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In vitro evaluation of antibacterial potential of *Aloe vera* against *Enterococcus faecalis*

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ABSTRACT

Background: Through *in vitro* study, antibacterial property of *Aloe vera* extract against Enterococcus faecalis, one of the most resistant endodontic pathogens to be isolated from an infected root canal was tested. **Objectives:** were, to assess the zone of inhibition of *Enterococcus faecalis* by aqueous and ethanolic extracts of *Aloe vera gel* by using disc diffusion method and to assess the Minimum Inhibitory Concentration (MIC) of aqueous and ethanolic extracts of *Aloe vera* gel against *Enterococcus faecalis* by two-fold serial dilution method. **Materials and Methods**: *Aloe vera* gel extract was prepared using sterile de-ionized water and 100% ethanol. A 10% stock solution was prepared. 3% Sodium hypochlorite solution and 2% Chlorhexidine solution were chosen as the positive controls while sterile deionized water was negative control. The antimicrobial potential of the control agents was tested against *Enterococcus faecalis* in a separate agar plate and were then compared with that of the test agent. The antibacterial efficacy was tested by disc diffusion method using Brain heart infusion agar media. **Results:** 50 µl and 75 µl of ethanolic extract of *Aloe vera* gel showed a zone of inhibition of 8 mm and 10 mm respectively against *Enterococcus faecalis.* Antibacterial property of *Aloe vera* extracts were much less when compared to the positive controls. **Conclusion**: Enterococcus faecalis was sensitive to 25% alcoholic extract of *Aloe vera* gel up to three dilutions and for 25% aqueous extract of *Aloe vera* gel only at first dilution.

Keywords: Antibacterial efficacy, *Aloe vera*, *Enterococcus faecalis*.

INTRODUCTION

India enjoys the privilege of housing one of the oldest civilizations in the world. She is a land of rich heritage, diverse culture, traditions, customs, values and beliefs. This background has enabled India to contribute enormously to the field of Medicine. For instance, Ayurveda and Siddha are two systems of Medicine that are purely Indian in origin [1]. Both these indigenous systems of Medicine use myriad of medicinal herbs to cure various diseases that are still an enigma to Allopathic medicine. Added to this, home remedy using commonly available herbs and spices for common ailments is a practice that is woven into Indian culture. Over a period of time, modern medicine and dentistry has duly acknowledged the potential of these traditional systems of medicine. In fact, the World Health Organization in 2008 gave a universal call to all member nations to promote traditional medicine in their countries [2].

Aloe vera, an herb bestowed with therapeutic and cosmetic properties, is a member of the *Asphodelaceae* family with its origin in the African continent [3]. Its therapeutic spectrum includes relief of gastric irritation, thermal burn, sunburn, as a laxative preceding rectal surgery, for treating hemorrhoid and to stimulate the immune system [4, 5]. Another important property of *Aloe vera* is its antimicrobial potential against various microorganisms such as *Staphylococcus aureus, Streptococcus pyogenes, Coryne bacterium xerose, Salmonella paratyphi and Mycobactrium tuberculosis* [6, 7].

In dentistry, antimicrobial agents are used extensively for various reasons. One such application is during root canal therapy. An infected root canal has complex morphology and mechanical instrumentation per se is insufficient to completely disinfect the root canal [8]. Antimicrobial agents in the form of intracanal medicament and/or endodontic irrigant are used along with mechanical instrumentation to achieve complete debridement of the root canal. Sodium hypochlorite and 2% Chlorhexidine are commonly used irrigating agents in endodontics. However, their usage has certain disadvantages. Sodium hypochlorite causes tissue toxicity, risk of emphysema when overfilled, allergic potential, disagreeable smell and taste [9]. On the other hand, 2% chlorhexidine causes teeth discolouration, loss of taste sensation, burning sensation and subjective dryness of the oral cavity [10].

In order to circumvent these problems, research have been conducted, to assess the antimicrobial property of herbal extracts like *Morinda citrifolia* [9], green *tea* [11], *Triphala* [11], *Arctium lappa* [12] and *Rosa damascene* [13] against endodontic pathogens. Review of dental literature shows that very few studies

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have tried the efficacy of *Aloe vera* as an antibacterial agent for dental use. Hence, in this project an attempt was made to assess the invitro antibacterial property of *Aloe vera* extract against *Enterococcus faecalis*, one of the most resistant endodontic pathogens to be isolated from an infected root canal [14, 15].

Objectives of the study were, 1) To assess the zone of inhibition of *Enterococcus faecalis* by aqueous and ethanolic extracts of *Aloe vera* gel by using disc diffusion method. 2) To assess the Minimum Inhibitory Concentration (MIC) of aqueous and ethanolic extracts of *Aloe vera* gel against *Enterococcus faecalis* by two-fold serial dilution method.

MATERIALS AND METHODS

Study design was *In-vitro* experimental study**.** The study protocol was submitted to the Institutional Review Board and was duly approved for its ethical integrity.

Preparation of the *Aloe vera* gel extracts

Aloe vera plants that grow commonly in the courtyard were identified based on its taxonomic features by a botanist. Fresh leaves from the identified plants were cut at the base and cleaned thoroughly using sterile water. *Aloe vera* gel extract was prepared using two solvents namely, sterile de-ionized water and 100% ethanol. For preparing the aqueous extract, vertical incisions were placed on the leaves and the incised leaves were arranged in a receptacle containing 200 ml of sterile de-ionized water for an hour. This was done to hasten the process of extraction of *Aloe vera* gel. Later, the content of the beaker was transferred to a clean screw capped glass bottle and the extraction was carried out by cold maceration technique with occasional stirring. After 24 hours, the contents of the bottle were subjected to filtration. The filtrate so obtained was reduced over a water bath set at 60^{o} Celsius to enable the complete evaporation of water. The crude extract so obtained was packed in a glass bottle, labeled as aqueous extract of *Aloe vera* gel and stored in a desiccator until further use. Ethanolic extract of *Aloe vera* gel was prepared in a similar manner but 100% ethanol was used instead of deionized water for extraction purpose.

Preparation of stock solution

A 10% stock solution was prepared by dissolving 1g each of aqueous and ethanolic extract of *Aloe vera* gel separately in 10 ml of sterile deionized water and was used for microbiological analysis.

Control agents

3% Sodium hypochlorite solution and 2% Chlorhexidine solution were chosen as the positive controls while sterile deionized water, which was used as the solvent to prepare the stock solutions, was selected as the negative control. The antimicrobial potential of the control agents were tested against *Enterococcus faecalis* in a separate agar plate and were then compared with that of the test agent.

Microbiological procedures

The antibacterial potential of aqueous and ethanolic extract of *Aloe vera* was assessed against *Enterococcus faecalis* (ATCC 35550) by disc diffusion method[1]. Brain heart infusion agar media was employed to culture *Enterococcus faecalis* (Hi Media Laboratories, Mumbai). The concentration of the organism was adjusted to 10,000 colony forming units/ml by using 0.5 McFarland Standards and was applied on the surface of the plate. The agar plates were incubated overnight at 37^{o} Celsius. Three such plates were prepared. In each plate, five wells of 8 mm diameter each were punched and were filled with 5 µl,10 µl, 25 µl, 50 µl, 75 µl of aqueous extract of *Aloe vera* gel. In the second agar plate, similar volumes of ethanolic extract of *Aloe vera* gel were filled in the wells and in the last plate, a fixed volume of the control agents were filled in the wells. The antibacterial activity was interpreted from the size (diameter) of inhibition zone observed as clear zone surrounding each well on the agar plates. The zone of inhibition was measured in millimeters using vernier caliper.

Two-fold serial dilution methods were employed to find out the Minimum Inhibitory Concentration (MIC) of aqueous and ethanolic extracts of *Aloe vera* gel against *Enterococcus faecalis*. A standard amount of *Enterococcus faecalis* culture was added to the diluted extract in sterile tubes and was incubated overnight at 37^{o} Celsius. The results were interpreted as sensitive if the supernatant was clear and as resistant if the suspension was turbid. A total of ten test tubes were used to assess the MIC. The last tube in the series showing clear supernatant was considered to be the MIC value of the tested agent against Enterococcus faecalis.

RESULTS

Zone of inhibition

Aqueous extract of *Aloe vera* gel did not demonstrate antibacterial property against Enterococcus faecalis by disc diffusion method. No zone of inhibition was appreciated surrounding the wells containing different volumes of aqueous extract of *Aloe vera* gel. On the other hand, 50 µl and 75 µl of ethanolic extract of *Aloe veran*gel showed a zone of inhibition of 8 mm and 10 mm respectively against Enterococcus faecalis. However, the antibacterial property of *Aloe vera* extracts were much less when compared to the positive controls as depicted in Table 1.

Minimum Inhibitory Concentration

10% *Aloe vera* gel extracts did not exhibit an appreciable antibacterial property as reflected by the zone of inhibition. Therefore, fresh stock solutions of aqueous and ethanolic extract of *Aloe vera* gel with 25% concentration was formulated and used to assess its Minimum Inhibitory Concentration against *Enterococcus faecalis*. *Enterococcus faecalis* was sensitive to 25% alcoholic extract of *Aloe vera* gel up to three dilutions and for 25% aqueous extract of *Aloe vera* gel only at first dilution as shown in Table 2 and 3.

Table 1: Zone of inhibition (in millimeters) for different volumes of *Aloe vera* extracts against *Enterococcus faecalis* compared to that of positive and negative controls.

AGENTS TESTED	VOLUME OF TEST AGENT				
	75 µl	50 µl	25 µl	10 µl	5 µl
10% Aqueous extract of *Aloe vera* gel	R	R	R	R	R
10% Ethanolic extract of *Aloe vera* gel	10	8	R	R	R
3% Sodium hypochlorite	20	15	12	10	<5
2% Chlorhexidine	22	20	18	15	12
Sterile deionized water	R	R	R	R	R

R = Resistance against *Enterococcus faecalis*

Table 2: Shows the Minimum Inhibitory Concentration of aqueous extract of *Aloe vera* gel against *Enterococcus faecalis*

Starting Concentration	Dilutions									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
25%	**S**	R	R	R	R	R	R	R	R	R
15%	R	R	R	R	R	R	R	R	R	R

R = Resistance against *Enterococcus faecalis*
S = Sensitive against *Enterococcus faecalis*

Table 3: Shows the Minimum Inhibitory Concentration of ethanolic extract of *Aloe vera* gel against *Enterococcus faecalis*

Starting Concentration	Dilutions									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
25%	**S**	**S**	**S**	R	R	R	R	R	R	R
15%	**S**	R	R	R	R	R	R	R	R	R

R = Resistance against *Enterococcus faecalis*
S = Sensitive against *Enterococcus faecalis*

DISCUSSION

The *in vitro* antibacterial property of aqueous and alcoholic extracts of *Aloe vera* gel against *Enterococcus faecalis* was assessed in the present study. The study assumes significance in the wake of growing trend in the usage of herbal products in dentistry. Moreover, the synthetic products that are currently in vogue as endodontic irrigants do suffer from certain disadvantages. The main tenet of studying the antimicrobial property of an herbal extract is that it is safe, abundantly available, culturally acceptable, economical and safer to the human body.

Aloe vera extracts were prepared by cold maceration technique in the present study so that the decomposition of heat sensitive active constituents can be avoided [16]. Review of literature revealed that aqueous and ethanolic extract of *Aloe vera* was effective in inhibiting the growth of microorganisms. However, ethanolic extract of *Aloe vera* was found to possess better antibacterial property than the aqueous extract against *Enterococcus faecalis*. The inner mucilaginous mass of *Aloe vera* contains *anthraquinone* which might be responsible for its antibacterial property [17].

Agar diffusion method is considered to be a standard method of assessing the antimicrobial property of a drug [18]. Aqueous extract (10%) of *Aloe vera* gel did not inhibit *Enterococcus faecalis* but 10%ethanolic extract of *Aloe vera* gel produced maximum inhibition zone of 10 mm. However, this was very less compared to the inhibition zone produced by the positive controls. The agar diffusion method is based on the ability of the test agent to diffuse through the solid agar medium and inhibit the microorganism. Probably, in this study the *Aloe vera* gel extracts did not possess the necessary diffusion property to inhibit the microorganism. Moreover, the pH of the test agent and the incubation period also dictates the activity of the test material. Previous studies have employed fresh extract of *Aloe vera* gel without dilution to study its antimicrobial property [19]. In the present study, a 10% extract of *Aloe vera* gel was prepared to study its efficacy as an antibacterial agent at a lower concentration.

In order to substantiate the results obtained in disc diffusion method, minimal inhibitory concentration of *Aloe vera* gel extracts against Enterococcus faecalis was also assessed in the present study. Minimal inhibitory concentration is considered to be more meaningful in assessing the antimicrobial property of a test agent [18]. *Enterococcus faecalis* was sensitive to 25% ethanolic extract of *Aloe vera* gel up to three dilutions.

The result of this study is in line with that conducted by Suresh Chandra *et al*. [20] In their study, chloroform extract of *Aloe vera* gel was found to produce an inhibition zone of 9 mm whereas methanolic extract of *Aloe vera* gel produced a zone of inhibition of 12 mm against *Enterococcus faecalis*. Further, Aloevera containing tooth gel was reported to inhibit *Enterococcus faecalis* with an inhibition zone of 22 mm in a study conducted by Dilip George *et al*. [21].

There exist no known agents that can mimic Sodium hypochlorite in its tissue dissolving property. *Aloe vera* is found to possess antimicrobial property, and hence it can substantially reduce the usage of Sodium hypochlorite during cleaning and shaping of root canals. Gel extract and the whole leaf extract of *Aloe vera* possess different spectrum of antibacterial property. It may be useful to evaluate and compare the antimicrobial potential of *Aloe vera* gel and whole leaf extract in future studies. Further research evaluating the potential of *Aloe vera* as an endodontic irrigant or as an intracanal medicament in biofilm models should be conducted before it is used *in vivo* conditions.

CONCLUSIONS

1. Aqueous extract of *Aloe vera* does not possess appreciable antibacterial property against *Enterococcus faecalis*
2. Ethanolic extract of *Aloe vera* possess antibacterial property against *Enterococcus faecalis*
3. Antibacterial property of *Aloe vera* gel extracts were less compared to the positive agents (2% Chlorhexidine and 5.25% Sodium hypochlorite)

Authors' contributions

1. Dr Puja C Yavagal: Conception and Design of the work, Preparation and Critical revision of the manuscript
2. Dr Sravanthi SV: Data Collection

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