Antimicrobial activity of _Aloe vera_ (L.) Burm. f. against pathogenic microorganisms

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Abstract

Plants play a major role in all the traditional system of medicine. Plants contain the rich source of natural products like vitamins, minerals and other immune-modulators. Most of which have been used for human welfare especially to cure disease caused by pathogenic microorganisms without any side effects. The present 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 (_B. subtilis, S. aureus_), Gram negative bacteria (_E. coli, K. pneumoniae, P. aeruginosa_). The disc diffusion method was used to test the antimicrobial activity. The result showed that more antimicrobial activity in ethyl acetate (1 – 9 mm) and ethanol extract (7 – 12 mm). The least inhibitory effect on petroleum ether extracts was 2 mm. This study was also estimate the amount of minerals present in fresh Aloe Vera juice by Atomic Absorption Spectroscopy. This is important to use of Aloe Vera for medications, cosmetics and food purpose.

Key words: Antimicrobial, pathogens, _Aloe vera_, medicinal plants

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Introduction

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. 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 (Mothana and Linglequist, 2005).

The use of plant product for pharmaceutical purpose has been gradually increased. According to World Health Organisation, medicinal plants would be the best source for obtaining a variety of drugs (Santos _et al._, 1995).

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 (Reddy _et al._, 2006; Erdo Urul, 2002; Atefl _et al._, 2003).
Aloe vera is a perennial, drought resisting, succulent plant. It has stiff green, lance-shaped leaves containing clear gel in a central mucilaginous pulp. Its thick leaves contain the water supply for the plant to survive long periods of drought (Foster, 1999). 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 in 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 fibres, 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 (Foster, 1999).

Concentrated extracts of Aloe leaves are used as laxative and as a haemorrhoid treatment. Aloe gel can help to stimulate the body’s immune system (Davis, 1997). The aloe plant contains different nutrient contents including vitamins, minerals, enzyme, sugars, phenolic compounds, lignin, saponins, sterol and aminoacid. Aloe vera contains many vitamins excluding vitamin D but including the important antioxidant vitamin A, C and F. Vitamin B (thiamine) B3 (Niacin), B2 (Riboflavin), choline and folic acid are also present. A trace of vitamin B12 also present (Coats, 1979). Vitamin B complex and C are to play an important role in reducing stress and inflammation. Aloe contains the enzymes such as amylase, lipase and carboxypeptidase. Lipase can digestion by breaking down fats and sugars. Amylase hydrolyse starch to liberate dextrin. The activity of serum amylase is increased in acute pancreatitis. The peak value of amylase is observed within 8 – 12 hours after the onset of disease which returns to normal by 3rd or 4th day. The pancreatic carboxypeptidase is metalloenzymes that are dependent on Zn⁺ for their catalytic activity i.e., also called Zn proteases. It inactivates bradykinins and produces an anti-inflammatory effect. During the inflammatory process, bradykinin produces pain associated with vasodilation and its hydrolysis to produce an analgesic effect (Obata, 1993; Shelton, 1991).

Aloe plant contains 25 per cent of solid fraction that contain sugars. The sugars are found in the mucilage layer of the plant surrounding the inner gel. It comprises both monosaccharides and polysaccharides. Sugar acts as immuno-modulators capable of enhancing and retarding the immune response (Green, 1996; Kahlon et al., 1991; Sheets, 1991). Anthroquinone is a phenolic compound found in the sap. The bitter Aloe consists of free anthraquinones and their derivatives like Barbaloain-IO-aloe- emodin-9-anthrone, Isobarbaloin, Anthrone-C-glycosides and chromones. These compounds exert a powerful purgative effect, which are potent antimicrobial agents and possess powerful analgesic effects (Lorenzetti et al., 1964; Sims et al., 1971). Aloe contain saponins which are soapy substances form 3 per cent of the gel and are general cleansers, having antiseptic and anticarcinogen properties (Hirat and Suga, 1983). Aloe contains Campesterol, F2 Sitosterol and Lupeol (Coats, 1979). It is an aspirin like compound present in Aloe plant possessing anti-inflammatory and anti-bacterial properties. Topically, it has a ketolytic effect which helps to debride a wound of necrotic tissue. Aloe vera gel provides 20 of the 22 necessary amino acids required by the human body. There are 7 of the 8, non-essential amino acids are Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Theronine and Valine. The 12 essential amino acids are Alamine, Arginine, Asparagine, Cystenine, Gycine, Glutamic Acid, Histidine, Proline, Serine, Tyrosine, Glutamine and Aspartic Acid. Minerals are defined as natural
components formed through geological processes needed in small amounts to regulate body functions. Minerals found in *Aloe vera* are calcium, zinc, chromium, potassium, etc. Magnesium lactate inhibits histidine decarboxylase and prevents the formation of histamine from the amino acid histidine (Shelton, 1991).

**Materials and Methods**

**Sample collection**

The plants were collected in Pavalamalaipatti near M. Pudupatti, Virudhunagar district, Tamil Nadu (INDIA). The area of investigation lies approximately between 77° 30' and 78° 20' longitude and 10° 05' – 10° 09' latitude. The elevation of the area of investigation range is 106m asl. Variations in the altitude and rainfall have a bearing on the vegetation in general. The floristic divisions of the area of investigation consists of dry deciduous forest, deciduous thorn forest.

**Collection of Plant Material**

The collected plant gel was freeze dried and then grinds to get crude extract. The crude extract is filtered through Whatmann filter paper. The plant extracts were prepared according to the method described by Ahmad *et al.*, (1998) with minor modifications. Briefly 1 gm gel extract was mixed in 5 ml of ethanol and mixed well and kept it under shaker for overnight (Lin *et al.*, 1999). After overnight incubation the mixture was filtered through Whatmann No. 1 paper and it was evaporated at room temperature. After evaporation, pellet was resuspended with 0.5 ml of Di Methyl Sulpho Oxide (DMSO) using micro syringe and recollect it for further use. Plant powder residue left after ethanol extraction was sequentially extracted with ethyl acetate, hexane and petroleum ether and obtained the extract by above mentioned method.

**Selection of Solvent**

Various solvents from polar to non-polar were chosen for the plant extraction. Non-polar solvents such as hexane (Sundar *et al.*, 2008), ethyl acetate (Sankaranarayanan *et al.*, 2008), petroleum ether, polar solvents such as ethanol (Gulluce *et al.*, 2008) were taken for this study.

**Bacterial Strains**

Bacterial strains were obtained from the Department of Microbiology, Ayya Nadar Janaki Ammal College (Autonomous), Sivakasi. The bacterial strains such as *E. coli*, *Bacillus*, *Klebsiella*, *Pseudomonas* and *Staphylococcus* were used for antimicrobial assay. All the strains were grown in LB medium contains beef extract, peptone, sodium, yeast, distilled water at pH 7.2 and incubated at 37°C for overnight. 1 OD culture of overnight grown cultures was used for antimicrobial assay.

**Disc Preparation**

The disc was used for evaluating the antimicrobial activity. The disc was prepared from the Whatmann No. 1 filter paper and it was stored at 4°C in dark place for further use. The size of the disc was 5 mm. The absorption capacity of the disc is 0.008 µl per dip in distilled water. This absorption capacity will vary according to the solvent density, dissolving capacity of extracts and compounds involved in it.

**Testing of Antimicrobial Activity**

The antimicrobial activity of *Aloe vera* extracted with different organic solvents was determined by disc diffusion method. 0.5 ml of each 0.1 OD overnight grown evaluated against *Aloe vera* plant extract by pour plate method. Briefly, 1% test culture was mixed with 100 ml nutrient agar at 40 to 45°C and dispensed into the sterile petriplates in aseptic conditions. The medium was allowed to solidify. Then the bacterial cultures were spread on the nutrient agar medium.
The disc was in the respective organic solvents for 1 or 2 seconds and evaporated in the air. Another disc was dipped in the plant extract of different solvent and allowed to evaporate in the air and then placed the petriplate.

The plates were incubated at 37°C for 24 hours and the clear zone developments were closely monitored. After incubation, the antibacterial activity of the Aloe vera extracts with different solvents against the microbes to be assessed by the diameter of the growth inhibition zone formed.

**Estimation of Mineral Content**

Take an Aloe plant and remove the outer green layer and get the central clear gel. Grind the gel very well. The homogenate was centrifuged at 5000 rpm for 15 minutes. Collect the supernatant and filtered through Whatmann filter paper. Get the purified sample without dust or turbidity and estimate the amount of mineral content by Atomic Absorption Spectrophotometer (AAS).

**Results and Discussion**

After standardization of various solvent extracts against Aloe vera the antibacterial activity of the Aloe vera extract with Hexane, Ethyl acetate, Petroleum ether, Ethanol was tested against E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Bacillus subtilis and Staphylococcus aureus. The maximum inhibition was observed against Klebsiella pneumoniae in all the four solvents (2 mm – 9 mm).

**Table-2 Antibacterial activity of the Aloe vera against bacterial pathogens by disc diffusion method**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Test organisms</th>
<th>Petroleum ether</th>
<th>Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>E. coli</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>K. pneumoniae</td>
<td>2 mm</td>
<td>8 mm</td>
</tr>
<tr>
<td>3.</td>
<td>P. aeruginosa</td>
<td>-</td>
<td>7 mm</td>
</tr>
<tr>
<td>4.</td>
<td>B. subtilis</td>
<td>-</td>
<td>11 mm</td>
</tr>
<tr>
<td>5.</td>
<td>S. aureus</td>
<td>-</td>
<td>12 mm</td>
</tr>
</tbody>
</table>

**Maximum Inhibition**

Among the two extracts of Aloe vera such as Ethyl acetate, Ethanol exhibited maximum antibacterial activity against the gram positive and gram negative bacteria. The minimum inhibition was observed against Klebsiella pneumoniae in all the four solvents (2 mm – 9 mm).

**Minimum Inhibition**

The minimum activity was exhibited by petroleum ether. All the organisms were resistant to petroleum ether extract except Klebsiella. Mostly all solvents exhibited negligible activity against E. coli.

The antimicrobial activities of Aloe vera with different solvent extracts against pathogenic microorganisms being discussed below:

The present study was undertaken to assay the antibacterial activity of Aloe vera, the medicinally important plant growing as a weed. It was used against some human pathogenic bacteria such as Bacillus subtilis, Escherchia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae.
In the present study, ethanol extract exhibited significant antibacterial activity against *B. subtilis*, *S. aureus* and moderate activity against *K. pneumoniae*, *P. aeruginosa*. The ethyl acetate and hexane extracts exhibited moderate antibacterial activity against all bacteria, and ethyl acetate showed least activity against *E. coli* (1 mm) and *B. subtilis*. Petroleum ether exhibited least antibacterial activity against only in *K. pneumoniae* (2 mm). In the titled study, the alcoholic extracts of *Aloe vera* gel possess significant inhibitory effect against the tested pathogens. *A. excelsa* leaf material appeared to contain some of the following either one of or a combination of very helpful enzymes, saponins, hormones and amino acids which can be absorbed into the human skin. One of these constituents is acemannan which has been isolated and tested for in *Aloe vera*. Acemannan is a complex carbohydrate and has immune stimulating and antiviral properties (Cappasso *et al*., 1998). Certain lectins which are found in the *Aloe* pith, are assumed to help in the stimulation of immune response by increasing the production of lymphocytes that are known to kill bacteria and some tumour cells (Imanishi *et al*., 1981).

The antimicrobial activity of the extracts and their potency was quantitatively assessed by the presence or absence of inhibition zone and zone diameter. Only alcoholic extract was found to be a better solvent for extraction of antimicrobially active substances compared to water and hexane (Ahmad *et al*., 1998).

Agarry *et al*., (2005) compared the antimicrobial activities of the gel and leaf of *Aloe vera* against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Trichophyton mentagrophytes*, *T. schoeleinii*, *Microsporum canis* and *Candida albicans*. The result showed that both the gel and the leaf inhibited the growth of *S. aureus* (18.0 & 4.0 mm). Only

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### Table 3. Estimation of Mineral content present in *Aloe vera* extract by Atomic Absorption Spectrophotometer (AAS)

<table>
<thead>
<tr>
<th>Minerals</th>
<th>Concentration (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium</td>
<td>640.5071</td>
</tr>
<tr>
<td>Calcium</td>
<td>118.7740</td>
</tr>
<tr>
<td>Copper</td>
<td>1.2822</td>
</tr>
<tr>
<td>Zinc</td>
<td>0.4498</td>
</tr>
<tr>
<td>Manganese</td>
<td>0.3605</td>
</tr>
<tr>
<td>Chromium</td>
<td>0.2754</td>
</tr>
<tr>
<td>Iron</td>
<td>0.2292</td>
</tr>
</tbody>
</table>

Using four different solvents viz., Ethyl acetate, Ethanol, Hexane, Petroleum ether. Of the four solvents, Ethyl acetate and Ethanol extract give the best result against all bacterial pathogens except *E. coli*. Ethanol shows the maximum inhibition (7 – 12 mm) and Ethyl acetate (1 – 9 mm).

Similarly antimicrobial assay of the three selected medicinally important plants, viz., *E. prostrara*, *Indigofera aspalathoides* and *I. tinctoria* were performed against two Gram positive and three Gram negative bacterial pathogens. Acetone and water were used as solvents for further antibacterial assay. Of the two solvents used, water extract of both mycorrhizal and non-mycorrhizal plants expressed more activity than acetone, against all the bacterial pathogens (Sundar *et al*., 2008).

An another experiment conducted with petroleum ether extract exhibited significant antibacterial activity against *B. subtilis*, *S. aureus* and moderate activity against *K. pneumoniae*, *P. aeruginosa*. The chloroform, methanol and ethanol extracts exhibited moderate antibacterial activity against all the seven types of bacteria. The aqueous extract exhibited least antibacterial activity against all the seven types of bacteria (Gavimath *et al*., 2008).
the gel inhibited the growth of *I. mentagrophytes* (20.0 mm), while the leaf possesses inhibitory effects on both *P. aeruginosa* and *C. albicans*.

Similarly, the antimicrobial activity of the *Aloe vera* juice against Gram-positive bacteria (*Mycobacterium smegmatis, Enterococcus faecalis, Micrococcus luteus* and *Bacillus sphericus*), Gram-negative bacteria (*Pseudomonas aeruginosa, Klebsiella pneumoniae, E. coli* and *Salmonella typhimurium*) and *Candida albicans* were also studied. The study showed that *Aloe vera* juice has antimicrobial activity against *M. smegmatis, K. pneumoniae, E. faecalis, M. luteus, C. albicans* and *B. sphericus*, but no inhibitory effect against the other bacterial strains. The least inhibitory effect was found against *M. luteus*, while *C. albicans* was detected to be the most sensitive strain (Suleyman Alemdar et al., 2009). Thus in the present study the antibacterial susceptibility test showed that, it was a growth inhibition on *K. pneumoniae* in all the solvent extract whereas in *P. aeruginosa, S. aureus* the inhibitory activity was observed in hexane, ethyl acetate and ethanol extract but not in petroleum ether. However, *B. subtilis* showed the inhibitory activity on ethyl acetate and ethanol, but has no response on petroleum ether and hexane. *E. coli* has least inhibitory activity (1 mm) on ethyl acetate but has no activity on other solvent extract. Regarding the elements analysed, the role of elements to the health of human is discussed here under.

**Potassium**

It regulates water balance, levels of acidity, blood pressure, controlling the activity of heart, muscles and the nervous system.

**Calcium**

It is important to bone growth and formation, blood clotting, nerve and the muscle functioning, regulate lower blood pressure, kidney function, reduce blood cholesterol level.

**Copper**

Copper is involved in the absorption, storage and metabolism of iron, formation of red blood cells and keeps bones, blood vessels, nerve and immune system healthy.

**Zinc**

It is vital to immune resistance, wound healing, digestion, reproduction, physical growth, diabetes control, maintaining normal vitamin A level.

**Manganese**

It is necessary for the metabolism of proteins and fat. It also supports the immune system, blood sugar balance and is involved in the production of cellular energy, reproduction and bone growth.

**Iron**

Its major function is to combine with protein and copper in making haemoglobin, the component of the blood that carries oxygen from the lungs to the tissues. It may result in weakness, fatigue, paleness of the skin, constipation and anemia.

**Chromium**

It supports the immune system, carcinogenic.

**References**


