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Antibacterial activity of *aloe vera* (*Aloe barbadensis*) leaf extracts

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Abstract

The antibacterial activity of *Aloe vera* extract with seven different solvents was evaluated against pathogenic bacteria like *Bacillus cereus*, *E. coli* and *Pseudomonas aeruginosa* by using standard disc diffusion method. Various solvents such as acetone, ethanol, ethyl acetate, chloroform, Dimethyl Sulfoxide (DMSO), Methanol and water were used for extracts. A gradual increase in the zone of inhibition with increase in the concentration of *Aloe vera* extract with different solvents was observed. The results showed that, *Aloe vera* – chloroform extract has showed significant antimicrobial activity against all the three pathogenic bacteria.

Keywords: *Aloe vera*, Antibacterial activity, Solvents

Introduction

Aloe vera is a medicinal plant universally confessed for its medicinal properties, exclusively for its anti-inflammatory and also has impact in wound-healing because of the presence of anti-microbial compounds such as lectins, anthraquinones, mannans, and polysaccharides. The *aloe vera* is used for curing digestive problems, constipation, poor appetite, colitis, irritable bowel syndrome as well as diabetes, immune system enhancement, pepticulcers (Brusick *et al.*, 1997; Mansour *et al.*, 2014; Ezuruike *et al.*, 2014; Kavyashree *et al.*, 2015; Pandey *et al.*, 2016) [4, 10, 7, 9, 11]. Traditionally various parts of *Aloe vera* have many medicinal properties due to its pharmacological activities and are utilized for many veterinary and human diseases (Blumenhal *et al.*, 1998) [5]. These types of herbs are capable to treat various infectious and non- infectious diseases. And also *Aloe Vera* proved to be it has anticancerous property when it was treated with rats affected by pleural tumour from hepatoma cells (Corri *et al.*, 1998) [6]. *Aloe vera* exhibits higher efficiency at lowering blood glucose levels among patients affected by Diabetes Mellitus (Fateme *et al.*, 2022) [8]. *Aloe barbadensis* Miller, commonly called as *Aloe vera*, is one among the 400 species of *Aloe* belonging to family Liliaceae and it originated from South Africa and has been a native species of sub-tropical and tropical climates, including the southern USA (Reynolds *et al.*, 1999) [14]. The use of this *aloe vera* extracts which has known antimicrobial properties can be used in the treatment of diverse microbial infections. A lot of studies have been administrated in various countries to prove that medicinal plants have greater efficiency of antimicrobial activity. Many studies are concentrated only with crude extracts (Reddy *et al.*, 2001; Erdo Urul, 2002; Atefl *et al.*, 2003) [13, 3].

Ultimately, this study seeks to provide a scientific basis for developing more effective and affordable natural antimicrobial agents, potentially serving as an alternative or complementary treatment to conventional antibiotics.

Materials and Methods

Collection of Plant Materials

The fresh and healthy succulent leaves of *Aloe barbadensis* were collected from the herbal garden maintained at the Department of Agriculture Microbiology, Faculty of Agriculture, Annamalai University, Chidambaram, Tamil Nadu, India. The succulent leaves were cut in fresh condition at the bottom near stem and thoroughly washed with running tap water. Then the leaves were dipped in sterilized double distilled water for the removal of dust and sand

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particles. Then the leaves were shade dried for an hour. These leaves were used as the raw materials for the extraction of antimicrobial compounds from the plant.

Extraction of the gel

Matured, healthy and fresh leaves of *Aloe vera* were washed in the running water for five minutes and rinsed with double distilled water. The leaves were dissected longitudinally and the colorless parenchymatous tissue (Aloe gel) was scrapped out using a sterile knife without the fibers. The gel was ground with DMSO (Pugh *et al.*, 2001) [12] using the mortar and pestle. The extracts were filtered using Whatman No - 1 filter paper and the extract was centrifuged at 5000 rpm for 5 minutes. The supernatant was collected and stored in refrigerator for 4 °C.

Preparation of the plant extract

Matured, healthy and fresh leaves of *Aloe vera* were washed in the running water for five minutes and rinsed with double distilled water. The leaves were placed in the sunlight. The dried sample was grinded in mortar and pestle to get their powder form. The sample's powder was individually mixed with different solvents in 1:10 ratio. After mixing, the solution was placed in dark for 48 hr. Then the sample was filtered with help of filter paper and transfer to a sterile petriplate. The weight of the empty petriplate was taken to calculate the differences after collecting the filtrate. The obtained filtrate was then placed in hot air oven and incubated for 24hrs for complete dry. The weight of the petriplate having the solid filtrate was then measured in order to calculate the difference. After that the DMSO was added (double the amount of DMSO to the filtrate) to the obtained filtrate.

Preparation of the Bacterial Inoculum

A loopful of (2 mm loop) bacterial culture viz., *Bacillus cereus*, *Escherichia coli* and *Pseudomonas aeruginosa* was suspended separately in one ml of sterilized water with vigorous shaking to prepare cell suspension. The cell suspension was added to 100 ml of sterile Trypticase soy broth and shaken vigorously in a temperature controlled shaker at 30 °C for 24 hrs. The cells were suspended in 0.1 M phosphate buffer to a cell concentration of 10^7 cell ml⁻¹ (O.D=0.5) and used as standard inoculums of test microorganisms.

Preparation of Filter Paper Disc

Discs of 5 mm diameter were prepared using Whatman No.1 filter paper and were sterilized in the hot air oven at 160 °C for 1 hour. The discs were impregnated with different solvent extracts and stored at 4 °C for the further use. Control paper discs were prepared by using 1% DMSO.

Antibacterial Activity of *Aloe vera* leaf extracts

Disc diffusion was carried out for the bacterial suspension (1ml of inoculums). Bacterial suspensions along with nutrient agar medium were poured in the petriplates and allowed for solidification. The disc diffused in *Aloe vera* leaf extracts with different concentrations of 250 ppm, 500 ppm and 1000 ppm were placed in quadrangular manner in different petridishes. Then petridishes were incubated at 30 ± 2 °C for 24 hours. After the incubation period, the results were noted and the zone of inhibition was measured in mm. For each test, three replicates were performed.

Measurement of antimicrobial activity Area of inhibition zone = $\pi (R+r) (R-r)$ Where

R=Radius of inhibition zone + filter disc r= Radius of filter paper disc

$$\pi=3.14$$

Statistical Analysis: Data were expressed as mean ± standard deviation. The data obtained were subjected to ANOVA test to determine whether there was significant difference between extract used and also between the lengths of incubation.

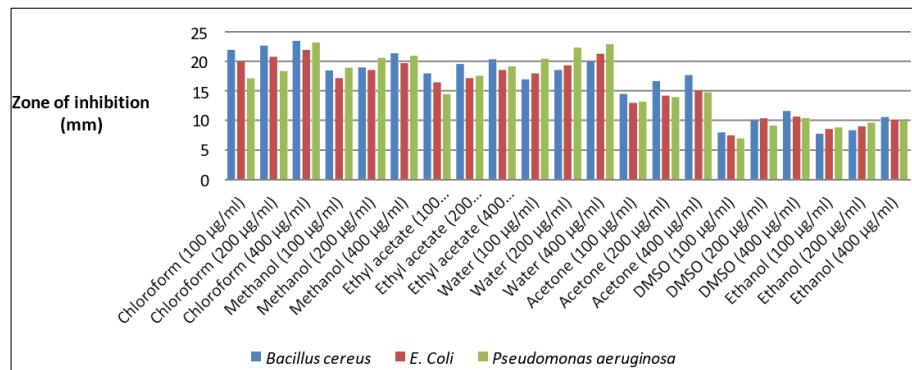
Results

The present study carried out on the *Aloe vera* revealed the antibacterial activities of various extracts of *Aloe vera* and the results are shown in Table 1 and Fig.1 & 2. The extracts using chloroform, methanol, ethyl acetate and water of *Aloe vera* showed active antibacterial activity against *Bacillus cereus*, *E.coli* and *Pseudomonas aeruginosa*. The chloroform and methanolic extract showed highest inhibition zone at higher concentration (i.e. 400 µg/ml). Overall the chloroform extracts showed greater inhibition of all pathogenic bacteria used when compared to other solvent extracts. The extracts of chloroform at the dose level of 400 µg/ml showed the inhibition zone of *Bacillus cereus* (23.5mm), *E.coli* (22mm), *Pseudomonas aeruginosa* (23.3mm) whereas the extracts of methanol at the dose level of 400 µg/ml showed the inhibition zone of *Bacillus cereus* (21.4mm), *E.coli* (19.7mm), *Pseudomonas aeruginosa* (21.0 mm), the extracts of ethyl acetate at the dose level of 400 µg/ml showed the inhibition zone of *Bacillus cereus* (20.4 mm), *E.coli* (18.6 mm), *Pseudomonas aeruginosa* (19.2 mm), the extracts of water at the dose level of 400 µg/ml showed the inhibition zone of *Bacillus cereus* (20.0 mm), *E.coli* (21.3 mm), *Pseudomonas aeruginosa* (23.0mm), the extracts of acetone at the dose level of 400 µg/ml showed the inhibition zone of *Bacillus cereus* (17.7 mm), *E.coli* (15.1 mm), *Pseudomonas aeruginosa* (14.8 mm), the extracts of DMSO at the dose level of 400 µg/ml showed the inhibition zone of *Bacillus cereus* (11.5 mm), *E.coli* (10.7 mm), *Pseudomonas aeruginosa* (10.5 mm) and the extracts of ethanol at the dose level of 400 µg/ml showed the inhibition zone of *Bacillus cereus* (10.6 mm), *E.coli* (10.2 mm), *Pseudomonas aeruginosa* (10.0 mm).

Table 1: Antibacterial Activity of Leaf Extract of *Aloe vera*

Leaf extracts	Zone of inhibition (mm)*		
	<i>Bacillus cereus</i>	<i>E. Coli</i>	<i>Pseudomonas aeruginosa</i>
Chloroform (100 µg/ml)	22.0	20.0	17.2
Chloroform (200 µg/ml)	22.7	20.8	18.4
Chloroform (400 µg/ml)	23.5	22.0	23.3
Methanol (100 µg/ml)	18.5	17.2	19.0
Methanol (200 µg/ml)	19.0	18.6	20.7
Methanol (400 µg/ml)	21.4	19.7	21.0
Ethyl acetate (100 µg/ml)	18.0	16.5	14.5
Ethyl acetate (200 µg/ml)	19.6	17.2	17.6
Ethyl acetate (400 µg/ml)	20.4	18.6	19.2
Water (100 µg/ml)	17.0	18.0	20.5
Water (200 µg/ml)	18.6	19.4	22.4
Water (400 µg/ml)	20.0	21.3	23.0
Acetone (100 µg/ml)	14.5	13.0	13.2
Acetone (200 µg/ml)	16.7	14.2	14.0
Acetone (400 µg/ml)	17.7	15.1	14.8
DMSO (100 µg/ml)	8.0	7.5	7.0
DMSO (200 µg/ml)	10.0	10.4	9.2
DMSO (400 µg/ml)	11.6	10.7	10.5
Ethanol (100 µg/ml)	7.8	8.6	8.9
Ethanol (200 µg/ml)	8.4	9.0	9.7
Ethanol (400 µg/ml)	10.6	10.2	10.0

Data represents average of three replicates. mm* = Mean of three replicates

**Fig 1:** Antibacterial Activity of Leaf Extract of *Aloe vera***Fig 2:** Plate Showing Zone of Inhibition

Discussion

In this study the chloroform extract has shown high zone of inhibition in *Bacillus cereus*, *E. coli* and *Pseudomonas aeruginosa* when compared with other solvents. The alcoholic extract was found to be a better solvent for

extraction of antimicrobial compounds by comparing with water and hexane (Ahmad *et al.*, 1998) [2]. Methanol and ethyl acetate extract has shown a high zone of inhibition in *Bacillus cereus* and *Pseudomonas aeruginosa* but moderate zone of inhibition in *E. coli*. Water extract has shown high zone of inhibition in *Pseudomonas aeruginosa* and *E. coli* but moderate zone in *Bacillus cereus*. The above findings support the scientific basis of *Aloe vera* usage in conventional treatment of bacterial diseases. The antibacterial activity of the extracts and their potential was quantitatively evaluated by the formation of inhibition zone and zone diameter. Agarry *et al.*, (2005) [1] compared the antimicrobial activities of *Aloe vera* against pathogenic microorganisms such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Microsporium canis*, *T. schoeleinii*, *Trichophyton mentagrophytes* and *Candida albicans*. Hence, it can be concluded that the leaf extracts of *Aloe Vera* can effectively act as an antibacterial agent.

Conclusion

The present study has revealed the importance of *Aloe vera* leaf extracts to control bacteria, which causes threat to human health and also found that the aloe vera extracts can be used for the treatment of various skin transmitted

infections. The results accepts that the *Aloe vera* showed traditional uses and *Aloe vera* could be exploited for new potent antimicrobial agents.

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