COMPARATIVE ANTIMICROBIAL ACTIVITY OF ALOE VERA GEL ON MICROORGANISMS OF PUBLIC HEALTH SIGNIFICANCE

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Summary

The comparative antimicrobial activity of different preparations of Aloe vera gel (fresh gel, preserved gel, cooling gel and acne cream) was studied against a number of microorganisms of public health significance by disc diffusion method. It was found that the fresh gel and preserved gel exhibited maximum zones of inhibition against *Bacillus subtilis* (24.7 & 34.5 respectively), where as cooling gel and acne cream against *Staphylococcus aureus* (30.3 & 26.3mm respectively) at 37^{o}C and similarly, minimum inhibition zones by all four preparations of Aloe vera gel were shown against *Aspergillus ficuum* (9.5, 15.5, 10.5 and 9.5mm respectively) after a period of 48h of incubation at 25^{o}C. However, its various preparations exhibited variable toxicity against one or more tested strains of *Aspergillus, Fusarium, Penicillium, Candida, Escherichia, Salmonella, Proteus, Staphylococcus* and *Bacillus*.

It was found that *Salmonella typhimurium and Bacillus cereus* showed a minimum 1.0% decrease from 48 to 96h in zones of inhibition whereas *Escherichia coli exhibited a* maximum decrease of 29.6% against acne cream after a period of 96h of incubation. It was concluded that the cumulative mean value of *Aspergillus niger, Aspergillus ficuum, Fusarium. solani, Penicillium digitatum, Candida utilus, Escherichia coli, Salmonella typhimurium, Proteus vulgaris, Staphylococcus aureus, Bacillus subtilis* and *Bacillus cereus* resulted in 14.25(I), 11.25(I), 15.66(S), 13.55(I), 20.28(S), 19.45(S), 13.37(I), 11.25(I), 26.22(S), 27.30(S) and 25.95(S) against fresh gel, preserved gel, cooling gel and acne cream respectively. The results of this study tend to give credence to the popular use of different preparations of Aloe vera gel as effective as modern medicine to combat pathogenic microorganisms.

Key words: Aloe vera, Fresh gel, Preserved gel, Cooling gel, Acne cream, Antimicrobial

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Introduction

Diseases due to pathogenic bacteria and fungi represent a critical problem to human health and they are one of the main causes of morbidity and mortality world wide [(1)]. The evolution of multiple drug resistant human pathogenic microorganisms has driven the search for new sources of antimicrobial substances, including plant metabolites [(2)]. Thus, the investigation of the efficacy of plant-based drugs in traditional medicine has been paid great attention because these drugs elicit few side effects, cheap and easily available, according to World Health Organization, 80% of the world population still relies mainly on plant drug [(3)].

Aloe, is a genus containing about four hundred species of flowering succulent plants belonging to *Lileaceae* family [(4)]. Aloe vera is a typical xerophyte with thick fleshy, strangely cuticularized spiny leaves. It has been promoted for large variety of conditions and has come to play a prominent role as a contemporary folk remedy [(5)]. True Aloe vera plant is called as *Aloe barbadensis Miller.* The plant contains 99% water and 1-0.5% solid matter at pH 4.5. The mucilaginous jelly from the parenchyma cells of the peeled, spineless leaves of the plant is referred as Aloe vera gel. The gel is a watery-thin, viscous, colorless liquid that contains anthraquinone glycosides, glycoprotein, gamma-lanoline acid, prostaglandins and mucopolysaccharides that are mainly responsible for the antibacterial, antifungal as well as its antiviral activity [(6)].

Aloe vera gel has been used since early times for the tropical treatment of burns and wounds. Studies have speculated that it is the glycoprotein fraction of the Aloe vera plant that is involved in wound healing effect [(7)]. The gel moisturizes the skin because of its water holding capacity and also stimulates cell growth thus enhancing the restoration of the damaged skin. Aloe gel is perhaps the most widely recognized herbal remedy in the United States today, it is used to relieve thermal burn, sunburn and promote wound healing [(8)]. In addition, research suggests that Aloe gel can help to stimulate the body's immune system [(9)]. Many studies have revealed the presence of many biologically active phytochemicals in the ethanolic extracts of Aloe vera gel [(10)], which may be responsible for its hypoglycemic and anti-oxidant properties [(11)]. Therefore, the present study was conducted to evaluate the anti-microbial activity of different preparations of Aloe vera gel such as fresh gel, preserved gel, cooling gel and acne cream against a number of microorganisms of public health significance.

Materials and Methods

The outermost whorls of leaves of mature Aloe vera plant of age almost four years were collected and washed with distilled water. The leaves were sliced across the width with a sharp knife as filleting a fish. The inner exposed surfaces revealed a transparent gooey-gel without the addition of sap. The gel was heated at 80^{o}C for 15 min. in a temperature controlled water bath followed by vacuum filtration to remove the fiber contents. Then filtrate was treated with activated carbon in order to remove aloin and at the same time improving its color and smell. Again the gel was heated to 50^{o}C for 10 min. and on

cooling the pH was adjusted to 4.5 with citric acid for its preservation. The different preparations such as fresh gel, preserved gel: (Methylparahydroxyl benzoate and Propylparahydroxyl benzoate 2%), acne cream: (4% bee wax) and cooling gel: (6% ethanol) were prepared and evaluated against a number of pathogens of public health significance [(12)].

Disc diffusion method [(13)] was used to detect the anti-microbial activity of different preparations of Aloe vera gel against a number of pathogens including bacteria (*Escherichia coli, Salmonella typhimurium, Proteus vulgaris, Staphylococcus aureus, Bacillus subtilis, Bacillus cereus*), fungi (*Aspergillus niger, Aspergillus ficuum, Fusarium solani, Penicillium digitatum*) and yeast (*Candida utilus*). Similarly discs impregnated with suitable concentrations of standard commercial antibiotics as described by Matsen [(14)] were also prepared and used for susceptibility test. Standard culture media (CM139, CM-69, CM201, CM7, CM145 and CM271) from Oxoid, UK, were employed throughout the present investigation for the purpose of culture maintained at their respective temperatures that is 25^{o}C for fungi and 37^{o}C for bacteria.

Sterile 4mm paper discs (Difco) were impregnated with filtered sterilized (0.45um Millipore filter) newly prepared different preparations of Aloe vera gel and placed on the freshly seeded microbial lawns (4 discs in each plate) with a control. Petri-plates were incubated at their respective temperatures for a period of 48 and 96h. The zones of inhibition against the tested organisms, thus developed, were measured with the help of a scale to the nearest in mm. The results of the antimicrobial activity of different preparations of Aloe vera gel against microorganisms were expressed as resistant, intermediate and susceptible [(15)].

Results & Discussion

The antimicrobial activity of different preparations of Aloe vera gel was investigated against different food borne pathogens by the disc diffusion method and the results are presented in table 1. It was found during the present period of study that the fresh gel, preserved gel, cooling gel and acne cream exhibited maximum zone of inhibition against *Bacillus subtilis, Bacillus subtilis, Staphylococcus aureus* and *Staphylococcus aureus* (24.70, 34.5, 30.3 and 26.3mm) respectively and the minimum zone of inhibition was shown by *Aspergillus ficuum* by all four preparations of Aloe vera gel i.e. 9.5, 10.5, 10.5 and 9.5mm respectively after a period of 48h of incubation at 37^{o}/25^{o}C. A same kind of observation was made by Agarry *et al.,* [(16)] representing that antimicrobial susceptibility testing of Aloe vera gel shows greatest inhibitory effect on the *Staphylococcus aureus* (18.0 mm). This result could be responsible for the popular use of Aloe vera gel and leaf to relieve many types of gastrointestinal irritations [(8)]. Kaithwas *et al.,* [(17)] studies of antimicrobial activity of Aloe vera gel using disc diffusion method also demonstrated that the gel was most effective against *Staphylococcus aureus*. In a similar study, Mangena [(18)] reported that the gel was found to be very effective against *Staphylococcus aureus*. As the Aloe vera gel is rich in a wide variety of secondary metabolites, such as anthraquinone glycosides, glycoproteins, gamma-lanoline acid, prostaglandins and

mucopolysaccharides, these are mainly responsible for the antimicrobial activity [6]. Especially the agents which are involved to prevent or destroy the bacterial or fungal cells and help to attain the normal growth and functions of the body are cinnamonic acid, salicylic acid, traumatic acid, phenol, allantoin, compesterol, lectins and gibberellins that are found to have effective as antimicrobial properties [12].

Table 1: Antimicrobial activity of four different preparations of Aloe vera gel against pathogens of public health significance.

Test organisms	Colony Morph/Temp °C / Media	Z ONE OF INHIBITION (mm) **								
		Fresh gel		Preserved gel		Cooling gel		Acne cream		C.M.V ***
		48h	96h	48h	96h	48h	96h	48h	96h	48h
Aspergillus niger	White, later green/ Black, 25°- CM139	12.5	10.8 12.9*	19.5	11.8 39.4*	19.5	15.8 18.9*	15.5	15.0 3.2*	14.25 I
Aspergillus ficuum	White, later green/ Black 25°- CM139	9.5	8.6 9.4*	15.5	15.0 3.2*	10.5	7.6 27.6*	9.5	8.12 14.5*	11.25 I
Fusarium solani	White cottony 25°- CM139	11.5	10.0 13.0*	19.0	17.65 7.1*	13.40	12.10 9.7*	18.75	18.0 4.0*	15.66 I
Penicillium digitatum	White, later blue-green 25°- CM139	9.8	9.5 3.0*	16.8	15.5 7.7*	12.8	12.5 2.34*	14.8	13.40 9.4*	13.55 I
Candida utilus	Creamy white 25°- CM139	16.8	16.0 5.0*	29.40	24.5 16.6*	18.9	14.5 23.8*	16.0	14.5 9.3*	20.28 S
Escherichia coli	Gram- rods 37°- CM69	11.5	10.2 11.3*	21.5	20.2 6.0*	13.5	10.0 25.9*	14.5	10.2 29.6*	19.45 S
Salmonella typhimurium	Gram- rods 37°- CM201	10.0	9.9 1.0*	16.0	11.9 25.6*	15.8	12.9 18.3*	11.7	9.8 16.2*	13.37 I
Proteus vulgaris	Gram- rods 37°- CM7	9.0	8.8 2.22*	14.0	11.8 15.7*	12.5	10.9 12.8*	9.5	8.3 12.6*	11.25 I
Staphylococcus aureus	Gram+ cocci 37°- CM145	22.3	19.0 14.7*	28.3	26.9 4.9*	30.3	29.8 1.6*	26.3	23.9 9.1*	26.22 S
Bacillus subtilis	Gram+ rods 37°- CM271	24.7	21.5 12.9*	34.5	32.5 5.7*	28	24.5 12.5*	22.0	19.5 11.3*	27.30 S
Bacillus cereus	Gram+ rods 37°- CM271	20.7	18.7 1.0*	31.7	28.5 10.0*	26.7	26.0 2.6*	24.7	24.0 2.8*	25.95 S

R= resistant, S= sensitive and I= intermediate.
CM139: Potato dextrose agar, CM69: Eosin methylene blue, CM201: Bismithsulphite agar, CM7: Macconkey's agar, CM145: Staphylococcus medium110, CM271: Blood agar base.
pH of the assay medium ranges from 6.4 to 7.3 depending upon the tested organisms.
* = Percentage decrease in zone of inhibition after an incubation period of 96h.
**= Zone of inhibition was examined after a period of 48h (First reading) and then after 96h (Second reading).
***= Cumulative mean value.

The percentage decrease in the zones of inhibition of different preparations of gel after a period of 96h of incubation at their respective temperatures ($25^{o}/37^{o}$C) against the pathogenic microorganisms of public health significance is also summarized in the same table. It was found that *Salmonella typhimurium, Bacillus cereus, Proteus vulgaris* and *Penicillium digitatum* showed 1.0, 1.0, 2.22 and 2.34% decrease, which was minimum, while *Aspergillus niger, Aspergillus ficuum* and *Escherichia coli* exhibited maximum decrease in its efficacy that was 39.4, 27.6 and 29.6% for fresh gel, preserved gel, cooling gel and acne cream respectively after a period of 96h of incubation. Thangam and Dhananjayan [19] conducted different experiments for the evaluation of different preparations of gel against pathogenic fungi. They also reported a decrease in the inhibitory zone from 15 to 26mm after a period of 96h of incubation, thus, resulting in a percentage decrease from 9.52 to 34.8 % against the tested strains. Saddique *et al.,* [20] also observed a decrease in inhibitory zone from 15 to 26mm after a period of 96 hours of incubation, thus, showing a percentage decrease from 9.52 to 34.8% against the tested pathogenic microorganisms. The low activity of sterile crude gel preparations might be due to a number of factors, such as time of collection of plant material, its storage, climate, which might, in turn, affect the amount of the active principal constituents in the plant material responsible for its minimum zone of inhibition [21].

The results of the antibacterial activity of different preparations of Aloe vera gel compared with antibiotics, Streptomycin and Gentamycin, as reference standards are presented in table 2. It was found that all the four 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.,* [22] 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 that Aloe vera is a potent antimicrobial agent compared with the conventional antibiotics. The results of the study by Coopoosamy and Magwa [23] also revealed that lowest concentrations of ethyl acetate and ethanol crude extracts of Aloe excelsa resulted in complete inhibition of visible growth of pathogenic bacteria compared with the control antibiotics, chloramphenicol and streptomycin sulfate.

Moreover, our study results showed the fact that the aseptic crude Aloe vera gel preparation had stronger retardation effective on gram positive test organisms (*Staphylococcus aureus, Bacillus subtilis* and *Bacillus cereus*) as compared to the gram negative ones (*Escherichia coli, Salmonella typhimurium* and *Proteus vulgaris*). Similarly, in another study, gram-positive test organisms were found to be more susceptible to the sterile Aloe vera gel preparation [24]. A same kind of observation was made by Ayoola *et al.,* [25] indicating that the undiluted extract of tangerine fruit volatile oil had potent activity against the gram positive bacteria but no significant activity against the gram negative bacteria. Johann *et al.,* [26] studies also indicated that the bioautography test of wax and hexane extracts form peels of the *citrus* species presented substantial antimicrobial properties against gram positive organisms.

Table 2: Antimicrobial activity of four different preparations of Aloe vera gel against different bacterial strains with antibiotics as reference standard

Tested Organisms	Different preparations of *Aloe vera* gel				Antibiotics	
	Fresh gel	Preserved gel	Cooling gel	Acne cream	Streptomycin	Gentamycin
Escherichia coli	-	++	-	+	-	++
Salmonella typhimurium	-	+	+	-	++	-
Proteus vulgaris	-	+	-	-	-	-
Staphylococcus aureus	+	++	++	++	++	+
Bacillus subtilis	++	++	++	+	++	++
Bacillus cereus	+	++	++	++	++	+

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

Conclusion

Scientists from divergent fields are investigating different plants extracts with an eye to their anti-microbial usefulness. Laboratories of the world have found literally thousands of physiochemical that have inhibitory effects on all types of microorganisms in vitro. From our results it can be concluded that Aloe vera gel extract possesses compounds with antimicrobial properties which can be used as antimicrobial agents in new drugs for therapy of infectious diseases in humans. The results of the present study thus explains the use of this plant in folk medicine for the treatment of various diseases whose symptoms might involve microbial infections and under line the importance of ethno botanical approach for the selection of Aloe vera in the discovery of new bioactive compounds. This plant could be a source of new antibiotic compounds being nontoxic and less expensive than the allopathic drugs.

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