at all concentrations shows an inhibitory effect against the *Staphylococcus aureus*. The highest inhibition zone obtained was 14.5mm with a concentration of 100%, concentrations of 25, and 50% showed inhibition zones of 5.9 and 9.4mm, respectively. Antibiotics extract of *Aloe vera* at all concentrations shows an inhibitory effect against the *Staphylococcus aureus*. The highest inhibition zone obtained was 16.5mm with a concentration of 100%, concentrations of 25 and 50% showed inhibition zones of 7.75 and 6mm, respectively.

The results depicted in (Table No.2 and Figure No.2) indicate that the high concentrations of *Aloe vera* aqueous extract (100%) used in this study had the highest inhibitory effects (16.65mm) against the tested microorganisms. However, this extract showed inhibition action of 10.5mm even at a minimal concentration (25%) used in this study. The other concentrations of the aqueous phase (50%) gave inhibition zones of 13mm. Ethanol extract of *Aloe vera* at all concentrations shows an inhibitory effect against the *Staphylococcus aureus*. The highest inhibition zone obtained was 12.5mm with a concentration of 100% concentrations of 25 and 50% showed inhibition zones of 5 and 9mm, respectively. Antibiotics extract of *Aloe vera* at all concentrations shows an inhibitory effect against the *Staphylococcus aureus*. The highest inhibition zone obtained was 16mm with a concentration of 100% concentrations of 25 and 50% showed inhibition zones of 5.95 and 5mm, respectively.

The results depicted in (Table No.3 and Figure No.3) indicate that the high concentrations of *Aloe Vera* aqueous extract (100%) used in this study had the highest inhibitory effects (16mm) against the tested microorganisms. However, this extract

showed inhibition action of 9mm even at a minimal concentration (25%) used in this study. The other concentrations of the aqueous phase (50%) gave inhibition zones of 11.5mm. Ethanol extract of *Aloe vera* at all concentrations shows an inhibitory effect against the *Staphylococcus aureus*. The highest inhibition zone obtained was 10mm with a concentration of 100% concentrations of 25 and 50% showed inhibition zones of 3.5 and 8mm, respectively. Antibiotics extract of *Aloe vera* at all concentrations shows an inhibitory effect against the *Staphylococcus aureus*. The highest inhibition zone obtained was 14mm with a concentration of 100% concentrations of 25 and 50% showed inhibition zones of 4.5 and 4.5mm, respectively.

It was found that all the Concentration preparations of *Aloe vera* gel exhibited reasonably good inhibitory activities compared with the standard reference antibiotics with the preserved gel being more potent compared with all others.

Table No.1: Effect of different Concentration of aqueous and alcoholic extracts of *Aloe vera* and Antibiotic on inhibition (mm) of *Staphylococcus aureus* using disc method at two days post-inoculation

S.No	Treatments	Concentration %	Inhibition zones(mm)			Mean
			R1	R2	R3	
1	Ethanol	25	7.5	6	5.8	5.9
		50	10	9	9.8	9.4
		100	16	14	15	14.5
2	Aqueous	25	10	13	12	12
		50	15.8	16	14	15
		100	19.2	18.4	18	18.2
3	Gentamycin	25	6.5	8.5	7	7.75
		50	9	7	5	6
		100	17	15	18	16.5

S.No	Summary	Count	Sum	Average	Variance
1	7.5	2	11.8	5.9	0.02
2	10	2	18.8	9.4	0.32
3	16	2	29	14.5	0.5
4	10	2	25	12.5	0.5
5	15.8	2	30	15	2
6	19.2	2	36.4	18.2	0.08
7	6.5	2	15.5	7.75	1.125
8	9	2	12	6	2
9	17	2	33	16.5	4.5
10	R2	9	106.9	11.87778	19.13444
11	R3	9	104.6	11.62222	25.12444

ANOVA							
S.No	Source of Variation	SS	Df	MS	F	P-value	F crit
1	Rows	343.32	8	42.915	31.93344	2.76E-05	3.438101
2	Columns	0.293889	1	0.293889	0.218685	0.652518	5.317655
3	Error	10.75111	8	1.343889			
4	Total	354.365	17				

Table No.2: Effect of different Concentrations of aqueous and alcoholic extracts of *Aloe vera* and Antibiotic on inhibition (mm) of *Staphylococcus aureus* using disc method at three days post-inoculation

S.No	Treatments	Concentration %	Inhibition zones(mm)			Mean
			R1	R2	R3	
1	Ethanol	25	7	5	5	5
		50	9	9	9	9
		100	14	13	12	12.5
2	Aqueous	25	10	11	10	10.5
		50	14	14	12	13
		100	18	16.4	16.9	16.65
3	Gentamycin	25	5	7.3	6.6	5.95
		50	8	6	5	5
		100	16	15	17	16

S.No	SUMMARY	Count	Sum	Average	Variance
1	7	2	10	5	0
2	9	2	18	9	0
3	14	2	25	12.5	0.5
4	10	2	21	10.5	0.5
5	14	2	26	13	2
6	18	2	33.3	16.65	0.125
7	5	2	13.9	6.95	0.245
8	8	2	11	5.5	0.5
9	16	2	32	16	2
10	R2	9	96.7	10.74444	17.03278
11	R3	9	93.5	10.38889	20.72611

ANOVA							
S.No	Source of Variation	SS	Df	MS	F	P-value	F crit
1	Rows	296.77	8	37.09625	55.9826	3.18E-06	3.438101
2	Columns	0.568889	1	0.568889	0.85852	0.381256	5.317655
3	Error	5.301111	8	0.662639			
4	Total	302.64	17				

Table No.3: Effect of different Concentrations of aqueous and alcoholic extracts of *Aloe vera* and Antibiotic on inhibition (mm) of *Staphylococcus aureus* using disc method at four days post-inoculation

S.No	Treatments	Concentration %	Inhibition zones(mm)			Mean
			R1	R2	R3	
1	Ethanol	25	5	3	4	3.5
		50	8	9	7	8
		100	12	10	10	10
2	Aqueous	25	9	10	8	9
		50	12.8	12	11	11.5
		100	17	16	16	16
3	Gentamycin	25	3.5	5	4	4.5
		50	7	5	4	4.5
		100	15	13	15	14

S.No	Summary	Count	Sum	Average	Variance
1	5	2	7	3.5	0.5
2	8	2	16	8	2
3	12	2	20	10	0
4	9	2	18	9	2
5	12.8	2	23	11.5	0.5
6	17	2	32	16	0
7	3.5	2	9	4.5	0.5
8	7	2	9	4.5	0.5
9	15	2	28	14	2
10	R2	9	83	9.222222	17.94444
11	R3	9	79	8.777778	21.19444

ANOVA							
S.No	Source of Variation	SS	Df	MS	F	P-value	F crit
1	Rows	306	8	38.25	43.03125	8.81E-06	3.438101
2	Columns	0.888889	1	0.888889	1	0.346594	5.317655
3	Error	7.111111	8	0.888889			
4	Total	314	17				

Table No.4: Antimicrobial activity of different Concentration preparations of *Aloe vera* gel aqueous and alcoholic extract on inhibition (mm) of *Staphylococcus aureus* with antibiotics as the reference standard

S.No	Treatments	Concentration %	Inhibition zones(mm)
1	Ethanol	25	+
		50	+
		100	++
2	Aqueous	25	+
		50	++
		100	++
3	Gentamycin	25	+
		50	++
		100	+

Antimicrobial activity: -, No inhibition +, Zone of inhibition \leq 8mm in diameter; ++, Zone of inhibition $>$ 8mm in diameter.

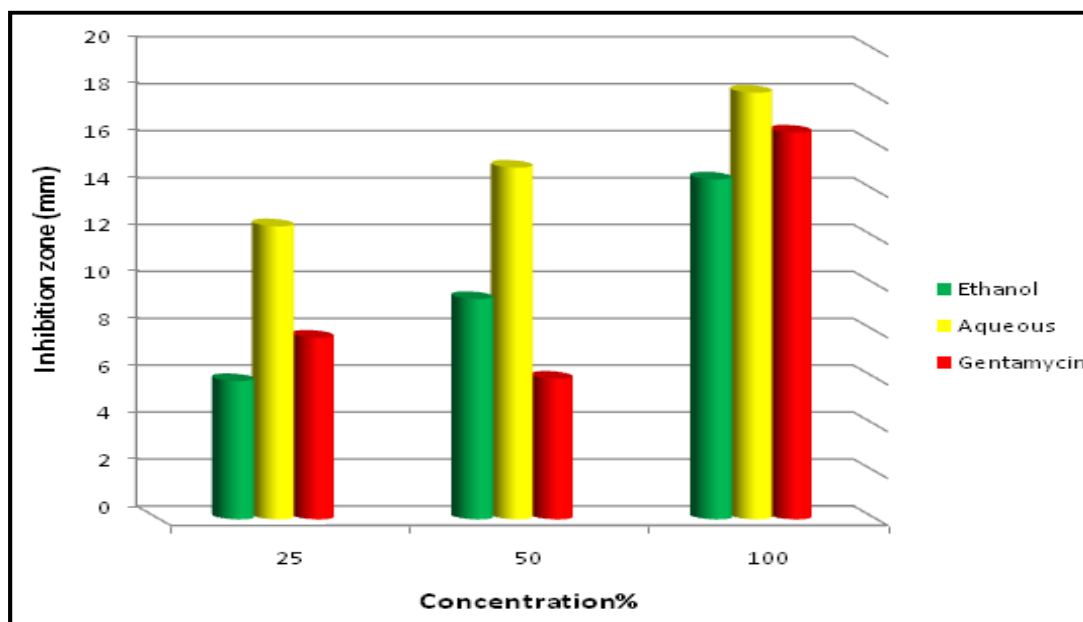


Figure No.1: Effect of different Concentration of aqueous and alcoholic extracts of *Aloe vera* and Antibiotic on inhibition (mm) of *Staphylococcus aureus* using disc method at two days post-inoculation

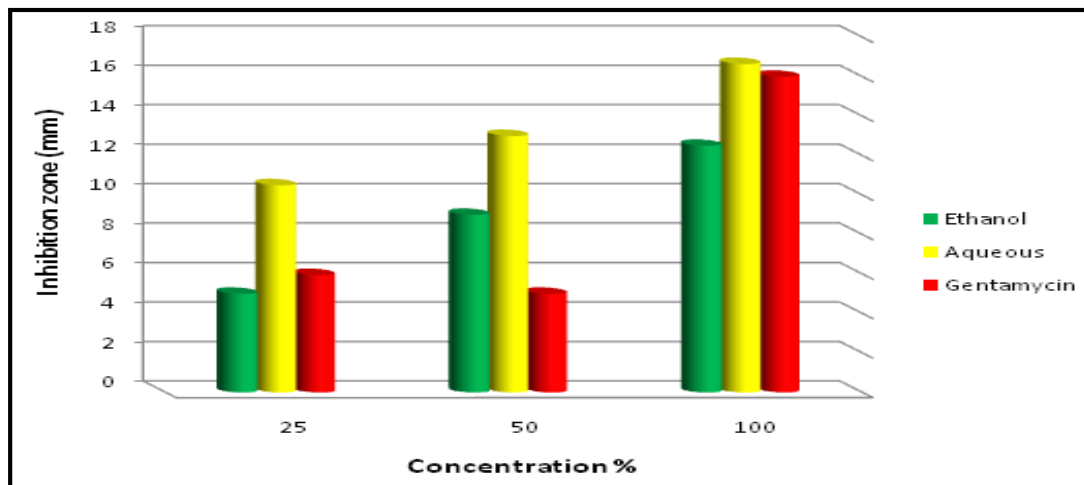


Figure No.2: Effect of different Concentrations of aqueous and alcoholic extracts of *Aloe vera* and Antibiotic on inhibition (mm) of *Staphylococcus aureus* using disc method at three days post-inoculation

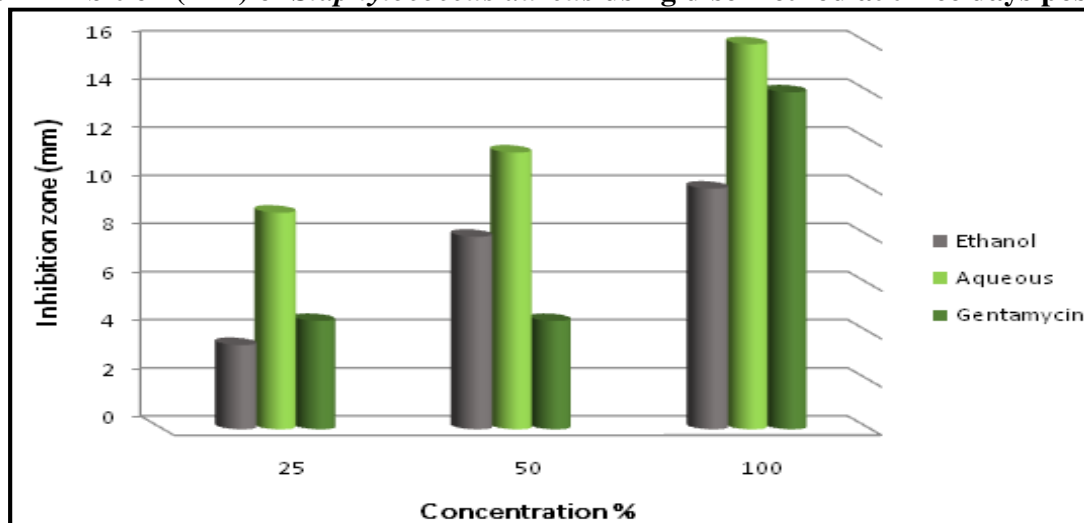


Figure No.3: Effect of different Concentrations of aqueous and alcoholic extracts of *Aloe vera* and Antibiotic on inhibition (mm) of *Staphylococcus aureus* using disc method at four days post-inoculation

CONCLUSION

This study showed that the aqueous extract phase of *Aloe vera* gave better results compared to the Ethanolic and Antibiotic phase of the same extract at this study at all concentrations tested. The broad antimicrobial action of the aqueous extract of the *Aloe vera* used in the study could be ascribed to the water-soluble components which are naturally occurring in the plant materials. According to⁹ remarkable antibacterial activities with Ethanolic extracts of *Aloe vera* gel even at low concentrations compared with the standard antibiotics and support the view that *Aloe vera* is a potent antimicrobial agent compared with the conventional antibiotics.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBLIOGRAPHY

1. He C L, Fu B D, Shen H Q, Jiang X L and Wei X B. Fumaric acid, an antibacterial component of *Aloe vera* L, *African Journal of Biotechnology*, 10(15), 2011, 2973-2977.

2. Hackbarth, C J and Chambers H F. Methicillin-resistant staphylococci: detection methods and treatment of infections, *Antimicrobial Agents and Chemotherapy*, 33(7), 1989, 995.
3. Fitzgerald J R, Sturdevant D E, Mackie S M, Gill S R and Musser J M. Evolutionary genomics of *Staphylococcus aureus*: insights into the origin of methicillin-resistant strains and the toxic shock syndrome epidemic, *Proceedings of the National Academy of Sciences*, 98(15), 2001, 8821-8826.
4. Ei-Jakee J, Marouf S A, Ata N S, Abdel-Rahman E H, El-Moez S A, Samy A A and El-Sayed W E. A rapid method for the detection of *Staphylococcus aureus* enters toxins in food, *Global Veterinaria*, 11(3), 2013, 335-341.
5. Rivandi M, Emami M R, Rad M, Kazemi Mehrjerdi H, Azizzadeh M and Ghasemi S. Bacteriological evaluation of aloe vera I. Fresh gel on experimental infected full-thickness open wounds induced with staphylococcus aureus in dogs, *Iranian Journal of Veterinary Surgery*, 7(1-2), 2012, 75-84.
6. Vinson J A, A I Kharrat H and Andreoli L. Effect of Aloe vera preparations on the human bioavailability of vitamins C and E, *Phytomedicine*, 12(10), 2005, 760-765.
7. Harrigan W F. Laboratory methods in food microbiology, *Gulf Professional Publishing*, 3rd Edition, 1998.
8. Irshad S, Butt M and Younus H. *In-vitro* antibacterial activity of *Aloe barbadensis* Miller (Aloe vera), *International Research Journal of Pharmaceuticals*, 1(2), 2011, 59-64.
9. Subramanian S, Kumar D S and Arulselvan P. Wound healing potential of *Aloe vera* leaf gel studied in experimental rabbits, *Asian J Biochem*, 1(2), 2006, 178-185.

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