Assessment of *in vitro* antibacterial activity of *Aloe barbadensis* leaf extracts

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Abstract

The antibiotics, 20th century wonder drug, were first developed in 1935 and thereafter, many antibiotics came in the market to treat, prevent and control the bacterial diseases. However, with the passage of time and following natural law of survival of the fittest, many bacteria developed resistance against these antibiotics and situation became so worrisome that at present many superbugs are present in the environment on which presently available antibiotic drugs are not having any effect. The antibiotic resistance to microbes leads to severe consequences. Infections caused by resistant microbes fail to respond to treatment resulting in prolonged illness and greater risk of death, longer periods of hospitalization and infections which increases the number of infected people moving in the community. When an infection becomes resistant to first line antibiotic, treatment has to be switched to second or third line drugs, which are always much more expensive and sometime more toxic as well. In poor countries, where many of the second or third line therapies for drug resistant infections are not available, making the potential of resistance to first line antibiotics considerably greater. The limited number of antibiotics in these countries are becoming increasingly inadequate for treating infections and necessary antibiotics to deal with infections caused by resistant pathogens are absent from essential drug list. Medicinal plants are a source of great economic value all over the world. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. In plants, these compounds are mostly secondary metabolites such as alkaloids, steroids, tannins, and phenol compounds, flavonoids, steroids, resins fatty acids gums which are capable of producing definite physiological action on body. Compounds extracted from different parts of the plants can be used to cure diarrhoea, dysentery, cough, cold, cholera, fever bronchitis etc. This study was planned for the *in vitro* assessment of various extracts of *Aloe barbadensis* plant leaves for their antibacterial activity. Extracts of the *Aloe barbadensis* were prepared in water, ethanol, methanol etc. It has been observed that these extracts showed very promising results as indicated by the zone of inhibition of bacterial culture through agar well diffusion method that varies from few mm to few cm. The bacteria used in this study were common pathogen of human and animals and were resistant to the commonly available antibiotic drugs. This study indicates the *in-vitro* antibacterial effects of *Aloe barbadensis* extracts which further needs confirmation and validation of its antibacterial effect both in *in-vitro* and *in-vivo*.

Key words: *Aloe barbadensis,* Leaf extracts, Antibacterial sensitivity, Agar gel diffusion test.

Introduction

Infectious diseases are the leading cause of death world-wide. Antibiotic resistance has become a global concern (Westh *et al.,* 2004). The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens (Bandow *et al.,*2003). Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads, because of the unmatched availability of chemical diversity. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases (Rojas *et al.,* 2003). Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections (Benkeblia, 2004). The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial

infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity (Colombo and Bosisio,1996).

Antibiotics are drugs that destroy or inhibit the growth of bacteria in concentrations that are safe for the host and can be used as chemotherapeutic agents to prevent or treat bacterial infections. Antibiotic resistance occurs when bacteria change in some way that reduces or eliminates the effectiveness of drugs, chemicals or other agents designed to cure or prevent the infection. Thus the bacteria survive and continue to multiply causing more harm. Widespread use of antibiotics promotes the spread of antibiotic resistance. Bacterial susceptibility to antibacterial agents is achieved by determining the minimum inhibitory concentration that inhibits the growth of bacteria. Resistance is defined as bacteria that are not inhibited by usually achievable systemic concentration of an agent with normal dosage schedule and/ or fall in the minimum inhibitory concentration ranges. Likewise the multiple drug resistance is defined as the resistance to two or more drugs or drug classes. Acquisition of resistance to one antibiotic conferring resistance to another antibiotic, to which the organism has not been exposed, is called cross resistance. An antibiotic has to go through a number of steps in order to exert its antibacterial action. First of all it has to enter the cells (influx). Once inside, it has to remain stable and accumulate to inhibitory concentrations. In some cases it has to be activated to an active form. Finally it has to locate and interact with its target(s) to exert its action. Alterations in any one or more of these processes can render the cells resistant to the antibiotic.

The antibiotic resistance to microbes leads to severe consequences. Infections caused by resistant microbes fail to respond to treatment resulting in prolonged illness and greater risk of death, longer periods of hospitalization and infections which increases the number of infected people moving in the community. When an infection becomes resistant to first line antibiotic, treatment has to be switched to second or third line drugs, which are always much more expensive and sometime more toxic as well. In poor countries, where many of the second or third line therapies for drug resistant infections are not available, making the potential of resistance to first line antibiotics considerably greater. The limited number of antibiotics in these countries are becoming increasingly inadequate for treating infections and necessary antibiotics to deal with infections caused by resistant pathogens are absent from essential drug list.

Medicinal plants are a source of great economic value all over the world. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country (Nadkarni *et. al.*,1976; Tandon *et.al.*,2004). Nowadays multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions (Chauhan and Singh, 2000). This situation forced scientists to search for new antimicrobial substances. Given the alarming incidence of antibiotic resistance in bacteria of medical importance, there is a constant need for new and effective therapeutic agents. According to World Health Organisation by 2020 most of the micro-organisms will become resistant against antibiotics so there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants. Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials (Chauhan *et al.*,2001).

Antibiotic resistance occur when bacteria change in some way that reduces or eliminates the effectiveness of drugs or agents designed to cure or prevent the infection, thus the bacteria survive and continue to multiply causing more harm. One of the factor for the cause is the increasing phenomenon of acquisition of resistance among micro-organism towards antimicrobial drugs is attributed due to their indiscriminate and improper use of current antimicrobial. Misuse and over usage of antibiotics is causing major downfall in our society. The defence mechanism of our body is so beautifully design that the cell present act itself against the micro-organism WBC often play ancillary role in engulfing and destroying of micro-organism. Likewise there are macrophages are capable of ingesting exogenous and endogenous matter but due to pollution of heavy metal, mycotoxins, and pesticides macrophage does not help in fighting against the disease and people easily

catches the infection. The drugs which we take has bacteriostatic effect it does not kill the micro-organism due to which recurrent infection takes place and the person become resistant against the antibiotic (Chauhan and Tripathi , 2002). The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. In plants, these compounds are mostly secondary metabolites such as alkaloids, steroids, tannins, and phenol compounds, flavonoids , steroids, resins fatty acids gums which are capable of producing definite physiological action on body (Chopra *et.al*., 1982). Compounds extracted from different parts of the plants can be used to cure diarrhoea, dysentery, cough, cold, cholera, fever bronchitis etc.

This study was planned for the *in vitro* assessment of various extracts of *Aloe barbadensis* plant leaves for their antibacterial activity.

Materials and methods

The leaves of *Aloe barbadensis* grown in the gamla without using any chemical fertiliser and/ or pesticide were collected and were washed under running tap water, air dried, cut into pieces and ground into fine powder and stored in airtight bottles. 5gms of powdered material was extracted by cold and hot maceration method successively with 150 ml of distilled water for 2 days in sterile conical flask. The extracts were filtered using Whatman filter paper No1. The filtrates were then evaporated at 40˚C and stored in air tight bottles. For solvent extraction, 5 gm of powdered material was taken in the solvents viz. hexane, acetone, chloroform, methanol and ethanol using the Soxhlet apparatus at a temperature of 30 to 35˚C. The filtrates were then evaporated at 40˚C and stored in separate air tight bottles. The plant extracts were weight in different concentration of 100mg/ml, 75mg/ml, 50mg/ml and 25mg/ml. The samples were dissolved in DMSO with the above concentration. The pyogenic bacterial cultures of *Staphylococcus aureus* were used for antibacterial activity. These bacterial strains were obtained from Institute of Microbial Technology, Chandigarh and were maintained by culturing them in nutrient agar weekly. A single colony from pure growth of test organism was transferred to 5 ml of Mueller-Hinton broth. The broth was incubated at 37ºC for 24 hours. The standardized inoculum suspension was inoculated within 15-20 minutes.

The antimicrobial assay of the plant leaf extracts were tested on microbial strain by agar gel diffusion method by Kirby-Bauer. In the agar well diffusion method 100µl of 24 hr broth culture of bacteria was aseptically and evenly swabbed on Mueller Hinton agar Wells of about 8mm diameter were aseptically cut using sterile cork borer. 100 µl of plant extracts of different concentration were then placed into the well. The plates were incubated at 37 ºC for 24hr. Microbial growth was determined by measuring the diameter of zone of inhibition (Rathore and Chauhan, 2008).

Results

This study shows that the leaf extracts of *Aloe barbadensis* have potent antibacterial activity. Antibacterial activities were observed using the agar well diffusion technique against the gram-positive bacteria *Staphylococcus aureus* and gram-negative bacteria, *Escherichia coli*. The sensitivity test was carried out with positive control i.e ciprofloxacin. The zones of inhibition were measured for each plant extract in mm using scale and were presented in table 1 and 2 along with different concentration of 100, 50 and 25mg/ml of the *Aloe barbadensis* plant leaf extracts.

The hot water extract of *Aloe barbadensis*, showed zone of inhibition 19,16 and 14mm for *E.coli* by using 100, 50 and 25mg/ml concentration of each extract, respectively. The cold water extract of *Aloe barbadensis* showed zone of inhibition 22, 19 and 15mm for *E.coli* by using 100, 50 and 25mg/ml concentration of each extract, respectively. The zone of inhibition as 26, 24 and 20 mm for *E.coli* by using 100, 50 and 25mg/ml concentration of ethanol extract of *Aloe barbadensis* were recorded. The methanol extract of *Aloe barbadensis* showed zone of inhibition 15,14 and 12mm for *E.coli* by using 100, 50 and 25mg/ml concentration of each extract. Similarly, the acetone extract of *Aloe barbadensis*, showed zone of inhibition 22, 20 and 18mm for *E.coli* by using 100, 50 and 25mg/ml concentration of each extract, respectively (Fig.1.).

The hot water extract of *Aloe barbadensis* showed zone of inhibition 18,17 and 16mm for *S. aureus* by using 100, 50 and 25mg/ml concentration of each, respectively. The cold water extract of *Aloe barbadensis* showed zone of inhibition 19,18 and 16mm for *S.aureus* by using 100, 50 and 25mg/ml concentration of each, respectively. The zone of inhibition 16,15 and 13mm for *S. aureus* were recorded by using 100, 50 and 25mg/ml concentration of ethanol extracts of *Aloe barbadensis*. The methanol extract of *Aloe barbadensis* showed zone of inhibition 18,17 and 17mm for *S. aureus* by using 100, 50 and 25mg/ml concentration of each extract, respectively while the acetone extract of *Aloe barbadensis* showed zone of inhibition 17,18 and 16 mm for *S. aureus* by using 100, 50 and 25mg/ml concentration of each extract, respectively.

Discussion

Aloe barbadensis belongs to the family *Lilaceae* and contain over 75 nutrients and 200 active compounds, including vitamins, enzymes, minerals, sugar, lignin, anthraquinones, saponins, salicylic acid and amino acids (Park & Jo, 2006). *Aloe barbadensis* has been shown to have anti-inflammatory (Afzal *et al*., 1991; Malterud *et al*., 1993), immunostimulatory (Ramamoorthy & Tizard, 1998) and cell growth stimulatory activity. Furthermore, activity against a variety of infectious agent has been attributed to *Aloe barbadensis*; for instance antiviral and antifungal. There are limited reports on the antimicrobial effects of isolated *Aloe barbadensis* components. Ferro *et al*., (2003) have shown that *Aloe barbadensis* leaf gel can inhibit the growth of Gram positive bacteria due to the presence of Gram positive bacteria specific plant compound anthraquinones (Gracia-Sosa *et al*., 2006; Dabai *et al*.,2007) and dihydroxyanthraquinones (Wu *et al*., 2006), as well as saponins (Reynolds and Dweck, 1999), which have been proposed to have direct antimicrobial activity. The anthraquinones have been reported to have antiviral activities also to some viruses, such as human cytomegalovirus, herpes simplex virus type 1 and poliovirus (Bernard *et al*., 1992; Semple *et al*., 2001).

During the process of evolution, nature has provided a defence mechanism in each and every living being, which protects them from the extraneous injuries and diseases. Among extraneous cause of diseases, bacteria constitutes a major part of human and animal diseases because of the fact that they directly cause the disease and also indirectly through their metabolites and as secondary and opportunistic pathogens. Normally, there are many infections cause diseases in man and animals but that is controlled by either through the para specific immunity present in the body, particularly in the form of macrophage phagocytic system or through the antibiotic therapy. The antibiotics, 20th century wonder drug, were first developed in 1935 and thereafter, many antibiotics came in the market to treat, prevent and control the bacterial diseases. However, with the passage of time and following natural law of survival of the fittest, many bacteria developed resistance against these antibiotics and situation became so worrisome that at present many superbugs are present in the environment on which presently available antibiotic drugs are not having any effect (Chauhan and Rana 2010).

There has been an increase in demand for the Phytopharmaceutical products of Ayurveda in the world particularly in western countries, due to the fact that the allopathic drugs might provide instant relief from the ailment but have serious side effects. Many pharmaceutical companies are now concentrating on manufacturing of Ayurvedic phytopharmaceutical products (Anpin Raja *et.al.*,2011). There are a wide range of infectious diseases which can be cured by using medicinal plants without any side-effect (Srinivasan *et.al*., 2001). The herbal drugs are easily available in the market and people can afford them easily as they are cost effective than the synthetic drugs which are high in cost.

In the year 1998, under the auspicious aegis of WHO, an international symposium was organized at Sir Eric Mann Institute of Public Health at Hague, The Netherlands in which the main theme of discussion was that "The 20th Century wonder drug antibiotics are no more effective, then what are the alternatives to control the bacterial infections in 21st century". Thereafter, this topic was very hotly discussed at may forums of National and International conferences to find a reasonable solution. As per experts, by the year 2020, most of the antibiotics will become useless because of the fact of development of resistance in the bacteria and severe side effects in the host. Besides, in general there is occurrence of immunodeficiencies, either in one or another component of the immune system which also lead to the weakness in macrophage phagocytic system

(Singh *et.al*., 2002; Saxena *et.al*., 2006). Needless to mention, the present available antibiotics are of two types, bacteriostatic and bactericidal. Bacteriostatic antibiotics check the growth of bacteria and do not kill them while the remaining bacteria are to be destroyed by the macrophage phagocytic system. However, this system becomes weak due to the presence of several kinds of contaminants and pollutants in the food chain (Chauhan and Singh, 2000) and is not able to cope up with the strength of the bacteria and that led to the severe bacterial infections, recurrent and persistent bacterial infections, occurrence of new diseases caused by very low pathogens or opportunistic pathogens, occurrence of severe secondary bacterial and viral infections etc (Chauhan and Singh, 2001). Considering the severity and magnitude of the problem, it was thought to initiate research programme on the development of such antibacterial preparation which can kill the bacteria or prevent their growth and simultaneously boost the immunity, including providing strength to the macrophages for both engulfment power as well as bactericidal power; the present communication is first step towards such study. Extracts of the *Aloe barbadensis* were prepared in water, ethanol, methanol etc. It has been observed that these extracts showed very promising results as indicated by the zone of inhibition of bacterial culture through agar well diffusion method that varies from few mm to few cm. The bacteria used in this study were common pathogen of human and animals and were resistant to the commonly available antibiotic drugs. This study indicates the *in-vitro* effect of *Aloe barbadensis* extracts which further needs confirmation and validation of its antibacterial effect both in *in-vitro* and *in-vivo*.

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Table:1. Antibacterial sensitivity (as mm zone of inhibition) of various extracts of *Aloe barbadensis* on *E coli*

Extracts/ Concentration of Aloe *barbadensis* (mg/ml)	100mg/ml	50mg/ml	25mg/ml
Hot water	19	16	14
Cold water	22	19	15
Ethanol	26	24	20
Methanol	15	14	12
Acetone	22	20	18

Fig. 1. Antibacterial sensitivity of various extracts of *Aloe barbadensis* on *E coli*

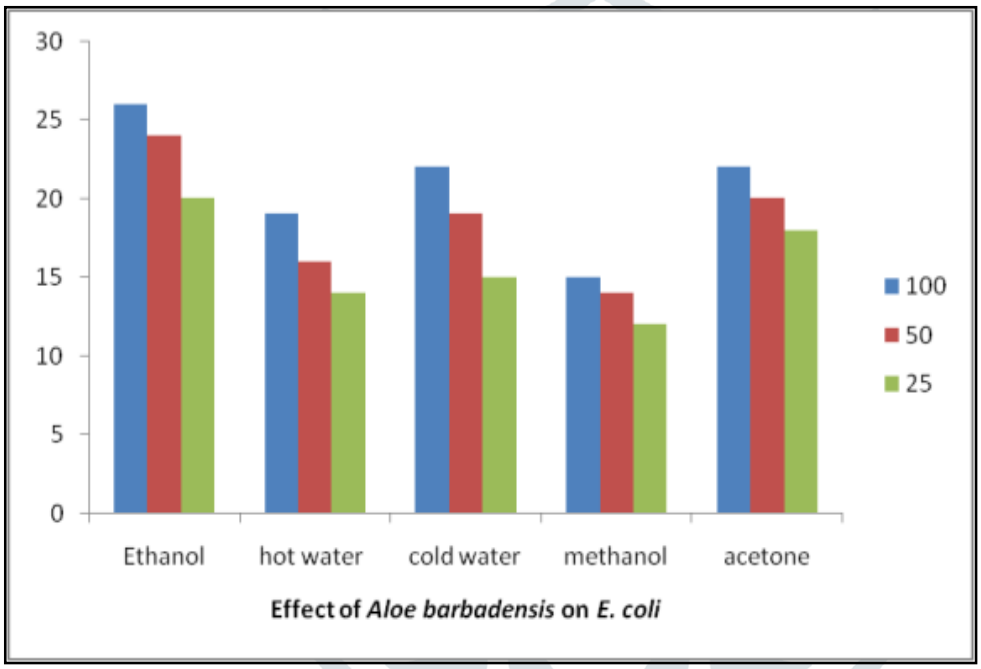

Table:2. Antibacterial sensitivity (as mm zone of inhibition) of various extracts of *Aloe barbadensis* on *Staphylococcus aureus*

Extracts/ Concentration of Aloe *barbadensis* (mg/ml)	100mg/ml	50mg/ml	25mg/ml
Hot water	18	17	16
Cold water	19	18	16
Ethanol	16	15	13
Methanol	18	17	17
Acetone	17	18	16

Fig.2. Antibacterial sensitivity of various extracts of *Aloe barbadensis* on *Staphylococcus aureus*