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ANTIBACTERIAL EFFECT OF AQUEOUS EXTRACT OF ALOE VERA AGAINST THE GRAM-POSITIVE BACTERIA STAPHYLOCOCCUS AUREUS IN MEDANI CITY - GEZIRA STATE – SUDAN- 2018

Yasir Hakim[1]*, Dalia Hamza[2], A. KH Khalil[3], Abdalla Khalid[4]

Assistant Professor of Pathology with MD Pathology, Head Unit of Microbiology, Department of Basic Medical Science, College of Medicine, Dar Al Uloom University, Riyadh, Kingdom of Saudi Arabia & Sennar University, International College of Medicine, Sudan.

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***Corresponding Author**
Yasir Hakim
Assistant Professor of Pathology with MD Pathology, Head Unit of Microbiology, Department of Basic Medical Science, College of Medicine, Dar Al Uloom University, Riyadh, Kingdom of Saudi Arabia & Sennar University, International College of Medicine, Sudan.

ABSTRACT

The plant *Aloe* vera was used historically as a topical to heal wounds, various skin conditions and orally as a laxative. The Gram-positive bacterium *Staphylococcus aureus* is considered to be the most pathogenic species of the genus Staphylococcus, being implicated in both community-acquired and nosocomial infections. The present investigation was undertaken at the University of Gezira, Center of Plant Pathology, during the year 2018. The aim of the study was to investigate the effect of Aloe vera on Staphylococcus aureus as antibacterial activity of aqueous and alcoholic extracts of *Aloe vera* on inhibiting the growth of the *Staphylococcus aureus* against a known Antibiotics (Gentamycin) as appositive control. Three concentrations of aqueous extract of *Aloe vera* and the Gentamycin, (25, 50 and 100%) were tested. The aqueous suspensions of the dried *Aloe vera* extracts were screened for their anti-*Staphylococcus aureus* activity using the agar-disc diffusion method. The results obtained indicated that the highest concentration of *Aloe vera* aqueous extract (100%) used in this study had the highest inhibitory effects (18.2 mm) against the tested bacterium, while the other two concentrations (50 % and 25%) showed an inhibition zones of 15 mm and 12 mm, respectively. For the positive control (Gentamycin) the highest inhibition zone 16.5 mm was obtained with the higher concentration (100 %). The

other concentrations (25 and 50 % showed inhibition zones of 7.75 and 6 mm, respectively. The study recommended that, further research should be done to clearly identify the active ingredients of *Aloe vera* and their other antimicrobial activities.

KEYWORDS: Aloe Vera, Antimicrobial activities, Gentamycin, Staphylococcus aureus.

INTRODUCTION

Aloe vera belongs to the Liliaceae family, of which there are about 360 species. It is a cactus-like plant that grows readily in hot, dry climates and currently, because of demand, is cultivated in large quantities. The gel of *A. vera* was used to treat stomach ailments, gastrointestinal problems, skin disease, constipation, radiation injury, inflammatory effect, healing wounds and burns, ulcer and diabetes. *A. vera* products are mainly for cosmetic, pharmaceutical, nutraceuticals and food industries. The gel stimulates cell growth and enhances the restoration of damaged skin. It moisturizes the skin because it has a water holding capacity. As a drink, it protects the mucous membrane of the stomach especially when irritated or damaged. A. vera juice is considered helpful for relieving many types of gastrointestinal irritation and juice products are widely available (Anderson and Phillipson., 1996).

Staphylococcus aureus poses 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 therapy (Hackbarth and Chambers,1989).Many hospital acquired MRSA strains are only susceptible to vancomycin (Fitzgerald *et al.*, 2001). Thus, there are strong concerns about the possible development and spread of vancomycin resistance in MRSA. Some vancomycin-resistant MRSA strains have been reported since 1996 (EI-Jakee *et al.*, 2014; ALian *et al.*, 2012). Some necrosis poisons cases occur by strong acids as H2SO4, it affects skin created necrosis or burns and these allow for bacteria growth. H_2SO_4 is one form of strong poison, because the poison's symptoms appear after five minutes from application on skin. If possible, treatment by Na_2Co_3 as antidote for H_2SO_4, but the necrosis caused by bacterial infection should be treated use drugs. The main constituents of *Aloe Vera* 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 water (Vinson *et al.*, 2005). *Aloe Vera* gel is obtained either from hand-filleted leaves of Aloe barbadensis or, by cold

processing of the whole leaf, in which case the product usually also contains appreciable quantities of the latex material and anthranoids. The anthranoids in whole leaf extracts of *Aloe Vera* can however, be reduced to levels below10mg/kg in the product (Reynolds and Dweck, 1999; Lee *et al.*, 2000; Hu *et al.*, 2003). Oliver (Oliver, 2012) indicates that *Aloe Vera* gel is used in veterinary medicine topically to promote wound healing on general skin wounds in all animals. It has also been recommended as a teat-dip in lactating cows, by intra mammary administration for (adjuvant) treatment of mastitis or high somatic cell counts, and by oral route in all food producing species as adjuvant treatment for a number of afflictions (ranging from anemia to infertility, mastitis and shock (Hu *et al.*, 2003;Oliver,2012). Medicinal plants according to the World Health Organization (WHO) defines them as herbal preparations made by introducing plant materials to extraction, fractionation, purification, concentration, or other physical or biological processes, which may be produced as a basis for herbal products or for immediate consumption. In human medicine *Aloe Vera* gel is used topically to promote wound healing. Oral use as a general tonic for a number of indications, where scientific proof is outstanding, has also been described. *Aloe Vera* gel is also widely used in cosmetics (Ramachandra and Rao, 2008; Subramanian *et al.*, 2006; Saravanan *et al.*, 2010; Kedarnath *et al.*, 2012). Moreover, *Aloe Vera* has ulcerogenic activity (Sai *et al.*, 2014).

OBJECTIVES

- To test the antimicrobial effects of *Aloe vera* aqueous and alcohol and Antibiotic (control) leaf extracts on Staphylococcus aureus.
- To determine the effect of A*loe vera* leaf extract different concentration on Staphylococcusaureus.

MATERIALS AND METHODS

Staphylococcus aureus

It was obtained from the microbiological laboratory of the Department of Pathology Medical lab, Faculty of Medicine, University of Gezira, Wad Medani, Sudan during the period from January to May, 2018.

Aloe vera plant

Were obtained from the University of Gezira fields during January to May, 2018.

Preparation of Nutrient agar

This was a general-purpose cultured medium for bacteria. It was obtained in a dehydrated form. The constituent of the medium were beef extract, yeast extract, peptone, sodium chloride and agar. It was prepared according to the manufactures instruction by suspending 28g in one liter distilled water. The medium was allowed to boil until it was completely dissolved. The pH of medium was adjusted to pH 7.4±0.2 and then the medium was sterilized in an autoclave at 121ºC (115b\in²) for 15 min (Harrigan, 1998).

Preparation of the crude extracts

Aloe vera leaf extract to prepare crude extract of fresh *Aloe vera* whole leaves were washed with distilled water, chopped into small pieces, air-dried and ground into powder. The *Aloe vera* mixed with 80% concentration of ethanol The pulp ethanol mix 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 using distilled water, serial dilutions of 25g/75ml, 50g/50ml and 100mg (w/v) were made in order to obtain 25%, 50% and 100% concentrations, respectively.

Preparation of test organism

The nutrient agar were mixed well and poured on the sterile petri plates. The agar media on petri plates were allowed to set for few minutes. nutrient agar plates were inoculated with respective bacteria (*S.aureus*), and then incubated at 37° C for overnight. Each time, a fresh bacterial culture was prepared.

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 the concentrations 25%, 50% and 100%.

Antibacterial activity

Antibacterial activity was measured using paper disc diffusion method, (Method of Saba *et al.*, 2011) was followed.

The following steps were involved in paper disc diffusion method. The normal agar were mixed well and poured on the sterile petri plates. The agar media on petri plates were allowed to set harden for few minutes. nutrient agar plates were inoculated with respective bacteria.

The small autoclaved discs of Whatmann filter paper were used. The test organism was spread on the petri plates by using sterilized glass spreader. During 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 para film.

Then, the petri plates were left at room temperature for 30 minute, to allow the diffusion of the test sample and then incubated at 37° C for overnight. The diameter of the zones of inhibition were measured in cm.

Statistical analysis

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

RESULTS

1. Two days post inoculation

The results depicted in Table (1) and figure (1) indicate that the high concentrations of *Aloe vera* aqueous extract (100%) used in this study had the highest inhibitory effects (18.2 mm) against the tested Staphylococcus aureus. However, this extract showed inhibition action of 12 mm even at minimal concentration (25%) used. The other concentrations of the aqueous phase (50 %) gave an inhibition zones of 15 mm.

Control (Gentomycin) extract of *Aloe vera* at all concentrations shows an inhibitory effect against the *Staphylococcus aureus.* The highest inhibition zone obtained was 16.5 mm with the concentration of 100 % Concentrations of 25 and 50 % showed an inhibition zones of 7.75 and 6 mm, respectively.

Table 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.

Treatments	Concentration %	Inhibition zones(mm)			Mean
		R1	R2	R3	
Ethanol	25	7.5	6	5.8	5.9
	50	10	9	9.8	9.4
	100	16	14	15	14.5
Aqueous	25	10	13	12	12
	50	15.8	16	14	15
	100	19.2	18.4	18	18.2
Gentamycin	25	6.5	8.5	7	7.75
	50	9	7	5	6
	100	17	15	18	16.5

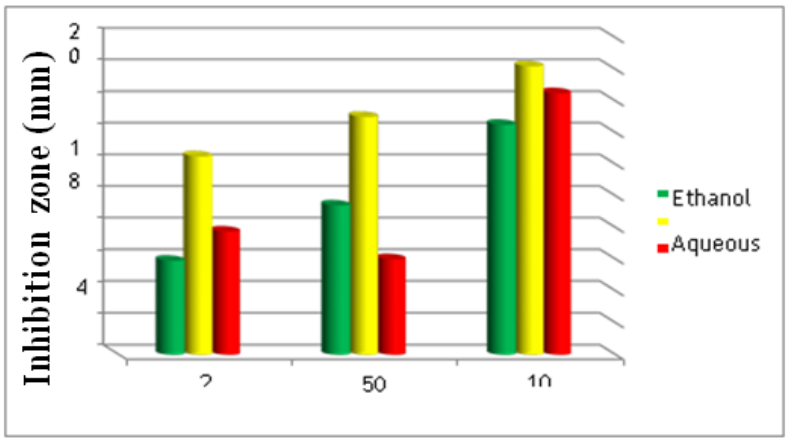

Figure 1: Effect of different Concentration of aqueous and alcoholic extracts of *Aloe vera* and Antibiotic on inhibition (mm) of *Staphylococcus aureus* using disc method at two days post inoculation.

2. Three days post inoculation

The results depicted in Table(2)and Figure (2) indicate that the high concentrations of *Aloe vera* aques extract (100%) used in this study had the highest inhibitory effects (16.65 mm) against the tested *Staphylococcus aureus*. However, this extract showed inhibition action of 10.5 mm even at minimal concentration (25%) used in this study. The other concentrations of the aqueous phase (50 %) gave an inhibition zones of 13 mm.

Control (gentamycin) extract of *Aloe vera* at all concentrations shows an inhibitory effect against the *Staphylococcus aureus.* The highest inhibition zone obtained was 16 mm with the concentration of 100 % Concentrations of 25 and 50 % showed an inhibition zones of 5.95 and 5 mm, respectively.

Table 2: Effect of different Concentration of aqueous and alcoholic extracts of *Aloe vera* and Antibiotic on inhibition (mm) of *Staphylococcus aureus* using disc method at three days post inoculation.

Treatments	Concentration %	Inhibition zones(mm)			Mean
		R1	R2	R3	
Aqueous	25	**10**	**11**	**10**	10.5
	50	**14**	**14**	**12**	13
	100	**18**	**16.4**	**16.9**	16.65
Gentamycin	25	**5**	**7.3**	**6.6**	5.95
	50	**8**	**6**	**5**	5
	100	16	15	17	16

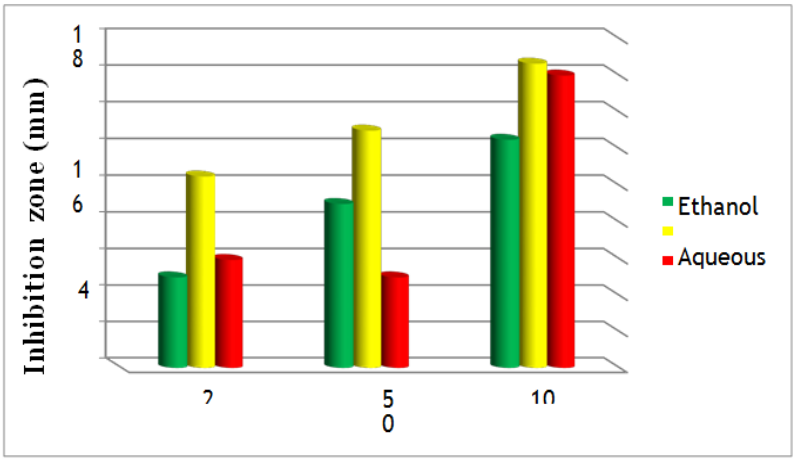

Figure 2: Effect of different Concentration of aqueous and alcoholic extracts of *Aloe vera* and Antibiotic on inhibition (mm) of *Staphylococcus aureus* using disc method at three days inculation.

3. Four days post inoculation

The results depicted in (Table 3 and Figure 3) indicate that the high concentrations of *Aloe vera* aqueous extract (100%) used in this study had the highest inhibitory effects (16 mm) against the tested microorganisms. However, this extract showed inhibition action of 9 mm even at minimal concentration (25%) used in this study. The other concentrations of the aqueous phase (50 %) gave an inhibition zones of 11.5 mm.

Control (Gentomycin) extract of *Aloe vera* at all concentrations shows an inhibitory effect against the *Staphylococcus aureus.* The highest inhibition zone obtained was 14 mm with the concentration of 100 % Concentrations of 25 and 50 % showed an inhibition zones of 4.5 and 4.5 mm, respectively.

Table 3: Effect of different Concentration of aqueous and alcoholic extracts of *Aloe vera* and Antibiotic on inhibition (mm) of *Staphylococcus aureus* using disc method at four days post inoculation.

Treatments	Concentration%	Inhibition zones(mm)			Mean
		R1	R2	R3	
Aqueous	**25**	**9**	**10**	**8**	9
	50	**12.8**	**12**	**11**	11.5
	100	**17**	**16**	**16**	16
Gentamycin	**25**	**3.5**	**5**	**4**	4.5
	50	**7**	**5**	**4**	4.5
	100	15	13	15	14

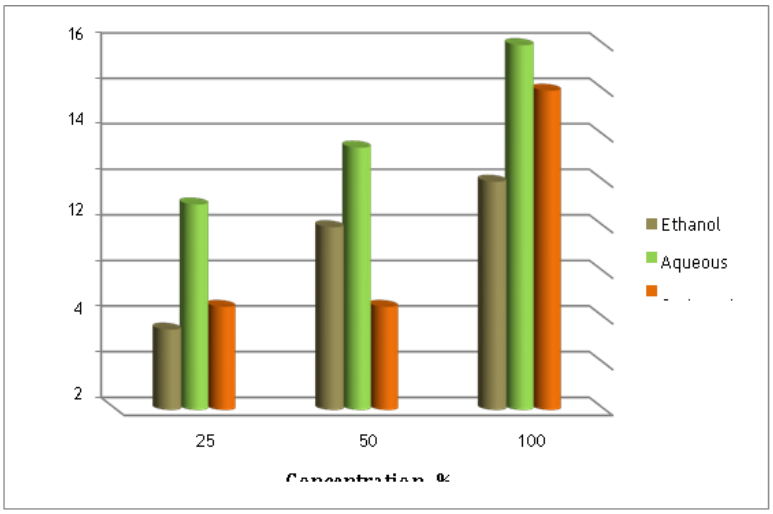

Figure 3: Effect of different Concentration of aqueous and alcoholic extracts of *Aloe vera* and Antibiotic on inhibition (mm) of *Staphylococcus aureus* using disc method at four days post inoculation.

Table 4: Antimicrobial activity of different Concentration preparations of *Aloe vera* gel aqueous and alcoholic extract on inhibition (mm) of *Staphylococcus aureus* with antibiotics as reference standard.

Treatments	Concentration%	Inhibition zones(mm)
Aqueous	25	+
	50	++
	100	++
Gentamycin	25	+
	50	++
	100	++

Antimicrobial activity: No inhibition +, Zone of inhibition ≤8mm in diameter; ++, Zone of inhibition > 8mm in diameter.

DISCUSSION

This study showed that aqueous exstract phase of *Aloe vera* gave better results compared to the antibiotic phase of the same extract at this study at all concentration 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**.** Other workers have reported There was no antimicrobial activity was reported using aqueous extract of *A. vera* leaves (Martineze *et al*.,1996).

The antimicrobial activity of the extracts and their potency was quantitatively assessed by the presence or absence of inhibition zone and zone diameter. Only alcoholic extract was found to be a better solvent for extraction of antimicrobially active substances compared to water and hexane (Ahmad *et al*., 1998).

(Subramanian *et al*., 2006) In other studies, the most effective antibiotic for gram positive is vancomycin than Gentamycin (Hoeger., 2004**).**

Aloe vera is a potent antimicrobial agent compared with the conventional antibiotics. The results of the study by Coopoosamy and Magwa., (2007) also revealed that lowest concentrations of ethyl acetate and ethanol crude extracts of Aloe excels resulted in complete inhibition of visible growth of pathogenic bacteria compared with the control antibiotics, chloramphenicol and streptomycin sulfate.

The results of this study disagree with earlier studies that showed that the antibacterial activity of some Iranian medicinal plants were more significant in the solvent extracts compared with aqueous extracts in all the plants, indicating that the active principle(s) responsible for antibacterial activity were more soluble in organic solvents (Babu *et al*., 2007). Similarly, the solvent extract of cloves flower was also found to exhibit a significantly higher inhibitory effect on the caries-inducing properties of *Streptococcus mutans* compared with the crude aqueous extracts (Abd Rahim and Gulam.,2006). In other studies the methanolic and petroleum spirit extracts of Pelargonium essential oils were more potent antibacterial agents than the steam distilled volatile samples (Lis- Balchin *et al.*,1998).

Finally, it was observed that the highest concentration of the aqueous and ethanolic extract of the plant has significant effect on the bactria isolates. They had liger zone of inhibition compared to the antibacterial agents used as control. Similar result was reported by Adeleke

et al. (2006).

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