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Research Article

*IN-VITRO*ANTIBACTERIAL EFFICACY OF *ALOE VERA* (*ALOE BARBADENSIS* MILLER, 1768) LEAF AND GEL IN DIFFERENT EXTRACTS AGAINST SOME SELECTED PATHOGENIC BACTERIA

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Abstract

*Aloe vera*leaf and gel is widely used as a traditional folk medicine for the treatment of different infectious diseases. The present study is to investigate the antibacterial efficacy of *A. vera*leaf and gel in different extracts like aqueous, chloroform and ethanol. They were tested against four Gram positive bacteria*Stapylococcusaureus*,*Staphylococcus epidermidis*, *Bacillus cereus* and *Bacillus subtilis* and four Gram negative bacteria*Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Salmonella typhi*.The susceptibility of the bacterial organisms to the three different extracts of *A. vera*leaf and gel revealed the ethanol extract of *A. vera*gel to possesses maximum growth inhibition onGram positive bacterium (*B. subtilis)*, than on the Gram negative bacteria.Hence, the present studysuggeststheethanol extract of *A.vera*gel to be used as an alternate antibacterial agent.

Keywords: *Aloe vera,* antibacterial activity, Gram positive and negative bacteria.

Introduction

Number of research investigations have demonstrated the antimicrobial efficacy of several constituents of higher plants (Rocio and Rion, 1982 andHabtemarian*et al.,* 1993).Active biological compounds present in the medicinal plants have always been of great interest to scientists to evaluate their antibacterial activity (Clark and Hufford, 1993). The best known traditional medicinal plant*A. vera*(*Aloe barbadensis*Miller) has legendary medicinal reputation dating back to thousands of years ago. It is one of the Ayurvedic medicinal plants that have been widely used to conserve human health. *A. vera* is also an "ornamental plantations" at every corner of the towns and villages. Conflicting reportsare also available on the therapeutic potentials of *A. vera* (Schmidt and Greenspoon, 1993), its toxicity, especially when used for both topically and parenterally (Balter, 1992 and Brusick and Menge, 1997).

*A.vera*is a member of Asphodelaceae(Lilliaceae) family (Basmatker*et al*., 2011).Itis a perennial succulent plant (Dinesh *et al*., 2012 and Ramesh *et al*., 2012) that is cactus like in its characteristics (Tyler, 1993). The plant has a long history as a multipurpose folk remedy (Reynolds and Dweck, 1999) and has been associated with myth, magic and medicine since pre-biblical times (Balter, 1992). The name *A. vera* was derived from the Arabic word "alloeh" meaning "shining" and "bitter" (Tyler *et al.,* 1976; Robbers *et al*., 1996;Adesuyi*et al*., 2012 and Ramesh *et al*., 2012) because of the bitter liquid found in the leaves. Antimicrobial activity of *A. vera* leaf and gel were reported to effectively kill or greatly reduce or eliminate the growth of *Staphylococcus aureus, Klebsiellapneumoniae, Streptococcus pyogenes, Pseudomonas aeruginosa, Escherichia coli* and *Salmonella typhi* (Reynolds and Dweck, 1999; Urch, 1999; Lawless and Allan, 2000 and Pugh *et al.,* 2001). Whole leaf components are proposed to have direct antibacterial properties includinganthraquinones and saponins (Reynolds and Dweck, 1999 andUrch, 1999).Polysaccharides of *A. vera*have been attributed with direct bacterial activity by stimulating phagocytic leucocytes to destroy bacteria (Lawless and Allan, 2000 and Pugh *et al.,* 2001). Due to the increasing development of antibiotic resistance, the emphasis of the present study is to suggest *A. vera* as an alternate natural remedy for the inhibition of variousbacterial infections.

Castillo *et al.* (2005) evaluated the inhibitory effect of the pulp and the liquid fraction of *A. vera*against the plant pathogenic fungi and other microorganisms affecting humans. The efficacy of the *A. vera* gel as an antimicrobial agent is shown to have a wide range of activity against Gram positive and Gram negative bacteria. Topical application of the gel has been effective in the treatment of various skin diseases including burns, cuts, eczema (Arunkumar and Muthuselvam, 2009) and alleviates. Oral administration has been found to be effective against stomach and intestinal disorder (Lawrence *et al.,* 2009). It accelerates the healing of mouth ulcer and aid the growth of new tissues. *A. vera* also has the ability to stimulate microphages (Davis, 1997). In the present investigation, the antibacterial efficacy of *A. vera* leaf and gel was screened on few selected bacterial species.

Materials and Methods

Collection of *A. vera* leaf

A. vera fresh leaves were collected in Thiruvallur District and authenticated by Dr. A. Manoharan, Associate Professor and Head of the Department of Plant Biology and Biotechnology, Presidency College, Chennai – 5.

Bacterial species used

Four species of Gram positive bacteria*Staphylococcus aureus*(ATCC6538), *Staphlococcusepidermidis*(ATCC 155),*Bacillus cereus* (ATCC 11778) and *Bacillus subtilis*(ATCC 6633) and four species of Gram negative bacteria *Escherichia coli* (ATCC 8739), *Klebsiella pneumonia* (ATCC 29665),*Pseudomonas aeruginosa*(ATCC 9027) and *Salmonella typhi*(ATCC 6539) were obtained from authorized laboratories and maintained in sterile growth medium.

Preparation of extracts

A. vera leaf and gel were extracted in different solvents like aqueous, chloroform and ethanol. After the extraction period, the extracts were filtered and concentrated in a water bath. These extracts were further used for the screening of antibacterial efficacy.

***In vitro* evaluation of antibacterial activity of *Aloe vera*leaf and gel**

In the present investigation, agar diffusion method (Agarry*et al.,* 2005) was used to evaluate the antibacterial activity of the *A. vera*leaf and gel extracts *in vitro.*

Standard drug used for antibacterial activity

Ciprofloxacin with a broad spectrum of antibiotic effect was used as the standard drug for the inhibition of bacterial activity.

Preparation of medium and sterilization

Ten grams of beef extract, 10gms of peptone, 5gms of sodium chloride and 1gm of agar were dissolved in 1 litre of water, to prepare the medium for diffusion.

Dissolution of the ingredients were aided with heat. The pH was adjusted to 8.0 to 8.4 with 5M sodium hydroxide and the medium was boiled for 10minutes. It was then filtered and sterilized by maintaining it at 115ºC for 30minutes in an autoclave. The pH was finally adjusted to 7.3±0.1.

Agar diffusion method

A suspension of Gram positive and negative organisms were separately added to the sterile nutrient agar medium at 45ºC. This mixture was transfered to sterile petridishes and allowed to solidify. Sterile discs of 5mm diameter (made from Whatman filter paper previously sterilized in U.V. lamp) were dipped in different concentrations of *A. vera* leaf (100, 150 and 200 µl) and *A. vera* gel (50, 100 and 150 µl) extracts. They were placed on the surface of agar plates. These plates were allowed to stand for 1hour at room temperature as a period of preincubation diffusion to minimize the effects of variation in time between the applications of different solutions. Then the plates were incubated for

24hours at 37±1ºC and observed for the antibacterial activity. The zone of inhibition diameter was observed and recorded.

Results and Discussions

The present investigation records the antibacterial efficacy of *A. vera* leaf and gel extractsagainst few selected Gram positive and negative pathogens. However, the degree of inhibition varied depending upon the concentration of the extracts. Hence, in the present investigation a common concentration of *A. vera* leaf and gel (150 µl) was selected and its efficacy against four Gram positive and negative organisms were discussed.*A. vera* gel extract exhibited maximum zone of inhibition against bacteria in comparison with *A. vera* leaf extract indicating the greater efficacy of *A. vera* gel than the leaf extract.

The results of the present investigation revealed the chloroform extract of *A. vera* leaf to possess maximal growth inhibition than the aqueous and ethanol extracts.*A. vera*was reported to act as an antiseptic agent against anumber of bacteria and fungi, like *Staphylococci, Streptococci*and *Candida*(Steinegger and Hansel, 1988 and Duke, 1997).Most probably the bioactive compounds present inthe extracts of *A. vera* leaf and gel are responsible for the inhibitory activity on bacterial species. As most of the antimicrobial properties of *A. vera* were depended upon the amount of the phytochemical compounds present in it, the phytochemical compounds of *A. vera* were analysed and reported (Tamilarasi, 2014).*A. vera* is rich in tannins, alkaloids, triterpenoids, flavonoids, saponins, acids and phenol. Each of these compounds has its own amazing effects on humans and other organisms. Most probably these bioactive compounds of*A. vera* might be responsible for the inhibition of microorganisms.

Antibacterial efficacy of *Aloe vera* leaf and gel in different extracts against selected bacterial organisms

(i)Antibacterial activity of *Aloe vera* leaf aqueous extract on selected microbes

Gram positive organisms

In the present investigation, the aqueous extracts of *A. vera*leaf (150µl) inhibited the growth of Gram positive bacteria like *Staphylococcus aureus, Staphylococcus epidermidis,Bacillus cereus* and *Bacillus subtilis*, in a wide range (42 to 55%). Among these bacterial organisms, the maximum zone of inhibition was observed with *B. cereus* (55%) and the minimum zone of inhibition was observed with *Bacillus subtilis*(42%). However, the other Gram positive organisms such as *S. aureus* (50%) and *S. epidermidis* (51%) were moderately inhibited by the aqueous extract of *A. vera* leaf.

Gram negative organisms

The inhibitory effect of *A. vera*leaf aqueous extract was observed on the Gram negative bacteria which ranged from 38 to 50% against *Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi*and*Klebsiellapneumoniae*. The maximum zone of inhibition was observed with *K. pneumonia*e (50%) and the minimum zone of inhibition with *P. aeruginosa*(38%). The other two microbes *E. coli* (39%) and *S. typhi* (41%) were moderately inhibited by the aqueous extract of *A. vera* leaf.

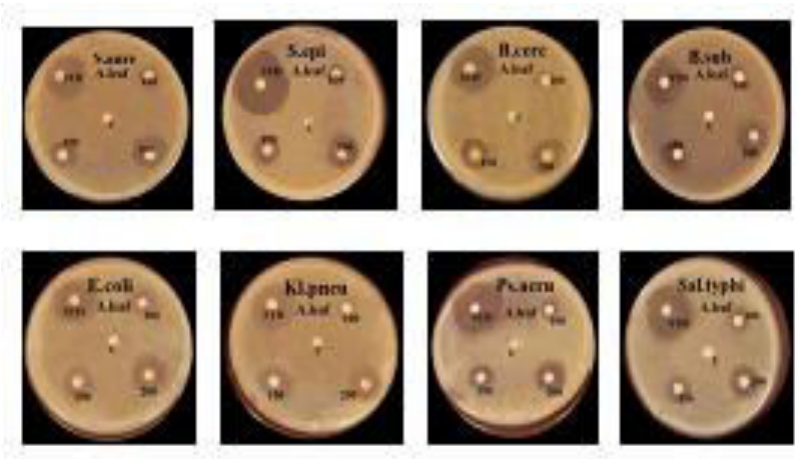
It can be observed that the Gram positive bacteria when exposed to *A. vera* leaf aqueous extract they were effectively inhibited than the Gram negative bacteria (Table 1 and Plate 1).

Table-1: Screening of*a*ntibacterial activity of *aloe vera*leaf aqueous extract.

			Zone of inhibition in mm		
Sl. No	**Organisms**	**STD**	**a100 µl**	**150 µl**	**200 µl**
	Gram positive bacteria				
1	*Staphylococcus aureus*	38	14 (37)**b**	19 (50)	23 (61)
2	*Staphylococcus epidermidis*	39	15 (38)	20 (51)	24 (62)
3	*Bacillus cereus*	38	16 (42)	21 (55)	25 (66)
4	*Bacillus subtilis*	38	13 (34)	16 (42)	20 (53)
	Gram negative bacteria				
5	*Escherichia coli*	38	12 (32)	15 (39)	22 (58)
6	*Klebsiellapneumoniae*	38	11(29)	19 (50)	21(55)
7	*Pseudomonas aeruginosa*	39	12(31)	15(38)	20(51)
8	*Salmonella typhi*	39	13(33)	16(41)	19(49)

a) Concentrations of extractb) Values in parentheses indicates the percentage of inhibition

Plate-1: Gram positive and negative bacterial organisms exhibiting sensitivity to aqueous extract of*A. Vera*leaf.



(ii)Antibacterial activity of *Aloe vera* gel aqueous extract on selected microbes

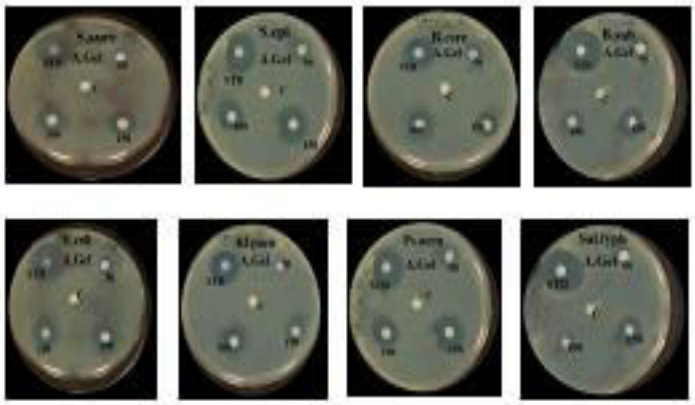
The results revealed that the Gram negative bacteria were more resistant to the aqueous extract of *A. vera*gel than the Gram positive bacteria (Table 2 and Plate 2).

Table-2: Screening ofantibacterial activity of *aloe vera* gel aqueous extract.

			Zone of inhibition in mm		
Sl. No	**Organisms**	**STD**	**a50 µl**	**100 µl**	**150 µl**
	Gram positive bacteria				
1	*Staphylococcus aureus*	38	11 (29)**b**	18 (47)	24 (63)
2	*Staphylococcus epidermidis*	38	15 (39)	19 (50)	22 (58)
3	*Bacillus cereus*	39	14 (36)	20 (51)	25 (64)
4	*Bacillus subtilis*	39	16(41)	21 (54)	26 (67)
	Gram negative bacteria				
5	*Escherichia coli*	38	13 (34)	18 (47)	24 (63)
6	*Klebsiella pneumonia*	38	11(29)	16(42)	20(53)
7	*Pseudomonas aeruginosa*	39	14(36)	21(54)	25(66)
8	*Salmonella typhi*	39	11(28)	14(36)	19(50)

a) Concentrations of extract b) Values in parentheses indicates the percentage of inhibition

Plate-2: Gram positive and negative bacterial organisms exhibiting sensitivity to aqueous extract of *a.vera* gel.



(iii)Antibacterial activity of *Aloe vera* leaf chloroform extract on selected microbes

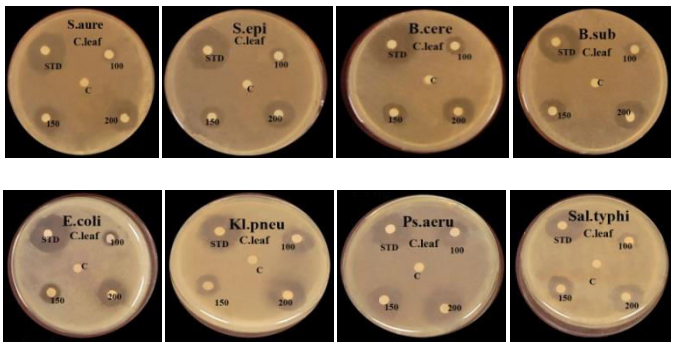
The chloroform extract of *A. vera*leaf showed maximum efficacy on the Gram negative bacteria than the Gram positive bacteria (Table 3 and Plate 3).

Table-3: Screening of antibacterial activity of *aloe vera*leaf chloroform extract.

			Zone of inhibition in mm		
Sl. No	**Organisms**	**STD**	**a100 µl**	**150 µl**	**200 µl**
	Gram positive bacteria				
1	*Staphylococcus aureus*	38	10(26)**b**	14(37)	20(53)
2	*Staphylococcus epidermidis*	38	11(29)	14(37)	21(55)
3	*Bacillus cereus*	39	13(33)	16(41)	23(59)
4	*Bacillus subtilis*	39	12(31)	16(41)	24(62)
	Gram negative bacteria				
5	*Escherichia coli*	39	12 (31)	16(41)	20(51)
6	*Klebsiella pneumonia*	38	16(42)	20(53)	23(61)
7	*Pseudomonas aeruginosa*	38	18(47)	22(58)	24(63)
8	*Salmonella typhi*	39	12(31)	16(41)	21(54)

a) Concentrations of extract b) Values in parentheses indicates the percentage of inhibition

Plate-3: Gram positive and negative bacterial organisms exhibiting sensitivity to chloroform extract of *a.vera* leaf.



(iv)Antibacterial activity of *Aloe vera* gel chloroform extract on selected microbes

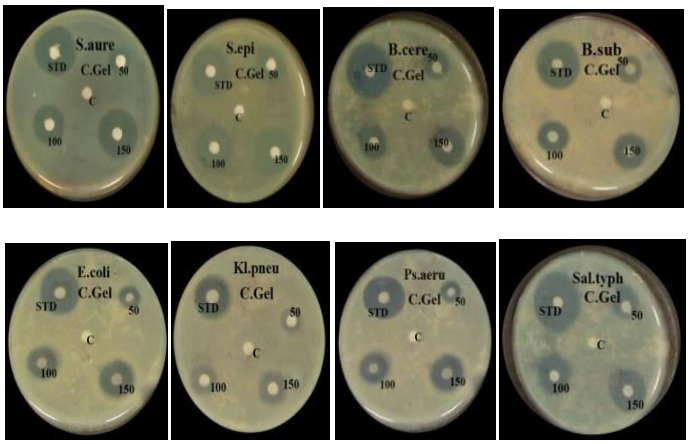
In the present investigation, the Gram positive bacteria were more sensitive to the chloroform extract of *A. vera* gel in contrast to the leaf extracts of *A. vera*(Table 4 and Plate 4).

Table-4: Screening of antibacterial activity of *aloe vera*gel chloroform extract.

		Zone of inhibition in mm			
Sl. No	**Organisms**	**STD**	**a50 µl**	**100 µl**	**150 µl**
	Gram positive bacteria				
1	*Staphylococcus aureus*	38	15(39)**b**	21(55)	25(66)
2	*Staphylococcus epidermidis*	38	20(51)	24(62)	28(72)
3	*Bacillus cereus*	39	19(49)	22(56)	27(69)
4	*Bacillus subtilis*	38	18(47)	23(61)	26(68)
	Gram negative bacteria				
5	*Escherichia coli*	39	16(41)	23(59)	23(67)
6	*Klebsiella pneumonia*	38	15(39)	20(53)	23(61)
7	*Pseudomonas aeruginosa*	38	16(42)	23(61)	28 (74)
8	*Salmonella typhi*	39	17(46)	24(66)	29(74)

a) Concentrations of extract b) Values in parentheses indicates the percentage of inhibition

Plate-4: Gram positive and negative bacterial organisms exhibiting sensitivity to chloroform extract of *a.vera* gel.



(v)Antibacterial activity of *Aloe vera* leaf ethanol extract on selected microbes

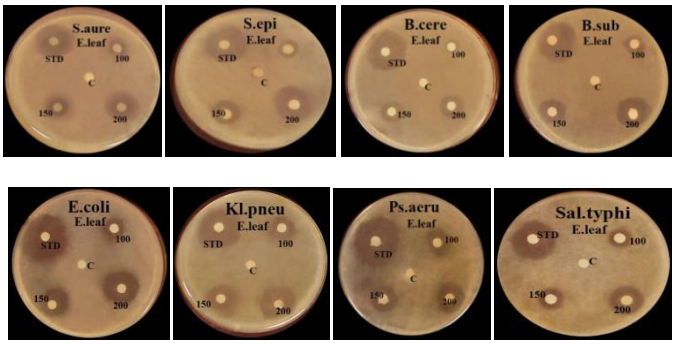
In the present investigation, like chloroform extract, the ethanolic extract of *A. vera*leaves showed a similar pattern of inhibition against Gram positive and Gram negative bacteria. It is evident from the present investigation that the Gram negative bacteria were more sensitive to *A. vera* leaf ethanol extract (Table 5 and Plate 5).

Table-5: Screening of antibacterial activity of *aloe vera*leaf ethanol extract

		Zone of inhibition in mm			
Sl. No	**Organisms**	**STD**	**a100 µl**	**150 µl**	**200 µl**
	Gram positive bacteria				
1	*Staphylococcus aureus*	38	11(29)**b**	13(34)	17(45)
2	*Staphylococcus epidermidis*	39	12(31)	15(38)	21(54)
3	*Bacillus cereus*	39	10(26)	14(36)	17(44)
4	*Bacillus subtilis*	39	13(33)	15(39)	22(57)
	Gram negative bacteria				
5	*Escherichia coli*	39	12 (31)	16(46)	22(56)
6	*Klebsiella pneumonia*	38	14(37)	16(42)	23(61)
7	*Pseudomonas aeruginosa*	39	15(39)	19(49)	22(56)
8	*Salmonella typhi*	38	13(34)	15(40)	20(53)

a) Concentrations of extractb) Values in parentheses indicates the percentage of inhibition

Plate-5: Gram positive and negative bacterial organisms exhibiting sensitivity to ethanol extract of *a.vera* leaf.



(vi)Antibacterial activity of *Aloe vera* gel ethanol extract on selected microbes

Among the Gram positive bacteria, *B. subtilis* (76%) was found to be maximally inhibited, which was more sensitive than the other bacterial organisms(Table 6 and Plate 6).

However, the antibacterial activity of *A. vera* gel extracts registered major differences with reference to the Gram positive and Gram negative organisms.

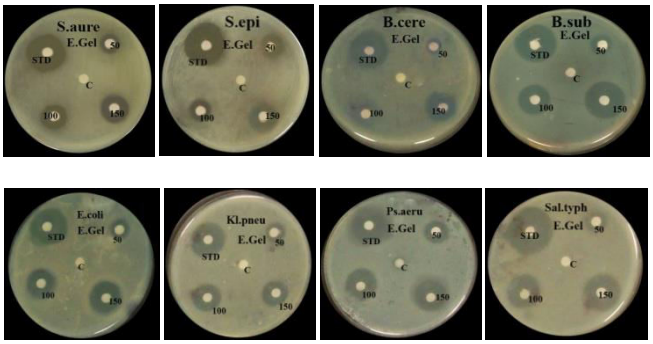
Subramanian *et al*. (2006) reported *A. vera* gel extract to posses compounds with antimicrobial properties which can be used as an antimicrobial agents in new drugs for the therapy of infectious diseases in humans.

Table-6: Screening of antibacterial activity of *aloe vera* gel ethanol extract.

		Zone of inhibition in mm			
Sl.no	**Organisms**	**STD**	**a50 µl**	**100 µl**	**150 µl**
	Gram positive bacteria				
1	*Staphylococcus aureus*	38	14(37)**b**	19(50)	23(61)
2	*Staphylococcus epidermidis*	39	15(39)	18(46)	24(62)
3	*Bacillus cereus*	38	13(34)	16(42)	23(61)
4	*Bacillus subtilis*	38	13(34)	23(61)	29(76)
	Gram negative bacteria				
5	*Escherichia coli*	39	15 (38)	21(54)	27(69)
6	*Klebsiella pneumonia*	38	16(42)	22(58)	25(66)
7	*Pseudomonas aeruginosa*	38	17(45)	21(55)	26(68)
8	*Salmonella typhi*	39	15(38)	24(62)	29(74)

a) Concentrations of extract b)Values in parentheses indicates the percentage of inhibition

Plate-6: Gram positive and negative bacterial organisms exhibiting sensitivity to ethanol extract of *a.vera* gel.



Previous studies revealed the antimicrobial activities of *A. vera* gel to kill or to greatly reduce the growth of *S. aureus*, *K. pneumoniae, S. pyogenes*, *P. aureginosa*, *E. coli* and*S. typhi* (Reynolds and Dweck, 1999; Urch, 1999; Lawless and Allan 2000 and Pugh *et al.,* 2001). Kadarnath*et al.,* 2012, also reported the capability of *A. vera*gel extract in inhibiting the growth of *Streptococcus* and *Klebsiella* species *in vitro*.

Studies of Fani and Kohanteb (2012), prove *A. vera*gel extract to exert strong bactericidal activity against both carcinogenic and periodontopathic bacteria.

Aloin, a bitter – tasting yellow compound of anthraquinone which is a c-glycoside derivative and a major active compound of *Aloe* plants, because of its polyphenolic structure inhibit protein synthesis of bacterial cells which could be the reason for the antimicrobial activity of *A. vera*(Saccu*et al*., 2001).

Thus, the present investigation recordsthe antibacterial activity of*A. vera* leaf and gel against the Gram positive and negative organisms. It is reported that the antibacterial activity of *A. vera*gel on Gram positive organisms is

significant and the percentage of efficacy can be arranged in the order of ethanol, chloroform followed by the aqueous extract of *A.vera*inspite of individual variation.However, the antibacterial activity of *A. vera* gel extracts registered major differences among them, with reference to the Gram positive and Gram negative organisms than the *A. vera*leaf extracts. Hence, the results of the present investigation registered the *A. vera* gel extracts to be more effective than *A. vera* leaf extracts.

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