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Research Article

Screening and Evaluation of Secondary Metabolites and Antimicrobial Activity of Saline and Non-Saline *Aloe barbadensis* Miller Plant

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Abstract

Background and Objective: *Aloe vera* was one of the oldest mentioned plants on record due to its medicinal properties and health benefits. The present study attempts to investigate qualitative and quantitative phytochemical constituents and antimicrobial activities in *Aloe barbadensis* Miller plant growing in saline and non-saline region. **Materials and Methods:** The qualitative phytochemical constituent detected in all extract and quantitative phytochemical constituent were determined and analyzed by using a spectrophotometer. Wherever the antimicrobial activities were determined by the agar disk diffusion method. **Results:** Phytochemical screening of the *Aloe barbadensis* Miller plant revealed the presence of some bioactive components, such as flavonoids, saponins, tannins, carbohydrates, reducing sugar, non-reducing sugar and protein. The major chemical constituents found in this plant were flavonoid, reducing and non-reducing sugar in both saline and non-saline region. The quantities of total carbohydrates were higher in both fresh *Aloe barbadensis* Miller gel (6.60%) and formulated *Aloe barbadensis* Miller powder (41.42%). The zone of inhibition is determined by the agar disk-diffusion method varied with the fresh *Aloe barbadensis* Miller gel. *Staphylococcus aureus* and *Escherichia coli* were resistant to the *Aloe barbadensis* Miller gel tested. Fresh *Aloe barbadensis* Miller gel showed good antibacterial potential against both types of bacterial strains, gram-positive *S. aureus* as well as gram-negative *E. coli*. **Conclusion:** Thus, the studied *Aloe barbadensis* Miller samples were concluded that as large-scale antibiotic resistance by bacteria was becoming an interesting public health concern and the race to discover the new antibacterial agent was on, *Aloe barbadensis* Miller gel along with its compounds with promising antibacterial activity could be used as an alternative herbal remedy.

Key words: *Aloe barbadensis* Miller, phytochemical screening, antimicrobial activity, agar disk diffusion method, herbal remedy

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Due to its cactus-like feel, *Aloe* was often mistakenly called a "Desert Cacti". There were over 400 species of *Aloe* grown around the world, but it is the *Aloe barbadensis* Miller (*Aloe vera* or "True Aloe") plant which had been of most of mankind uses because of the good medicinal properties it displays. *Aloe vera* (*Aloe barbadensis*) was commonly used to treat different skin diseases, sunburn, minor cuts, insect bites and used as wound healing, anti-inflammatory, antiviral, antitumor, laxative and psoriasis¹.

The resistance of microorganisms against antimicrobial drugs was a major problem of recent times, which was increasing day by day. Microorganisms play vital roles in human life for causing so many diseases. Medicinal plants are a rich source of antimicrobial agents^{2,3}. *Staphylococcus aureus* was considered to be a major pathogen that colonizes and infected both hospitalized patients with decreased immunity and healthy immune-competent people in the community. This bacterium was found naturally on the skin and in the nasopharynx of the human body. It can cause local infections of the skin, nose, urethra, vagina and gastrointestinal tract, most of which are minor and not life-threatening⁴. *E. coli* was the prominent cause of enteritis, urinary tract infection, septicemia and other clinical infections, such as neonatal meningitis⁵. Antonisamy *et al.*⁶ studied the antimicrobial potential of DMSO crude extracts of *Aloe barbadensis* Miller (*Aloe vera*) gel against the selected pathogens *Bacillus subtilis*, *Salmonella typhi*, *Escherichia coli*, *Staphylococcus aureus*, *Proteus vulgaris*, *Aspergillus fumigatus*, *Candida albicans* and *Penicillium* sp.

Aloe vera is highly traditionally used to cure various diseases⁷. Medicinal plants were considered as the greatest pharmaceutical stores existing on the earth as they can produce eternal secondary phytochemicals having bioactive properties. The *A. vera* plants having a strong relationship between medicinal importance and the presence and absence of secondary metabolites⁸. These phytochemicals work efficiently to cure various diseases and illnesses since ancient times⁹. The role of phytochemical is important to provide them medicinal properties¹⁰. Herbal medicinal treatment is popular in Pakistan and a large population, especially in rural areas, prefers the traditional treatment because of no side effects, efficacy and economy¹¹. The *Aloe vera* plant leaves and inner gel contains numerous help it has the potential to cure sunburns and minor cuts and even skin cancer and acts as also acts as an extremely powerful laxative. Various parts of the plant had different effects on the body¹².

The research carried out in saline and non-saline regions, wherever the saline region refers to the amount of dissolved salt that is present in water and soil, the concentration of magnesium, calcium and sulfate ions are also substantial.

MATERIALS AND METHODS

Sample collection: *Aloe barbadensis* Miller plant was collected in the non-saline area of Agriculture University of Junagadh, Gujarat and saline area of Lodhwa village, Kodinar, Gujarat. Taxonomic identification and confirmation were carried out at the Bhakta Kavi Narsinh Mehta University, Junagadh, Gujarat. The study was carried out from July, 2018 to April, 2019. The bacteria *Staphylococcus aureus* and *Escherichia coli* were sourced from GMERS Medical College, Junagadh, Gujarat.

Preparation of plant extract: Powdered plant material was soaked in distilled water at 80°C for 4 hrs in soxhlet apparatus and chloroform for 24 hrs in airtight bottles and plant extracts were filtered through whatman filter paper. To prepared fresh gel, *A. barbadensis* Miller leaves gels were homogenized twice in a blender at a high speed for 5 min. The extracted gel was subjected to straining through a strainer to remove traces of cellular matter. The *A. vera* clear gel was then subjected to further processing.

Qualitative phytochemical analysis: Detection of active phytochemical constituents was carried out for all the extracts using the standard procedures¹³.

Quantitative phytochemical analysis: Depending on the qualitative results the quantitative assay is carried out for carbohydrates, proteins, reducing sugars, total sugars and non-reducing sugar. The carbohydrates were determined by the phenol sulfuric acid (colourimetric) method¹⁴, the protein was determined by Lowry's method¹⁵ and reducing and non-reducing sugar were determined by the cupric reduction method¹⁶.

Anti-microbial activity: The antibacterial activity of *A. vera* inner gel against both gram-positive and gram-negative bacteria had been demonstrated by the agar disk diffusion method¹⁷.

RESULTS

Qualitative phytochemical analysis measurements: In a qualitative phytochemical study of fresh *A. vera* gel and formulated *A. vera* gel in different saline and non-saline

Table 1: Qualitative phytochemical analysis of *A. vera* gel of saline and non-saline area

Phyto-chemical constitute	Test names	Non-saline area			Saline area		
		Fresh gel	Aqueous extract	Chloroform extract	Fresh gel	Aqueous extract	Chloroform extract
Flavonoids	Alkaline reagent test	+	+	+	+	+	+
	Lead acetate test	+	+	+	+	+	+
	HCl test	-	-	-	-	-	-
Terpenoids	Salkowski's test	-	-	-	-	-	-
Saponins	Lead acetate test	+	+	-	+	+	-
Tannins	Ferric chloride test	-	-	-	-	+	-
Anthraquinones	Borntrager's test	-	-	-	-	-	-
Carbohydrates	Molisch test	-	-	+	-	-	+
Reducing sugar	Fehling test	+	+	+	+	+	+
Non-reducing sugar	Benedict's test	+	+	+	+	+	+
Protein	Xanthoproteic test	+	+	-	+	-	-

+: Present and -: Absent

Table 2: Quantitative phytochemical analysis of fresh and formulated *Aloe vera* gel

Quantitative analysis	Fresh <i>A. vera</i> gel (%)	Formulated <i>A. vera</i> powder (%)
Total carbohydrates	6.60	41.42
Proteins	1.2	3.2
Reducing sugars	0.95	13.25
Non-reducing sugars	0.61	4.64
Total sugars	1.56	17.89

regions, different qualitative tests were performed for some major groups of phytochemical constituents. In this analysis, *A. vera* gel showed the presence of various groups of phytochemical constituents like proteins, flavonoids, saponins, reducing sugar, non-reducing sugar, carbohydrates and tannins in Table 1.

From the results, it was revealed that there was no major difference showed in the phytochemical analysis of both areas. In the saline region, flavonoids, saponins, tannins, carbohydrates, reducing sugar and non-reducing sugar and proteins were present whereas, terpenoids and anthraquinones were completely absent. In the saline region, the flavonoids, reducing and non-reducing sugar was present in all three extracts of *A. vera*, fresh *A. vera* gel, aqueous extract and chloroform extract wherever the saponins completely absent in chloroform extract and tannin were present in the aqueous extract and absent in fresh gel and chloroform extract and proteins were completely absent in chloroform extract and aqueous extract wherever present in fresh *Aloe* gel. In the non-saline region, flavonoids, saponins, carbohydrates, reducing sugar and non-reducing sugar and proteins were present whereas, tannins, terpenoids and anthraquinones were completely absent. The saponins and proteins were present in fresh *A. vera* gel and aqueous extract wherever completely absent in chloroform extract and carbohydrates were present in chloroform extract wherever completely absent in aqueous extract and fresh *A. vera* gel.

From the results, it was revealed that there was no major difference showed in the phytochemical analysis of both areas. Tannins were present in the saline area whereas, absent

in the non-saline area. Saponins were present in fresh and aqueous extract of *A. vera* gel, whereas absent in chloroform extract. Carbohydrates were only present in chloroform extract of *A. vera* gel, whereas absent in both fresh and aqueous extract of *A. vera* gel. Proteins were present in fresh gel and aqueous extract of *A. vera* gel, whereas absent in chloroform extract of *A. vera* gel. Tannins were only present in an aqueous extract of *A. vera* gel of the non-saline area. Flavonoids were highly present in all the extracts of *A. vera* gel of both the areas from tests, alkaline reagent test and lead acetate test. Whereas absent in the HCL test.

Quantitative phytochemical analysis observations: There were several forms of *A. vera* gel like lyophilized powder, pure gel, formulations, etc., available commercially. Quantitative analysis of phytochemicals in different forms of *Aloe vera* gel showed that the both fresh *Aloe* and formulated *Aloe* exhibited a good amount of phytochemicals in them. The highest amount was found in carbohydrates, which was 41.42% in formulated *A. vera* gel. The lowest amount was found in non-reducing sugars, which was 0.61% in fresh *A. vera* gel in Table 2. From the present results, it was clear that the highest amount of phytochemicals were found in formulated *A. vera* gel (*A. vera* powder) compare to the fresh gel. The quantitative analysis of plants is commercially important being a great interest of pharmaceutical and food industries.

Anti-microbial activity effects: The results revealed the antibacterial activity of the *Aloe vera* gel against the bacteria, *Staphylococcus aureus* and *Escherichia coli*. The antibacterial

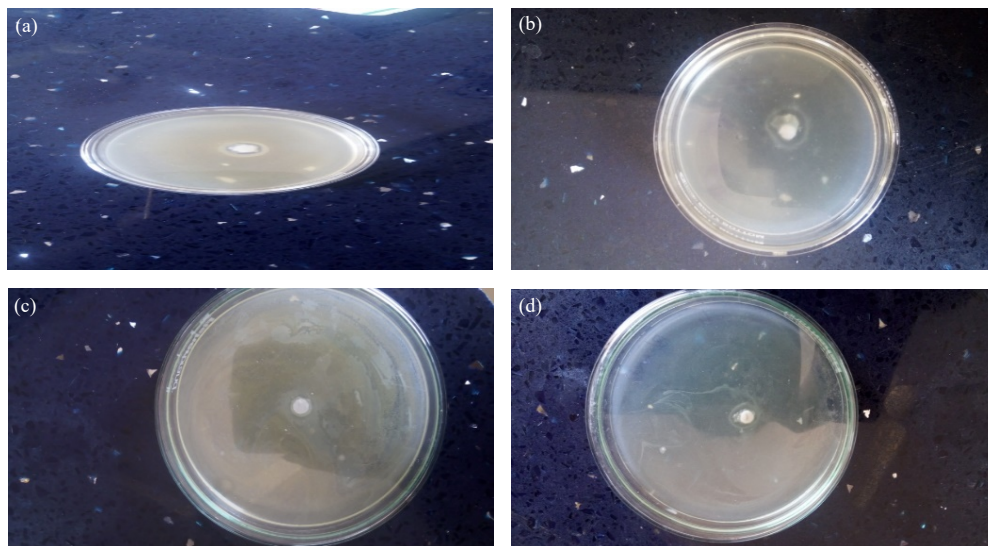


Fig. 1: Antibacterial activity of *Aloe vera* plant zone of inhibition against, (a) *Staphylococcus aureus* of non-saline *Aloe vera* gel, (b) *Escherichia coli* of non-saline *Aloe vera* gel, (c) *Staphylococcus aureus* of saline *Aloe vera* gel and (d) *Escherichia coli* of saline *Aloe vera* gel

Table 3: Antibacterial activity of agar disk diffusion method of fresh *A. vera* gel

Microorganisms	Value of zone of inhibitions in fresh <i>A. vera</i> gel (mm)	
	Non-saline area	Saline area
<i>Staphylococcus aureus</i>	11.33	5
<i>Escherichia coli</i>	9	8

activity of fresh *A. vera* gel's results were given in Table 3. From the results, it was revealed that the zone of inhibition of fresh *A. vera* gel of non-saline area against *Staphylococcus aureus* was 11.33 mm and the zone of inhibition of fresh *A. vera* gel of saline area against *Staphylococcus aureus* was 5 mm as well as the zone of inhibition of fresh *A. vera* gel of non-saline area against *Escherichia coli* was 9 mm and the zone of inhibition of fresh *A. vera* gel of saline area against *Escherichia coli* was 8 mm. The antibacterial activity of the *A. vera* gel against choose skin pathogens showed in Fig.1a-d zone of inhibition in different variations which range from 5-18 mm. But there were no major differences found between the two organisms of both areas.

The maximum zone of inhibition was found against the zone of inhibition of fresh *Aloe vera* gel of non-saline area against *Staphylococcus aureus*, which was 11.33 mm. The minimum zone of inhibition was found against the zone of inhibition of fresh *Aloe vera* gel of saline area against *Staphylococcus aureus*, which was 5 mm. From the results, it was revealed that the fresh gel of the non-saline area showed a higher zone of inhibition against the organisms, *Staphylococcus aureus* and *Escherichia coli*.

DISCUSSION

This study evaluated the screening of phytochemical constitute in fresh gel and leaves extracts and the preliminary antimicrobial assay was conducted in the fresh gel of *Aloe barbadensis* Miller leaves. As the results show in Table 1, the phytochemical results showed that the extracts and fresh gel were very rich in flavonoids, saponins, reducing and non-reducing sugar in both saline and non-saline region. Whereas, various studies have shown that saponins, flavonoids, glycosides and alkaloids are good antidiabetic metabolites¹⁸⁻²⁰. The phytochemical compound amounts depend on lots of factors as, climate, soil, water core, varieties, season, harvest date, geographic region, storage, bitter pit and irradiation moreover additional conditions²¹.

The fresh *Aloe vera* gel of both the areas, non-saline and saline areas showed antibacterial activity against common skin pathogens. The fresh gel of the non-saline area showed a higher zone of inhibition against the organisms, *Staphylococcus aureus* and *Escherichia coli*. The inhibitory activities evaluated of *A. barbadensis* or *Aloe vera* leave against some bacteria viz., *Mycobacterium smegmatis*,

Staphylococcus aureus, *Enterococcus faecalis*, *Micrococcus luteus*, *Bacillus sphaericus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Escherichia coli* and *Salmonella typhimurium*²². Yebpella *et al.*²³ revealed that the screening of the *A. vera* plant was potentially working against the treatment of pathogenic infections. In some studies of the *A. vera* plant investigated the inhibitory activities like cariogenic (*Streptococcus mutans*), periodontopathic (*Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*) activities against some disease²⁴.

Diseases had been managed traditionally using medicinal plants, as they contain components of therapeutic value. Because of this, in the present investigation, phytochemical analysis of fresh *A. vera* gel, aqueous extracts and chloroform extracts of *A. vera* gel of saline and non-saline regions were investigated. There was no major difference showed in the phytochemical analysis of both areas. Although *A. vera* has been used for a long time in history, it lacks compatible scientific evidence to support the therapeutic claim. The researcher identifies that components of *A. vera* exhibit their therapeutic values and the exact mechanism by which they act.

CONCLUSION

The study on *A. vera* leaves had begun with the phytochemical analysis and antimicrobial activity of the *A. vera* leaves of the different regions, non-saline and saline. The gel had potential in the manipulation and development of drugs for the treatment of diseases caused by these skin pathogens. The fresh *A. vera* gel of both the areas, non-saline and saline areas showed antibacterial activity against common skin pathogens. The fresh gel of the non-saline area showed a higher zone of inhibition against organisms, *Staphylococcus aureus* and *Escherichia coli*. From the results, it was concluded that simply pure *A. vera* gel was sufficient to treat several skin disorders.

SIGNIFICANCE STATEMENT

This study discovered the medicinal value of the *A. vera* plant, it can be discovered the number of secondary metabolites and antimicrobial properties present in the *A. vera* plant, that can be beneficial for medicinally and traditionally uses of *A. vera* plant as well as it can be beneficial for many types of research. This study will help the researchers to uncover the critical areas of saline and non-saline *A. vera* plant chemical constitute and their different environmental conditions that many researchers were not able to explore. Thus a new theory on the *A. vera* plant may be arrived at.

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