

Studies on Antibacterial Activity and Biochemical Analysis of *Aloe Vera* and *Moringa Oleifera*

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Abstract

Inhibitory effect and medicinal properties of distilled water, Methanol, Ethanol, Acetone, Petroleum ether of *Aloe vera* and *Moringa oleifera* on different standard strains of bacteria were determined by zone of inhibition. The values of zone of inhibition was measured and compared with standard values. In the present study the crude extract of *Aloe vera* and *Moringa oleifera* showed significant zone inhibition against *Bacillus sp*, *E.coli*, *Micrococcus sp*, *Staphylococcus aureus* and *Streptococcus sp*. The petroleum ether and distilled water extract of leaves showed better results than Acetone extracts against all the pathogenic bacteria. There was no activity found with methanol and ethanol extract against all the test isolates. The distilled water and petroleum ether extract of all treated plant leaves stored good inhibitory activity.

Introduction

In India, medicinal plants have made a good contribution to the development of ancient Indian Materia Medica. The primitive men have used different plant, as therapeutical agents for remedial measure with the study of disease and their treatment must have been accumulated in the course of many centuries. Resistance towards prevailing antibiotics having become wide spread among bacteria and fungi; new classes of antimicrobial substance are usually required.

During the past one century has been a rapid extension of the allopathic system treatment in India. It generated commercial demand for pharmaceutical drugs and their products in India. Efforts have been made to introduce many of these drug plants farmers. Several research institutes have undertaken studies on the cultivation practices of medicinal plants, which were found suitable and remunerative for commercial cultivation. The agronomic practice for growing poppy, isabgol, cinchona, ipecac, belladonna, ergot and others have been developed and there is now localized cultivation of these medicinal plants commercially.

Medicinal and aromatic plants are found in forest areas throughout south Asia, from the plains to the high Himalayas, with the greatest concentration in the tropical and subtropical belts and arid region of desert. India recognizes more than 2,500 plant species having medicinal value, Sri Lanka about 1,400 and Nepal around 700. Some of these, found at high altitudes in particularly stressful environments, grow very slowly and cannot live elsewhere. Others are more broadly distributed and adapt more easily to different ecological conditions.

The Indian pharmacopoeia recognized eighty-five drug plants whose ingredients are used in various pharmaceutical preparations. We have however, confine our test to few important commercially grown medicinal plants whose cultivation deserves priority in our national economy.

Aloe vera and *Moringa oleifera* are important medicinal plants. Its medicinal importance was mentioned very well, in siddha medicinal literature of India. Herbest is also used in Ayurvedic systems as an effective medicine for anti inflammatory.

Aloe vera cleans the morbid matter from the stomach, liver, kidneys, spleen, bladder and it's the finest colon cleanser known. In turn, this process purify the blood. It is healing and something in the relief of indigestion, stomach distress and ulcers. Others claim relief from arthritis, bladder and kidney infection; leg cramps, constipation, hemorrhoids insomnia, and for vaginitis, it is an excellent vaginal douche.

Moringa oleifera seeds in water purification learn to eat *Moringa* has loads of recipes. To date, most research on economic uses has focused on *Moringa oleifera* and the other species have been almost completely ignored. The present study was designed to screen for the antibacterial activities presence of photochemical in the leaves of these plants.

Materials and Methods

Plant Collection

The fresh healthy leaves of *Aloe vera* and *Moringa oleifera* were collected from the herbal garden at Ponnaiyah Ramajayam

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College, Thanjavur, India. *Aloe vera* leaves are freshly collected and *Moringa oleifera* leaves were air dried. The leaves were cleared by washing with tap water.

Aloe Vera

Aloe vera is commonly known as a succulent plant with amazing healing properties. In botanical plant latin *Aloe vera* is known as *Aloe barbadensis*. Aloe being the genus and barbdensis being the species. *Aloe barbadebsis* is named after the place where the plant was first documented Barbados.

Aloes are grown very easily at home, but most home gardens do not know what is required to take care of this succulent.

Moringa oleifera

Moringa oleifera (Murungai) is one of the world's most useful plants. Though apparently native only to restricted areas in the southern foothills of the Himalayas. *M.oleifera* is cultivated in all the countries of the tropics. *M.oleifera* is cultivated for its leaves, fruits and roots for a variety of food and medicinal purpose.

Sterilization of Plant Materials

The disease free and fresh plants were selected. About 2 g of fresh and healthy leaves were taken for each solvent extraction. They were washed with distilled water for three times. Then surface sterilized with 0.1% mercuric chloride for 20 seconds. Again the leaves were washed thoroughly with distilled water (three times).

Preparation of Plant Extracts

Two grams of sterilized plant leaves were kept in the 10ml organic solvents such as ethanol, methanol, acetone, petroleum ether and distilled water. Then they were ground well with the help of mortar and pestle. The plant material was subjected to centrifugation for 15 min at 10000 rpm. Again, it was filtered through Whatermann No.1 filter paper. The supernatant was collected and made to known volume, by adding sterile distilled water and stored for further antimicrobial screening purposes.

Selection of Micro Organism

The test organism such as *Staphylococcus aureus*, *Streptococcus sp*, *Bacillus sp*, *E-coli* and *Micrococcus sp* were used further investigation.

Staphylococcus aureus

The cells are spherical measuring 0.5 to 1.5 μm in diameter. The bacterium is Gram positive, non-motile and facultative anaerobes. The colonies are usually opaque and may be green or white and some times yellow to orange. The Optimal temperature is 30-37°C. Some Species of the genus are opportunistic pathogens of humans.

Streptococcus sp.

Streptococcus is Gram positive, arranged in chains of varying length. Streptococcus forms part of the normal flora of man and

animals. Cocci arranged in chain were first described by Billoth (1874). The individual Cocci are spherical or ovoid, 0.5 to 1 μm in diameter arranged in chains. Some species (Str. Pyogenes) are important human pathogens causing pyogenic infections.

Escherichia coli

The cells are straight rods with 1.1 – 1.5 μm x 2.0 – 6.0 μm in size. Capsule or microcapsules are present in many strains. They are Gram negative and motile by peritrichous flagella or are non-motile. They can grow in the facultatively annerobic condition. The optional temperature for growth is 37°C. They occur as normal flora in the lower part of the intenzyme. *E.coli* strain that contains enterotoxins and cause diarrhea disease.

Bacillus sp.

Sporogenous rod-shaped gram-positive bacteria are divided into two classes based on oxygen requirement, the aerobic Bacilli and the anaerobic Clostridia. Members of the genus Bacillus are ubiquitous saprophytes present in soil, dust, air and water expect *B.anthraxis*, the causative agent of anthrax, in animals and men.

Micrococcus sp.

Gram positive free living cocci are aerobes. The spherical bacterium is called coccus. Coccus means berry. The individual spherical bacterium is called micrococcus.

Inoculum preparation

The culture media used to study the colony morphology and cultured characteristics of microorganisms. The nutrient agar medium is used for bacteria.

<u>Ingredients</u>	-	<u>gm/liter</u>
Peptone	-	5 gm
Beef extract	-	3 gm
Sodium chloride	-	5 gm
Agar	-	15 gm
Distilled water	-	1000 ml
pH	-	6.8

A clear 1000 ml conical flask with 1000 ml distilled water was taken. All ingredients were weighed and dissolved in 1000ml of distilled water. The pH was adjusted to 6.8 and sterilized at 120°C for 15 minutes under the pressure 15 lbs.

Screening for antibacterial assay- activity assay

The antibacterial activity was demonstrated by disc diffusion assay (Baure *et al*, 1996). Nutrient agar medium was poured in to sterile petriplate. Then bacterial cell culture swaps to agar plate. 100 μl of plant extract were applied on sterile filter paper disc (5 mm diameter). The disc were placed on the surface of inoculated agar plates. The plates were incubated for 24 hrs at 37°C. Solvent and distilled water is used as a negative control. Inhibition zone

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diameter around each of the disc were measured and recorded at the end of the incubation time. An average zone of inhibition was calculated for the three replicates.

Estimation of phyto compounds (Quantitative test)

Estimation of Carbohydrates

The amount of carbohydrate was estimated by Anthrone method.

Reagent

(i) 0.5% of TCA

5gms of TCA was dissolved in 100 ml of distilled water.

(ii) Anthrone Reagent

50 mg of Anthrone powder was dissolved in 100 ml of 60% Sulphuric acid. To this 1 gm of Thio Urea was added to stabilize the colour.

Standard

1 mg of glucose was dissolved in 10 ml of saturated benzoic acid.

Procedure

0.5 ml of test sample was taken. They were homogenized with 1 ml of 5% TCA and centrifuged at 300 rpm for 5 minutes. 0.5 ml of the colour supernatant was taken to represent the standard and blank respectively. To each of these test tubes were kept in boiling water for 15 minutes. Then they were cooled in dark and their optical density were measured colorimeter at 620 nm.

Estimation of protein – (Biuret Method)

The total protein concentration of the plant extract were determined by biuret method (Gornal *et al*, 1949).

Reagents

1. NaOH

4 gms of NaOH was dissolved in 100 ml of distilled water.

2. Deproteinizing agent 5% TCA

5gms of TCA was dissolved in 100ml of distilled water.

3. Biuret Reagent

1.5gms of Cupric sulphate and 6 gms of Sodium Potassium tartarate were dissolved in 500 ml of distilled water to which 300 ml of 10% NaOH was added. The solution was made up to 1000 ml with distilled water.

Procedure

0.5ml of test sample was taken. Homogenized well with 1 ml of 5% TCA and centrifuged at 3000 rpm for 5 minutes. The supernatant was discarded. The precipitate was dissolved in 2 ml

of NaOH. 8ml of biuret reagent was added to all. After half an hour using blank for zero adjustment optical density was measured at 530 nm in a colorimeter.

Estimation of lipids (Folch *et al*, 1957)

Reagents

(i) Chloroform - Methanol Mixture (2:1).

(ii) 0.9% Sodium Chloride

(iii) Phospho – Vanillin reagent

0.2 gm of vanillin was dissolved in 80% Orthophosphoric Acid.

(iv) Concentrated Sulphuric acid

(v) Cholesterol Standard

5gm of dry Cholesterol powder was dissolved in 25 ml of Chloroform

Methanol

Mixture (0.2mg/ml).

Procedure

About 0.5ml of test sample was taken to each sample. 2.5ml of Chloroform Methanol Mixture was added. The samples, were homogenized 0.4ml of NaCl was added to each of these test tubes, to remove non – lipid contaminants and to release bound acidic lipids. The samples were centrifuged. The upper phase and the middle protein precipitate were discarded. The lower phase was made up to 1 ml with chloroform methanol mixture. 0.5 ml of sulphuric acid was added to these samples and were kept in boiling water bath for 10 minutes.

A blank, constituting the distilled water and 1 ml of cholesterol standard were taken simultaneously along with the samples and kept in the water bath for 10 minutes. After cooling the mixture for 30 minutes. 5ml of vanillin reagent was added 10 each and the O.D was read at 520nm.

Result and Discussion

The present investigation showed the inhibitory effect of Distilled water, Methanol, Ethanol, Acetone, Petroleum ether extract of *Aloe vera* and *Moringa oleifera* against different bacteria such as *E.coli*, *Bacillus sp*, *Micrococcus sp*, *Staphylococcus sp* and *Streptococcus sp*.

The previous study was designed to obtain preliminary information on the antimicrobial effect of three palestinian medicinal plants on certain pathogenic microorganisms. The hole plate diffusion method was preferred to be used in this study since it was found to be better than the disc diffusion method. (Essawi *et al* 2000).

The most significant antibacterial activity and the maximum zone of inhibition observed were 17 mm, 16 mm, 15 mm and 14 mm against *Bacillus sp*, *Micrococcus sp*, and *E-coli* (Table 1 & 2). The resistant zone of inhibition of 10mm and 11mm were observed against *Staphylococcus sp*, *Streptococcus sp* and *Micrococcus sp*. The most significant antibacterial activity was observed against *Bacillus sp* and *E.coli* by *Moringa oleifera* (Plate 2). Hence the present study indicate the most significant inhibitory effect of extract of *Moringa oleifera* against *Bacillus sp* and *E.coli*. Futher the study reveals the most significant inhibitory

effect of crude extract of *Aloe vera* against *Micrococcus sp* and *E.coli*. This antibacterial activity by the extracts of *Aloe vera* and *Moringa oleifera* may be due to the presence of antibacterial substances (Arora and Bhardwaj, 1997).

In addition to the present investigation biochemical investigation was done for the extract of the *Aloe vera* and *Moringa oleifera*. The results were tabulated (Tabel 3).

Moringa oleifera has more amounts of protein, lipids and carbohydrates than *Aloe vera*. Now a day the *Moringa oleifera*

S.No	Name of Organism	Zone of Inhibition (Diameter in mm)				
		Dist. H ₂ O	Petroleum ether	Acetone	Methanol	Ethanol
1.	<i>Micrococcus sp.</i>	16	14	12	-	-
2.	<i>E.coli</i>	14	12	11	-	-
3.	<i>Staphylococcus sp.</i>	12	09	-	-	-
4.	<i>Streptococcus sp.</i>	08	06	05	-	-
5.	<i>Bacillus sp.</i>	06	04	03	-	-

Table 1. Antibacterial activities of *Aloe vera*

S.No	Name of Organism	Zone of Inhibition (Diameter in mm)				
		Dist. H ₂ O	Petroleum ether	Acetone	Methanol	Ethanol
1.	<i>Micrococcus sp.</i>	11	09	08	-	-
2.	<i>E.coli</i>	15	13	12	-	-
3.	<i>Staphylococcus sp.</i>	10	08	07	-	-
4.	<i>Streptococcus sp.</i>	10	08	-	-	-
5.	<i>Bacillus sp.</i>	17	15	13	-	-

Table 2. Antibacterial activities of *Moringa oleifera*

S.No	Plant Name	<i>Aloe vera</i>	<i>Moringa oleifera</i>
1.	Carbohydrates	12 mg/100ml	193.3mg/100ml
2.	Protein	15.71 mg/ 100ml	71.42mg/100ml
3.	Lipids	20 mg / 100ml	16.5 mg / 100ml

Table 3. Biochemical analysis of plant extract

have been used as nutrient for human beings. The present investigation indicates that both the tested plants have nutrient sources as well as antibacterial activity. The antibacterial activity of *Aloe vera* and *Moringa oleifera* and the availability of plants would make economical benefit in the field medicine especially in bacteriology.

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