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

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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 alcoholic extracts of *Aloe vera* and the Gentamycin, (25, 50 and 100%) were tested. The alcoholic 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 different concentrations of alcoholic extract of *Aloe vera* at all its concentrations showed an inhibitory effect against the *Staphylococcus aureus but* The highest inhibition zone did not exceed 14.5 mm at the higher concentration (100 %). However, the other concentrations (25 and 50 %) showed inhibition zones of 5.9 and 9.4 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

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

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

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 Jaunary ,2018.

Aloe vera plant

Were obtained from the University of Gezira fields during February to Jaunary, 2018.

Methods

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 Gentamycin was dissolved in distilled water. Further dilutions were made using the same solvent according to CLSI document M100-S18. Gentamycin 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

Ethanol extract of *Aloe vera* at all concentrations shows an inhibitory effect against the *Staphylococcus aureus.* The highest inhibition zone obtained was 14.5 mm with the concentration of 100 % Concentrations of 25 and 50 % showed an inhibition zones of 5.9 and 9.4 mm, respectively. 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 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
Gentamycin	25	6.5	8.5	7	7.75
	50	9	7	5	6
	100	17	15	18	16.5

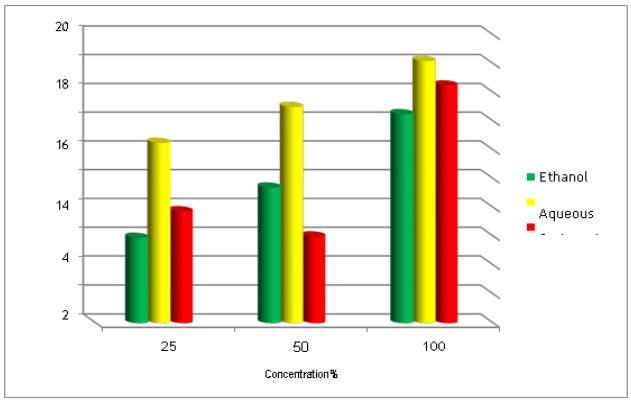

Figure 1: Effect of different Concentration of alcoholic extracts of *Aloe vera* and Antibiotic on inhibition (mm) of *Staphylococcus aureus* using disc method at two days post inoculation.

2. Three days post inoculation

The results depicted in Tabl (2) and Figure (2) indicate that the Ethanol extract of *Aloe vera* at all concentrations shows an inhibitory effect against the *Staphylococcus aureus.* The highest inhibition zone obtained was 12.5 mm with the concentration of 100 % Concentrations of 25 and 50 % showed an inhibition zones of 5 and 9 mm, respectively. 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 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	
Ethanol	25	7	5	5	5
	50	9	9	9	9
	100	14	13	12	12.5
Gentamycin	25	5	7.3	6.6	5.95
	50	8	6	5	5
	100	16	15	17	16

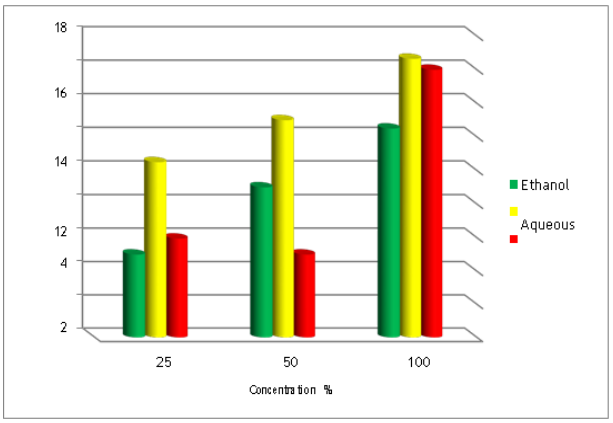

Figure 2: Effect of different Concentration of aqueous and alcoholic extracts of *Aloe vera* and Antibiotic on inhibition (mm) of *Staphylococcus aureus* using disc method at three days inculation.

3. Four days post inoculation

The results depicted in (Table 3 and Figure 3) indicate that the Ethanol extract of *Aloe vera* at all concentrations shows an inhibitory effect against the *Staphylococcus aureus.* The highest inhibition zone obtained was 10 mm with the concentration of 100% Concentrations of 25 and 50 % showed an inhibition zone of 3.5 and 8 mm, respectively.

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 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	
Ethanol	25	5	3	4	3.5
	50	8	9	7	8
	100	12	10	10	10
Gentamycin	25	3.5	5	4	4.5
	50	7	5	4	4.5
	100	15	13	15	14

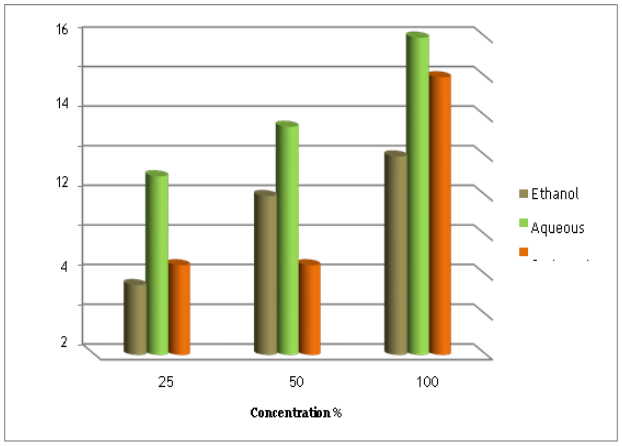

Figure 3: Effect of different Concentration of aqueous and alcoholic extracts of *Aloe vera* and Antibiotic on inhibition (mm) of *Staphylococcus aureus* using disc method at four days post inoculation.

DISCUSSION

This study showed that aqueous exstract phase of *Aloe vera* gave better results compared to the ethanolic and antibiotic phase of the same extract at this study at all concentration tested .

The ethanolic extract shows lower action compared to the aqueous and antibiotic extract (resuspension) as antimicrobial agents. This may be due to little diffusion properties of the extract in the agar or because fresh plants contain active substances which may be affected or attributed by the used solvent.

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).

The results of the antibacterial activity of different concentration preparations of *Aloe vera* gel compared with antibiotics, Gentamycin. 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. (Subramanian *et al*., 2006) In other studies, the most effective antibiotic for gram positive is Vancomycin than Gentamycin (Hoeger., 2004). also observed remarkable antibacterial activities with ethanolic extracts of *Aloe vera* gel even at low concentrations compared with the standard antibiotics and support the view. *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 other experiment conducted with petroleum ether extract exhibited significant antibacterial activity against *Pseudomonas aeruginosa*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Escherichia coli* and moderate activity against *Staphylococcus aureus* and *Bacillus subtilis*. The chloroform, methanol and ethanol extracts exhibited moderate antibacterial activity against all the seven types of bacteria. The aqueous extract exhibited least antibacterial activity against all the seven types of bacteria (Gavimath *et al*., 2008).

Their study results showed that the methanol gel extract preparation had stronger retardation effect on gram positive test organisms (*S. aureus,* and *S.epidermidis*) as compared to the gram negative bacteria (*E. coli, K. pneumonia, P. aeruginosa, P. vulgaris and P mirabilis*). Similar results were documented, in an earlier study, (Agarry and Olaleye., 2005). where the gram-positive test organisms were found to be more susceptible to the sterile *Aloe vera* gel preparation and the antimicrobial susceptibility testing of *Aloe vera* gel has a greatest inhibitory effect on the *S. aureus* with 18.0 mm diameter of zone of inhibition. Results of the present research also correlates with the earlier findings by Kaithwas *et al.(*2008) as well as studies conducted by Mangena.(1999) where it was demonstrated that the *Aloe vera* gel being rich in a wide variety of secondary metabolites, such as polysaccharides, anthraquinone glycosides, glycoproteins, gamma-linolenic acid, prostaglandins which was found to be very effective against Gram positive in particular against *S. aureus*

The ethanol and aqueous extracts were active in inhibiting the growth of *Escherichia coli Staphylococcus aureus and Candida albicans* though *Candida albicans* had the least zone of inhibition. This result conformed to the result of investigators on similar studies such as Johnson *et al*., (2012; Joshua *et al.(*2010). On other study, it was observed that the ethanolic extracts had a significantly higher antimicrobial activity than the aqueous extract this difference is attributed to the solubility of the active component in different solvents. (Karou *et al.* (2007), This result disagree the findings of Anani *et al*. (2000), It was observed that different isolates exhibited varying degree of resistance to the ethanolic extract of the *Sisda acuta*. This result did not supports the findings of Anani *et al*.(2000), who noted that methanolic extract of *Sida acuta* had a significant activity on *S. aureus*, *E. coli*, *B. subtilis* and *Mycobacterium phlei* and against no inhibition effect recorded on *Streptococcus faecalis and Klebsiella pneumoniae*. Similar results were obtained by Rajakaruna *et al*. (2002), Saganuwan and Gulumbe (2006) with methanolic extract of *Sida acuta*. This difference in susceptibility can be attributed to two factors, the inherent resistant factor of the different species of the isolates and the previous exposure of the organism to other antimicrobial drugs.

Although the ethanolic extracts produced some inhibitory effects on the clinical isolates, the aqueous extracts were observed to produce high inhibitory effects. This confirmed the report of Okwu and Josiah., (2006) who reported that the aqueous best solvent for extraction over ethanol when working with plants of medicinal importance.

Finally, it was observed that the highest concentration of the aqueous and ethanolic extract of the plant has significant effect on the bacteria 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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