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# ANTIBACTERIAL ACTIVITY OF *ALOE VERA* EXTRACT ON MULTI-DRUG RESISTANT HUMAN PATHOGENS

**Shahnaz Anjum[1*], Urfeya Mirza[2], Shayista Yousuf[3]**

*[1]MSc Scholar, Department of Botany, School of Life Sciences,*
*Baba Farid Institute of Technology, Suddhowala Dehradun, Uttarakhand (India)*
*[2]PhD Scholar, Department of Veterinary Surgery and Radiology,*
*Sher-e-Kashmir University of Agricultural Sciences and Technology, Kashmir (India)*
*[3]MSc Scholar, Department of Zoology, School of Life Sciences,*
*Baba Farid Institute of Technology, Suddhowala Dehradun, Uttarakhand (India)*

**ABSTRACT**

*Aloe vera is a cactus-like perennial, drought resistant and succulent herbal medicinal plant reported to possess anti-microbial, anti-neoplastic, anti-inflammatory, anti-diabetic and immuno-modulatory properties. Considering the vast potentiality of this plant as a source of antimicrobial drugs, this study was aimed to evaluate the therapeutic potentials of Aloe vera plant extract against multi-drug resistant human clinical bacterial isolates - Escherichia coli and Staphylococcus aureus by agar well diffusion method. Ethanolic leaf extract of Aloe vera exhibited broad-spectrum anti-bacterial activity with inhibition zones ranging from 5 to 20 mm in the two separate readings taken. Zone of inhibition varied with five different dilutions (0.25%, 0.50%, 0.75%, 1.0% and a control) and organisms tested. The effectiveness was more against the gram-negative bacteria Escherichia coli than the gram-positive bacteria Staphylococcus aureus. The outcome demonstrates antimicrobial potential of the plant and hence lends support for its use in traditional medicine in the treatment of bacterial infections mostly by gram-negative bacteria. The results of this study were encouraging, despite the need for clinical studies to determine the real effectiveness and potential toxic effects in vivo.*

***Key words: Antimicrobial activity, plant extract, human pathogens, multi-drug resistant, Agar well diffusion method.***

## I. INTRODUCTION

In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world [9,10,11,8,5]. However, the situation is alarming in developing as well as developed countries due to indiscriminate use of antibiotics. Therefore, alternative antimicrobial strategies are urgently needed, and thus this situation has led to a re-evaluation of the therapeutic use of ancient remedies, such as plants [3,20]. Plants are the largest drug stores ever known on earth, by producing endless bioactive chemical compounds which have direct effects on animal and human health [6]. Today, most of the modern drugs (synthetic or semi-

synthetic) are initially produced from natural products such as medicinal plants prescribed in the ancient traditional medicine [19]. Traditional medicine is in practice for many centuries by a substantial proportion of the population of many centuries. It is recognized that in some developing countries, plants are the main medicinal source to treat various infectious diseases. The use of plant extract for medicinal treatment has become popular when people realized that the effective life span of antibiotic is limited and over prescription and misuse of traditional antibiotics are causing microbial resistance [12]. Plant extracts represent a continuous effort to find new compound against pathogens. Approximately 20% of the plants are found in the world have been submitted to pharmacological or biological test, and a substantial number of new antibiotics introduced on the market are obtained from natural or semisynthetic resources [15]. According to World Health Organisation, medicinal plants would be the best source for obtaining a variety of drugs [13]. The use of plant extracts, with known antimicrobial properties, can be of great significance in the treatment of various microbial infections. In the last decade, numerous studies have been conducted in different countries to prove such efficiency in number of medicinal plants. Most of the studies are restricted with crude extracts [4,14].

Atropine, Ephedrine, Digoxin, Morphine, Quinine, Reserpine and Tubocurarine are few examples of medicines invented from the knowledges of the traditional medicine [2]. *Aloe vera* (*A. vera*) is one of these well-known medicinal plants [18]. It is a cactus-like perennial, drought resistant, succulent plant belonging to the Liliaceae family, of which there are over 360 known species. The name is derived from the Arabic word 'alloeh' which means 'bitter', referring to the taste of the liquid contained in the leaves. *Aloe vera* is believed to have originated in the Sudan. It grows in arid climates and is widely distributed in Africa, India and other arid areas. The elongated and pointed leaves of plant contain two distinct products: yellow latex (exudate) and clear mucilaginous gel (*A. vera* gel). *A. vera* gel is revealed after removal of the thick outer cuticle [1]. Its thick leaves contain the water supply for the plant to survive long periods of drought [16]. The leaves have a high capacity of retaining water also in very warm dry climates and it can survive very harsh circumstances. When a leaf is cut, an orange-yellow sap drips from the open end. When the green skin of a leaf is removed a clear mucilaginous substances appears that contains fibers, water and the ingredient to retain the water in the leaf. The gel contains 99.3% of water, the remaining 0.7% is made up of solids with carbohydrates constituting for a large components [16]. Concentrated extracts of Aloe leaves are used as laxative and as a hemorrhoid treatment. *Aloe* gel can help to stimulate the body's immune system [7].

*Aloe vera* was first used in the 1930s to heal radiation burns [21]. To date, there are many reports of its beneficial properties in human, so that it is used for pharmaceutical, food, and cosmetic industries. *Aloe vera* gel has been used in gastrointestinal disorders, sunburn, and wounds since ancient times. Furthermore, various *in vitro* and *in vivo* studies on *A. vera* have demonstrated that it possesses several biological activities, such as anti-inflammatory, antioxidant, immune modulating, and cell growth stimulatory activity as well as antibacterial, antiviral, and antifungal properties [17]. The use of plant product for pharmaceutical purpose has been gradually increased. Many scientific studies of the use of *Aloe vera* have been undertaken, some of them conflicting. Despite these limitations, there is some preliminary evidence that *Aloe vera* extracts may be useful in the treatment of minor skin infections, sebaceous cysts, diabetes, and elevated blood lipids in humans. These positive effects are thought to be due to the presence of compounds such as polysaccharides, mannans,

anthraquinones, and lectins. Considering the vast potentiality of plants as sources for antimicrobial drugs, this study aimed to detect the antibacterial activities of *Aloe vera* plant extract against multi-drug resistant human clinical bacterial isolates (*Escherichia coli* and *Staphylococcus aureus*).

## II.MATERIALS AND METHODS

### 1. Collection of plant material

*Aloe vera* leaves were collected during the month of March 2016 from Suddhowala Dehradun (Uttarakhand, India). The plant was deposited in the respective department of botany for proper identification and was authenticated by a taxonomist.

**Fig.1:** Plant of *Aloe vera*

### 2. Preparation of extract

The collected mature and fresh leaves of *A. vera* were thoroughly washed with distilled water to remove soil and unwanted dust particles. The leaves were shade dried, and then powdered using a blender. 1.5 grams of the sample were taken in a beaker with 3.5 ml of 96% ethyl alcohol. Ratio of leaf powder and alcohol was 1:3 (w/v). Then the beaker was kept at normal room temperature. After 48 hrs, the extract was filtered and stored in a sterile volumetric flask and preserved in refrigerator for further use.

**Fig.2:** Preparation of plant extract

### 3. Collection of bacterial isolates

Human clinical bacterial isolates of presumptive *Staphylococcus aureus* and *Escherichia coli* were collected from Synergy Hospital, Dehradun, India and aseptically transferred to the project Laboratory. After collection, all the isolates were labeled, sub cultured and placed in an incubator for further use.

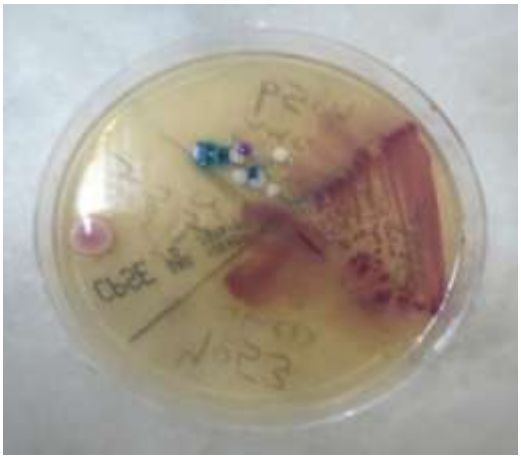

**Fig.3:** Cultured *Escherichia coli*

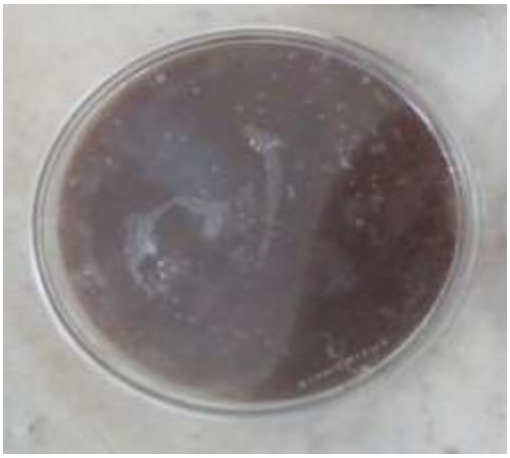

**Fig.4:** Cultured *Staphylococcus aureus*

### 4. Preparation of the nutrient agar medium and plating

500 ml of the nutrient agar medium was prepared from nutrient broth (6.5 gram) and agar powder (12.5 grams). The medium was then transferred into an Erlenmeyer flask and autoclaved for 15 minutes. After autoclaving, the nutrient agar medium was plated onto the petri-plates carefully. The petri-plates were kept as such for few minutes till the medium began to solidify for further use.

**Fig.5:** Plating of the nutrient-agar medium

### 5. Agar well diffusion method

Nutrient agar medium plates were swabbed with broth culture of respective bacteria (spreading). Wells (10 mm diameter and about 2 cm apart) were made in each of these plates using sterile cork borer. Five different dilutions (0.25%, 0.50%, 0.75%, 1.0% and a control) were made from the plant extract and then placed in test tubes. About 0.3ml of each dilution of the plant solvent extract was added into each well using a sterile syringe and allowed to diffuse at room temperature for 2 hours. The control experiments comprising inoculums without plant extract were set up. The plates were inverted and placed in an incubator at $37^0$c for 18-24 hrs for bacterial pathogens. The diameter of the inhibition zone (mm) was measured. Duplicates were maintained and the experiment was repeated.

**Fig.6:** Creation of wells using a cork borer

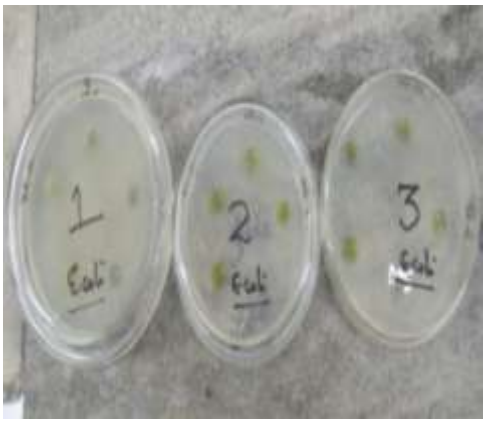


**Fig.7:** Wells filled with five dilutions of the extract

### 6. Antimicrobial activity of plant extract to clinical isolates

The antimicrobial potential of the experimental plant was evaluated according to its zone of inhibition against various pathogens. The results revealed that *Aloe vera* extract is a potent antimicrobial against both the microorganisms studied. The diameter of the zone of inhibition was measured in millimeter (mm) using a measuring scale.

## III.RESULTS AND DISCUSSIONS

Antibacterial activity of *Aloe vera* belonging to Liliaceae family was evaluated in vitro against two multi-drug resistant clinical bacteria which are known to cause wide range of diseases and infections in humans like pneumonia, bacteremia, peritonitis, cholecystitis, septic wounds and bedsores including meningeal, gastrointestinal, urinary tract and bacteremia infections. This plant is known to have healing properties against various diseases in humans and was found to exhibit potential antimicrobial properties against the isolated human clinical bacterial isolates.

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**Measurement of antimicrobial activity using Agar well diffusion method**

The antimicrobial potential of *Aloe vera* was evaluated according to its zones of inhibition against the two pathogens. The results revealed that the extract is a potent antimicrobial against all the microorganisms (*Escherichia coli* and *Staphylococcus aureus*) studied. The antibacterial activity of the extract and its potency was assessed by the presence or absence of inhibition zones. Results of the study are shown in table 1.

**Table 1: Diameter of zone of inhibition of *Aloe vera* extract against *Escherichia coli* and *Staphylococcus aureus* at different dilutions.**

| | **Zone of inhibition (mm) shown by *Aloe vera*** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | ***Escherichia coli*** | | | | | ***Staphylococcus aureus*** | | | | |
| | **Dilutions** | | | | | **Dilutions** | | | | |
| | **0.25** | **0.50** | **0.75** | **1.0** | **C** | **0.25** | **0.50** | **0.75** | **1.0** | **C** |
| Reading 1 | 9.0 | 11.0 | 13.0 | 16.0 | 5.0 | 5.0 | 6.0 | 7.0 | 9.0 | 5.0 |
| Reading 2 | 10.0 | 15.0 | 18.0 | 20.0 | 5.0 | 10.0 | 12.0 | 14.0 | 15.0 | 5.0 |

**Fig.8: Chart showing the antibacterial activity of *Aloe vera* extract against *Escherichia coli* (Reading 1)**

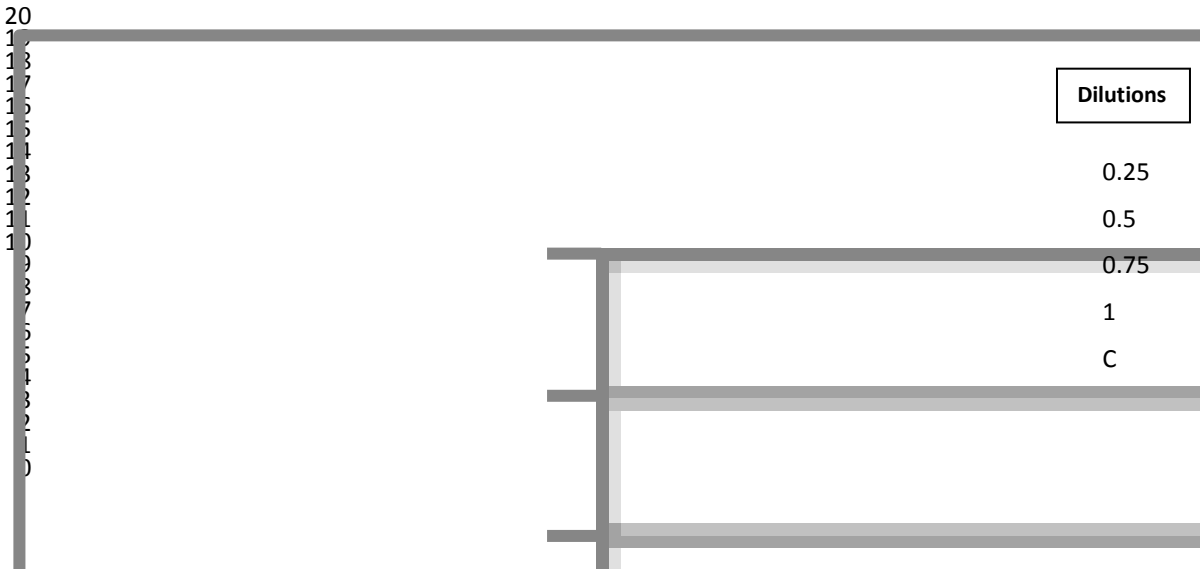


**Fig.9: Chart showing the antibacterial activity of *Aloe vera* extract against *Staphylococcus aureus* (Reading 1)**

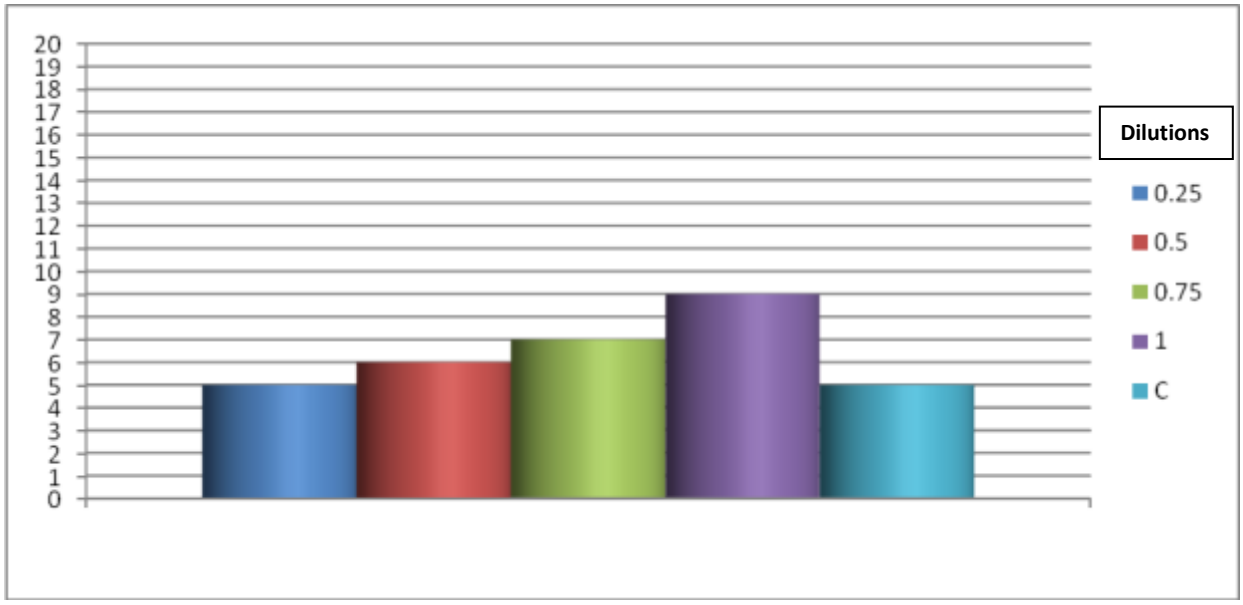



**Fig.10: Chart showing the antibacterial activity of *Aloe vera* extract against *Escherichia coli* (Reading 2)**

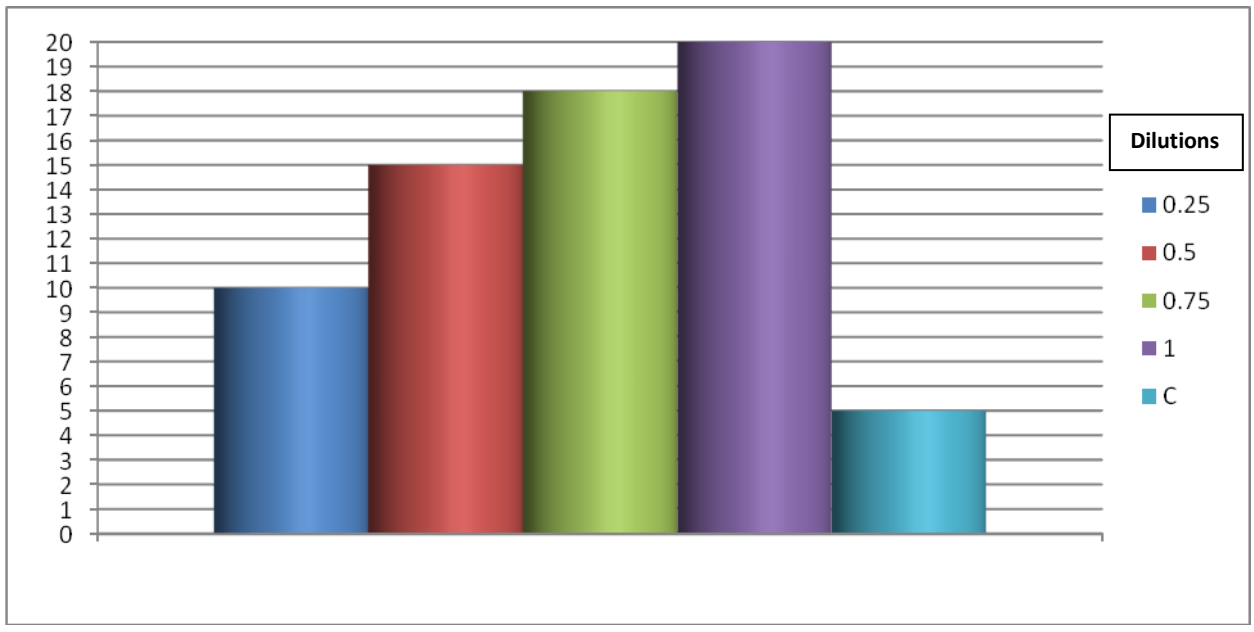
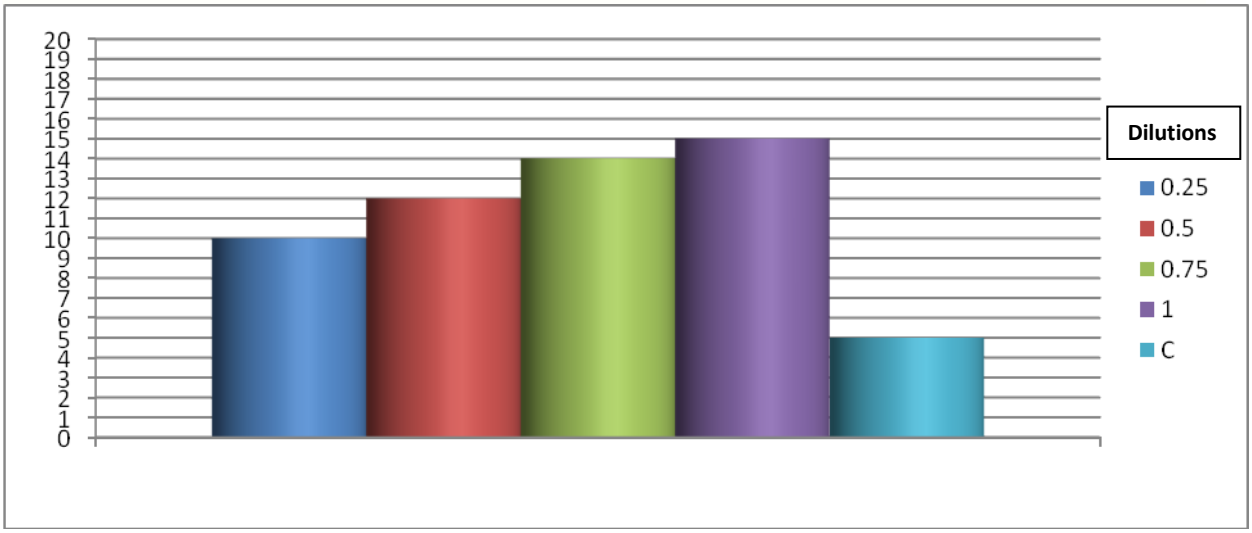



**Fig.11: Chart showing the antibacterial activity of *Aloe vera* extract against *Staphylococcus aureus* (Reading 2)**




From the above results, it is clear that *Aloe vera* leaf extract exhibits antibacterial activitiy against both gram-positive as well as gram-negative bacteria. And also it is evident that the inhibition was concentration dependent. In both the readings, maximum inhibition zones were shown by the Aloe extract at 1.0 dilution. The maximum zone of inhibition (20mm) was shown against *Escherichia coli* (Reading 2). All the dilutions of plant extract showed positive effects against both the pathogens; however, 1.0 dilution appeared to be highly active. This study ascertains the value of *Aloe vera* used in ayurveda, which could be of considerable interest to the development of new drugs.

## IV.CONCLUSION

The present study has revealed the importance of natural products to control antibiotic resistant bacteria, which have been a threat to human health. It is, therefore highly essential that medicinal plants whose properties have not been fully characterized should form a top agenda of top management in developing nations whose citizens are sometimes unable to afford expensive orthodox medicine. This study has confirmed that the *Aloe vera* extract could be used for the treatment of various infections including skin transmitted infections. The results lend credence to the folkloric use, if this plant in treating microbial infection and shows that *Aloe vera* could be exploited for new potent antimicrobial agents. Hence, it can be concluded that the leaf extract of *Aloe vera* can effectively act as an antimicrobial agent and also has the ability to replace most of medium medicines of this era.

Further investigations are required to identify bioactive components present in *A. vera* and its effect on wide range of bacteria and fungus including the pathogenic strains. It is hoped that this study would lead to the development of aloe plant usage as a main medicinal source to treat various infectious diseases.

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