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# Investigation of Antimicrobial Activity of *Aloe vera* and it's Application

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***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

*Aloe vera* consists of about 420 species, and has been used as a traditional medicine for about 3000 years. The preliminary phytochemical screening of the *aloe vera* gel powder revealed the presence of steroids, phenolics, glycosides, alkaloids, flavonoids, saponins and tannins. Invitro antibacterial studies on the leaf extract were carried out on medically important bacterial strains, including *Bacillus subtilis, Candida albicans* using agar disc diffusion method. The bacterial strains were exposed to the following four different concentrations of extracts: 25µl, 50 µl, 75 µl and 100 µl. The result of our antibacterial assay revealed that the extract showed good inhibitory activity against all the tested pathogens.

*Keywords: Aloe barbadensis miller; phytochemical screening; disc diffusion; antibacterial; antidiabetic activity.*

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## 1. INTRODUCTION

The perennial plant known as *Aloe vera* is *Aloe barbadensis miller*, which is a well-known pharmaceutical herb that has long been used in traditional Chinese medicine for the treatment of various diseases. It is widely distributed in the semitropical regions and cultivated in many provinces of China [15]. Aloe gels have an important role in food preservation as edible coatings. Edible coatings generally provide a thin layer on the fruit surface, which acts as a barrier to atmospheric gases and moisture" [1]. "Aloe gels help to reduce the respiration and transpiration of fresh produce and delay postharvest deterioration of foods, promoting food preservation. Edible coatings are generally applied by dipping the foods, spraying, or brushing. To date, numerous studies have been conducted into the postharvest use of *Aloe vera* gel as an edible coating" [1]

*Aloe vera* is reputed to have medicinal properties. For centuries, it has been used for an array of ailments such as mild fever, wounds and burns, gastrointestinal disorders, diabetes, sexual vitality and fertility problems to cancer, immune modulation, AIDS and various skin infections [16].

"Medicinal plants of the lily family (Liliaceae), genus Aloe, have been used for the treatment of skin diseases for more than 5,000 years. Among more than 360 Aloe species, *aloe vera* (*Aloe barbadensis* miller) has been the most popular in both folk and officinal medicine. *Aloe vera* extracts are widely used in a variety of over-the-counter and dermatological products. Many studies report the effective use of this plant when applied topically for the treatment of burns, sunburns, inflammatory skin disorders and wounds" [2].

"*Aloe vera* is made up of many complex ingredients including polysaccharides, glycoproteins, phenolic compounds, salicylic acid, lignin, hormones, amino acids, vitamins, saponins, and enzymes, which give *Aloe vera* its many beneficial properties including anti-inflammatory, antibacterial, antioxidant, immune-boosting, and hypoglycemic properties [21]. Even though several effective antifungal agents are available for oral candida infections, the failure is not uncommon because isolates of *C. albicans* may exhibit resistance to the drug during therapy" [3]. Hence, the present study was conducted with an aim to assess the antimicrobial activity of ethanolic extracts of *Aloe vera* leaf and gel against *C. albicans* and *Bacillus subtilis In vitro*.

## 2. MATERIALS AND METHODS

### 2.1 Collection of Plant Material

Fresh leaves weighing 1kg collected from the *Aloe vera* plants (*A. Barbadensis miller*, belonging to *Liliaceae* family) naturally grown in Coorg. after washing the leaves with tap water and then again. "The leaves were dissected with a sterile knife into longitudinal sections and gel was scooped out with a sterile sharp spatula, care being taken to avoid the mixture of the fibres into the gel [4]. *Aloe vera* gel was macerated in an electric blender to obtain a fine paste by adding few drops of methanol was added to all the conical flasks. subsequently, the flasks were kept in rotatory shaker for 3days, after 3 days, then a gel was filtered using Whatman filter paper no.1.it was then evaporated in a heating mantle stored in a screw cap test tube at 4℃" [5].

### 2.2 Test Culture

The organisms used were collected from the Department of Microbiology, The American College, Madurai. Bacterial culture maintained in nutrient agar and the fungal culture maintained in potato dextrose agar. Agar well diffusion method**,** 500 µL of the RPMI and culture was collected in cuvettes to which gel extracts of different dilutions (25, 50, 75, and 100 µL) were added on a well of Muller Hinton agar. The cultures were then incubated for 24 hours and add DMSO in control [20].

### 2.3 Minimum Bactericidal and Fungicidal Concentration

The minimum inhibitory concentration of ethanol extracts was prepared by adding 0.2g of extracts in 2ml, 4ml, 8ml and 1ml of distilled water to obtain 0.125mg/ml, 0.25mg/ml, 0.5mg/ml and 1mg/ml were introduced into wells and measured the concentration [6]. To each of these dilutions, a loop full of culture adjusted to 0.5 McFarland standard, was inoculated and all the tubes were incubated at 37ºC for 24 hrs.

### 2.4 Biochemical Assay for Phytochemical Analysis

Phytochemical analysis for major phytoconstituents of the plant extracts was undertaken using qualitative methods. The plant

extracts were screened for the presence of biologically active compounds like glycosides, phenolics, alkaloids, tannins, flavonoids, saponin and steroids [17].

### 2.5 Identification Test

"2ml filtrate of the plant extract was mixed with 2ml of HCL and about 6 drops of Mayor's reagents. A creamish or pale -yellow precipitate indicates the presence of alkaloids" [7].

"2ml of the extract was dissolved in 2ml of acetic anhydride which is then added to 2ml of sulphuric acid. The colour changes from violet to blue or green in some samples indicating the presence of steroids" [4]. "1ml of the extract was treated with few drops of one percentage ferric chloride solution was observed of brownish green or a blue-black coloration confirming presence of tannins" [8].

"Few of filtrate was added to five-six drops of concentrated hydrochloric acid and 1.5ml of methanol solution. Pink tomato red color indicates the presence of flavonoids. 2ml of extract is mixed with 2ml of chloroform [9]. Then 2ml acetic anhydride and 2 drops of concentrate sulphuric acid were added from the side of test tube first red then blue finally green color appears indicating presence of glycosides [24]. 2ml of ethanol was added to the test solution and few drops of ferric chloride solution. Blue coloration indicates the presence of phenolics" [10]. "2ml of distilled water was added to 2ml of the solution and Shaked well and observed for frothing" [11].

### 2.6 High Performance Liquid Chromatography (HPLC)

"*Aloe barbadensis miller* crude extract in hexane, ethyl acetate fraction (III), Quercetin in Methanol were used in HPLC [19]. The HPLC was performed on YMC C18 column, 5¼m, 250×4.6mm, using a gradient consisting of solvent A (water/formic acid,90/10) and solvent (methanol), at a flow rate of 0.7ml/minute and separation was monitored by absorbance at 254nm" [2].

### 2.7 FTIR

Fourier Transform Infrared Spectroscopy, also known as FTIR Analysis or FTIR Spectroscopy, is an analytical technique used to identify organic, polymeric, and, in some cases, inorganic materials [12]. The FTIR analysis method uses infrared light to scan test samples and observe chemical properties. FTIR spectra reveal the composition of solids, liquids, and gases [18]. The most common use is in the identification of unknown materials and confirmation of production materials (incoming or outgoing) [23].

## 3. RESULTS AND DISCUSSION

### 3.1 Preparation of Plant Extract

*Aloe vera* sample (*A. liliaceae*) is collected from a garden near Avaniyapuram, Madurai and washed it with tap water and again rinsed with distilled water then, the gel was scrapped with the help of sterile spatula

The collected gel was mixed with equal amount of methanol, covered it with aluminium foil with small holes on it after 3 to 4 days methanol gets evaporated and powder of *aloe vera* was scrapped and collected in an Eppendorf tube.

### 3.2 Culture Preparation

Pure culture of *C. albicans* strain was cultured in potato dextrose agar and *B. subtilis* was cultured in trypticase soy agar then, the inoculum was isolated after 24hrs and adjusted to match the 0.5 McFarland [13]. Test sample was prepared by dissolving 4g of *aloe vera* powder in 4ml of DMSO (Dimethyl Sulfoxide) [9].

### 3.3 Antimicrobial Activity

Test sample was added in different dilutions (25, 50, 75 and 100$\mu l$) on a well of muller hinton agar and the control well consists only DMSO then, incubate it for 24hrs.after 24hours observe the zone of inhibition *aloe vera* powder shows more zone of inhibition in *C. albicans* than *B. subtilis* [14]. Antimicrobial activity of *Candida albicans* were ranging from 13mm to 19mm and for *Bacillus subtilis*, 10 to 12mm are measured and tabulated.

### 3.4 Phytochemical Analysis

Result of phytochemical analysis shows the presence of alkaloids, steroids, tannins, flavonoids and saponins and indicates the absence of phenolics and glycosides [22]. The detailed results are tabulated below.

HPLC represented the difference in retention time of Aleo vera and all the peaks ranged from 2 to 4min. FTIR analysis of gel extract represented peak at 3456.55cm$^{-1}$ with heterocyclic amine, organic sulfates, organic nitrates and alkyl substituted ether [25].

**Table 1. Antibacterial and Antifungal activity of Aloe vera gel powder against *C. albicans* and *B. subtilis***

| S.No | Media | Culture | Concentration | Zone of Inhibition |
|---|---|---|---|---|
| 1 | MHA | *C. albicans* | 25µl | 13mm |
| | | | 50µl | 14mm |
| | | | 75µl | 14mm |
| | | | 100µl | 19mm |
| 2 | MHA | *B. subtilis* | 25µl | - |
| | | | 50µl | - |
| | | | 75µl | 10mm |
| | | | 100µl | 12mm |

**Table 2. Phytochemical analysis**

| S.No | Phytochemical analysis | Observation |
|---|---|---|
| 1 | Test for alkaloids | Pale yellow precipitates form it indicate the presence of alkaloids |
| 2 | Test for steroids | Brown colour ring forms it indicates the presence of steroids |
| 3 | Test for tannins | Black color was observed it indicates the presence of tannins |
| 4 | Test for Flavonoids | Pink tomato red colour was observed it indicates the presence of flavonoids |
| 5 | Test for glycosides | Absence of glycosides |
| 6 | Test for Phenolics | Absence of phenolics |
| 7 | Test for saponins | Presence of saponins |

**Table 3. FTIR**

| Frequency $cm^{-1}$ | Absorption $cm^{-1}$ | Appearance | Group | Compound |
|---|---|---|---|---|
| 4000-3000 | 3434.6 | Strong | >NH -stretch | Heterocyclic amine |
| 2000-1000 | 1109.83 | Medium | C-O-Stretch | Alkyl substituted ether |
| | 1411.64 | Medium | -oxy compounds | Organic sulfates |
| | 1629.55 | Strong | Hetero-oxy compounds | Organic nitrates |

**Fig. 1. Aloe vera sample**

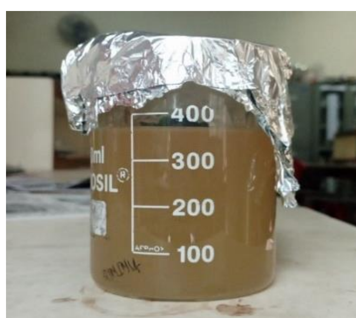


**Fig. 2. Methanol extract**

**Fig. 3. *B. subtilis***

**Fig. 4. *C. albicans***

**Fig. 5. Aloe vera powder**

**Fig. 6. DMSO**

Fig. 7. Test sample

**Fig. 8. Antifungal activity of aloe vera against *C. albicans***

**Fig. 9. Antibacterial activity of aloe vera against *B. subtilis***

**Chromatogram**

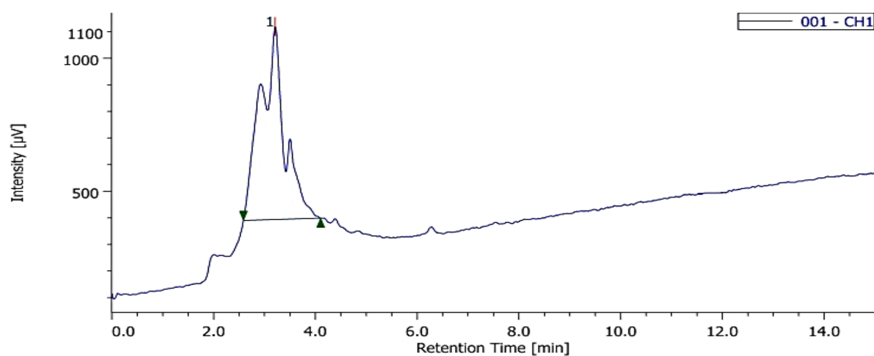


**Fig. 10. JASCO HPLC**

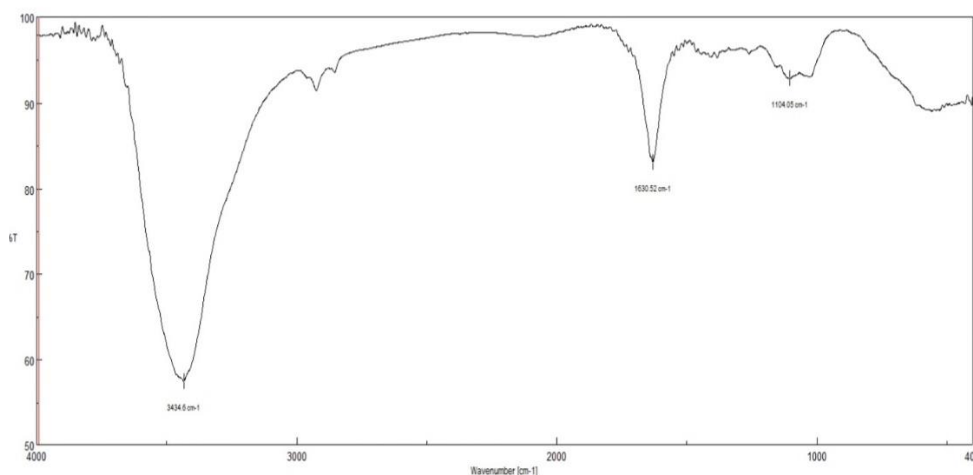


**Fig. 11. FTIR**

## 4. SUMMARY

The preliminary phytochemical screening of the leaves revealed the presence of alkaloids, steroids, tannins, flavonoids and saponins. In vitro antibacterial studies on the leaf extract were carried out on medically important bacterial strains, including *Bacillus subtilis, Candida*

*albicans* using agar disc diffusion method. The bacterial strains were exposed to the following four different concentrations of extracts:25µl; 50µl, 75µland 100µl. The result of our antimicrobial assay revealed that the extract showed good inhibitory activity against all the tested pathogens. The present study reveals that some compounds are used to formulates new and antimicrobial drug can be originate with more potent.

## 5. CONCLUSION

The work was checked based on its antimicrobial activity, as well as the phytochemical analysis were also done which clearly states that the extracts is with the presence of alkaloids, steroids, tannins, flavonoids and saponins Analysis was successful. Future studies will be based on clinical trial of *A. Barbadensis miller* which has its antimicrobial property.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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