



ISSN 2048-4143

International Research
Journal of
PHARMACEUTICALS

Research Paper

In-Vitro antibacterial activity of *Aloe Barbadensis* Miller (Aloe Vera)

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Abstract

Medicinal plants play an important role for health care. Medicinal plants have ability to cure both infectious and non-infectious diseases. According to an estimate about 25% of medicines are derived from plants. The objective of the present study was to evaluate the antibacterial activity of *Aloe barbadensis* Miller (Aloe Vera) by using agar diffusion assay and gel filtration chromatography. The bacterial strains used in this research work were *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhi*, *Pseudomonas*, *Klebsiella pneumoniae*, *Staphylococcus epidermidis*. Aloe Vera plant leaves and gel were macerated in different organic solvents including ethanol, methanol and distilled water. Then, by using agar diffusion assay antibacterial activity was estimated. The zones of inhibition were measured by scaling and represented by tables and graphs. The Aloe Vera extract of Methanol showed the maximum antibacterial activity as compared to other solvent extracts. Then, distilled water macerated form of Aloe Vera leaves were used for gel filtration chromatography technique in order to determine the fraction containing the active components. Fraction 8 showed maximum antibacterial activity against all above mentioned bacterial strains. This study reveals the plausibility of the presence of some bioactive components in Aloe Vera. The further investigation on crude extracts would characterize bioactive components of Aloe Vera which would be done by using High-performance liquid chromatography (HPLC).

Keywords: *Aloe Vera*, Antibacterial Activity, Agar Diffusion Assay, Gel Filtration Chromatography

1. Introduction

The use of herbs and medicinal plants is a universal phenomenon. Every culture on earth has relied on the huge variety of natural chemistry found in healing plants for their therapeutic properties. As per World Health Organization (WHO), about 80% of world population use medicinal plants to treat human disease (Serrentino, 1991). Aloe Vera is a stem less or sometime very short-stemmed succulent plant growing up to 60-100 cm tall. The leaves are thick and fleshy green with some varieties showing white flecks on the upper and lower stem surfaces. The margin of the leaf is serrated and has small white teeth. The flowers are produced in summer. Each flower is pendulous, with a yellow tubular corolla 2–3 cm long. Aloe Vera forms arbuscular mycorrhiza, a symbiosis that allows the plant better access to mineral nutrients in soil (Gong et al, 2002). Vera gel consists of 99.3% water. The remaining 0.7% is made up of solids with glucose and

mannose constituting for a large part. These sugars together with enzymes and amino acids give the special properties as a skin care product (Crew et al, 1939; Borrelli & Izzo, 2000 and Agarry et al, 2005). The Aloe Vera gel is extensively used in gastrointestinal disorders including peptic ulcer (Thirupathi et al, 2010 and Johnsonan et al, 2011).

The recent researches on Aloe Vera are appreciable. In the previous study, A. Vera aqueous and alcoholic extracts were prepared by decoction and hot percolation process. Alcoholic extracts displayed higher antibacterial and anti fungal activity than aqueous extract (Choi et al, 2001).

Another study was conducted to determine the antimicrobial activity of Aloe Vera juice with different solvents viz; hexane, ethyl acetate, petroleum ether and

ethanol against Gram positive bacteria and Gram negative bacteria. The disc diffusion method was used. That study estimated the amount of minerals present in fresh Aloe Vera juice by Atomic Absorption Spectroscopy. It is important for medications, cosmetics and food purpose (Khaing, 2011). Aloe Vera has been used worldwide for pharmaceutical, food and cosmetic industries. The Aloe extract showed the significant antioxidant activity by the DPPH radical scavenging method (Bland et al, 1985).

2. Materials and Methods

The present study was designed to evaluate antibacterial activity of Aloe Vera by using its leaf and gel extract. The study was performed at Institute of Biochemistry and Biotechnology, University of the Punjab, Lahore.

In order to see the antibacterial activity of Aloe Vera agar disc-diffusion assay and gel filtration chromatography was used. In the present work, there were the main steps involved; Collection and identification of Aloe Vera, Collection and identification of bacterial strains, Cold extraction of Aloe Vera (Maceration), Agar diffusion assay with bacterial strains and Gel filtration chromatography.

The Aloe Vera gel and leaves were collected from different areas in Lahore, Punjab. The bacterial strains used in this research project were *Escherichia coli*, *Bacillus subtilius*, *Salmonella typhi*, *Pseudomonas*, *Klebsiella pneumoniae*, *Staphylococcus epidermidis*. These bacterial strains were collected from Jinnah Hospital, Sheikh Zayed Hospital and the Department of Microbiology and Molecular Genetics, University of the Punjab, Lahore. Bacterial strains were preserved in glycerol stock solution at -70° C in Institute of Biochemistry and Biotechnology. Dr. Abdul Rehman Niazi (taxonomist) from the Department of Botany, University of the Punjab, Lahore identified the medicinal plants. The Aloe Vera leaves were sterilized properly. 10 g fresh Aloe Vera leaf with gel was dried in the oven at 80° C for 48 hours and then powdered. In the process of maceration, 10g of crushed plant part was dissolved in 100 ml of organic solvent i.e. ethanol, methanol and distilled water. The conical flask was covered by cotton plugs to avoid solvent evaporation. The extract was placed in shaking incubator at 250 rpm for 48 hours. After shaking, it was filtered with muslin cloth. The filtered extract was centrifuged at 8000 g for 20 minutes. The supernatant was collected in sterile flask. Then, it was stored at 4° C.

The agar disc-diffusion assay was performed by diffusing the Aloe Vera extract from a paper disc that contains test microorganisms. The following steps were involved in agar diffusion assay. The conical flask of 100 ml of L.B broth was inoculated with each test organism and incubated at 37° C for overnight. The 10 ml of M.H agar

was mixed well and poured on the sterile Petri plates. The agar media on Petri plates were allowed to set and harden for few minutes. The small autoclaved discs of Whatmann filter paper were used. The 100 μ l of nutrient L.B broth culture of each test organisms was poured in the centre of each agar Petri plates. The nutrient broth culture of test organisms was spread on the Petri plates by using sterilized glass spreader. During agar-disc diffusion assay, the sterile discs were dipped in the different crude extracts of medicinal plants with the help of sterilized forceps and placed at the centre of the Petri plates. The antibiotic Ampicillin drug was loaded on Petri plates as a control to check the comparison of antibacterial activity with different crude extracts of medicinal plants. The maximum antibacterial activity observed by ampicillin against *E. coli* was 22 mm. The Petri plates were sealed with Para film. Then, the Petri plates were left at room temperature for 2 hours, to allow the diffusion of the test sample and then incubated at 37° C for overnight. The diameter of the zones of inhibition was measured in mm.

Gel filtration chromatography was used to separate the fractions involved in the inhibition of bacterial growth. The column (0.25x30cm) was packed by using the swollen gel Sephadex G-100. The packed column was equilibrated with 50 mM Tris-Cl buffer (pH 7.5). Supernatant (2ml) having extract of Aloe Vera was loaded onto a gel filtration column. The flow rate of column was 1.0 ml/minute. Total 10 fractions (2 ml of each) were collected. Remained fractions were stored at 4° C for further analysis. The antibacterial activity of fractions was checked by agar disc-diffusion assay. The zones of inhibition were measured by scaling and maximum zone of inhibition in (mm) was further analyzed by thin layer chromatography.

3. Results

We conducted a prospective observational study of antibacterial activity of Aloe Vera extracts. The Aqueous, Ethanol and Methanol extracts of Aloe Vera were screened against bacterial strains i.e. *Escherichia coli* and *Bacillus subtilius* by using agar disc-diffusion assay as shown in Figures 1-3 and Table 1.

Graphical presentation of the average of zones of inhibition of Aqueous, Ethanol and Methanol extracts of Aloe Vera is shown in Figure 4. The Aloe Vera fractions (1-10) obtained from gel filtration chromatography. Fraction 1 and 2 can not be tested further because these fractions are colourless and contain only buffer. These fractions (3-10) were screened against six bacterial strains i.e. *Escherichia coli*, *Bacillus subtilius*, *Salmonella typhi*, *Pseudomonas*, *Klebsiella pneumoniae*, *Staphylococcus epidermidis*. The zones of inhibition were clearly shown in

the Figures (5-10) and measured of zone of inhibition is mentioned in Table 2. Graphical presentation of comparison of different column fractions of Aloe Vera is shown in Figure 11.



Figure 1. Aloe Vera Aqueous Extract with *E.coli*



Figure 2. Aloe Vera Ethanol and Methanol Extracts with *E.coli*



Figure 3. Aloe Vera Aqueous Extract with *B.Subtillus*

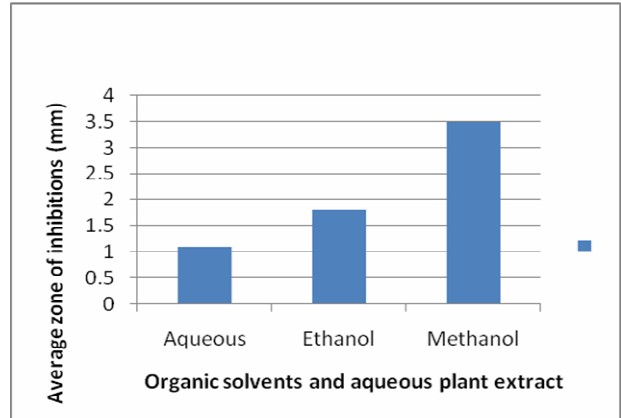


Figure 4. Average of Zone of Inhibition Organic Extracts and Aqueous Extract of *Aloe Bardensis Miller*



Figure 5. Aloe Vera Column Fractions 7, 8, 9 and 10 with *E.coli*



Figure 6. Aloe Vera Column Fractions 5, 6, 7 and 8 with *B. subtilus*

4. Discussion

The Aloe Vera is common in both traditional Chinese and Ayurvedic medicine (Boudreau & Beland, 2006). The

Aloe Vera leaves were used to check the antibacterial activity during this study.



Figure 7. Aloe Vera column fractions 6, 7, 8 and 9 with *S. typhi*



Figure 8. Aloe Vera Column Fractions 5, 6, 7 and 8 with *Pseudomonas*



Figure 9. Aloe Vera column fractions 7, 8, 9 and 10 with *Klebsiella*



Figure 10. Aloe Vera Column Fractions 3, 4, 5 and 6 with *S. epidermitis*

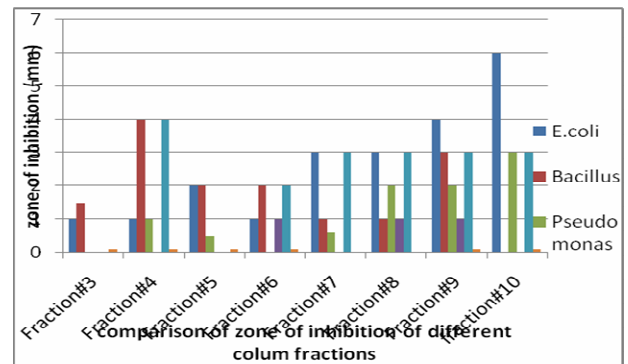


Figure 11. Comparison of different column fractions (3-10) of Aloe Vera

Table 1. Results for Aloe Vera Extracts Showing Measurement of Zone of Inhibition

Bacterial Strains	Aqueous	Ethanol	Methanol
<i>E. coli</i>	1 mm	2 mm	8 mm
<i>B. subtilis</i>	1.5 mm	2 mm	3 mm

The antibacterial activity of Aloe Vera plant fractions were evaluated against bacterial strains i.e. *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhi*, *Pseudomonas*, *Klebsiella pneumoniae*, *Staphylococcus epidermidis*.

The three macerated forms i.e. ethanol, methanol and distilled water of Aloe Vera were tested and methanol extract was most effective than other macerated forms of Aloe Vera leaves as shown in Table 1 and Figures 1-3. The maximum zone of inhibition was observed with Methanol extract of macerated Aloe Vera leaf. This observation was correlated with Cock et al, (2008).

Table 2. Results of Aloe Vera Fractions, Isolated by Gel Filtration Chromatography

Bacterial Strains	Fraction 3	Fraction 4	Fraction 5	Fraction 6	Fraction 7	Fraction 8	Fraction 9	Fraction 10
<i>E. coli</i>	1 mm	1 mm	2 mm	1 mm	3 mm	3 mm	4 mm	6 mm
<i>B. subtilis</i>	1.5 mm	4 mm	2 mm	2 mm	1 mm	1 mm	3 mm	-
<i>S. typhi</i>	-	-	-	1 mm	-	1 mm	1 mm	-
<i>Pseudomonas</i>	-	1 mm	0.5 mm	-	0.6 mm	2 mm	2 mm	3 mm
<i>Klebsiella</i>	0.1 mm	0.1 mm	0.1 mm	0.1 mm	-	-	0.1 mm	0.1 mm
<i>S. epidermitis</i>	-	4 mm	-	2 mm	3 mm	3 mm	3 mm	3 mm

Aloe Vera leaves contain a range of biologically active compounds, the best-studied being acetylated mannans, polymannans, anthraquinone C-glycosides, anthrones and anthraquinones, and various lectins. Aloe Vera has multiple uses as laxative, antihelminthic, hemorrhoid remedy, and uterine stimulant. It is used often in combination with licorice root, to treat eczema or psoriasis (Robson et al, 1982). A. Vera possesses antifungal, antiviral and antibacterial activity against skin infections such as acne, herpes and scabies (Haller et al, 1991 and Mantle et al, 2001). The bacterial strains involved in this project may cause many type of ailments in humans. Therefore, in many countries there are many folks using medicinal plants to treat such type of diseases caused by bacteria.

5. Conclusion

The present study has showed the possibility of the presence of some bioactive components in crude extracts of Aloe Vera due to which it has showed strong antibacterial activity. Moreover, the further analysis on bioactive components of Aloe Vera would be suggested by High-performance liquid chromatography (HPLC).

Acknowledgements

Authors wish to thank the staff of Jinnah Hospital, Sheikh Zayed Hospital, Department of Microbiology and Molecular Genetics, University of the Punjab, Lahore for providing bacterial strains and their identification. Special thanks to Dr. Abdul Rehman Niazi (taxonomist) from the department of Botany, University of the Punjab, Lahore.

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