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## Comparative antimicrobial evaluation of socotrine aloe and aloe barbadensis miller

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### Abstract

The utilization of herbal medicines, derived from plants and their parts, has experienced a significant resurgence globally. This is attributed to their perceived cost-effectiveness and minimal side effects, particularly in underdeveloped regions where they continue to serve as the primary source of healthcare for a substantial portion of the population. In response to the growing demand, a multitude of companies have emerged to produce herbal remedies. Traditional medicinal systems, such as Ayurveda and Siddha, have long relied on various plant species to combat a wide range of illnesses, driven by concerns about the toxicity and side effects of allopathic treatments.

The surge in antibiotic use was anticipated to vanquish infectious diseases, but it inadvertently led to the proliferation of drug-resistant bacteria. Multi-drug resistant strains of pathogens like *E. coli*, *K. pneumoniae*, and *Candida albicans* now pose a grave challenge to healthcare institutions worldwide, resulting in escalating treatment costs and patient fatalities.

This study focused on *Aloe vera*, specifically *Aloe barbadensis miller* and *Socotrine aloe*, to assess their antimicrobial properties. *Aloe vera* gel was extracted and subjected to physicochemical tests to evaluate its bioactivity. The antimicrobial activity of these extracts was examined against a range of clinical bacterial and fungal strains.

The findings revealed that both *Aloe barbadensis miller* and *Socotrine aloe* possessed noteworthy antimicrobial activity, presenting a potential resource for treating bacterial and fungal infections. *Socotrine aloe* exhibited superior antimicrobial activity against *E. coli*, *B. subtilis*, and *S. typhi*, while *Aloe barbadensis miller* excelled against *P. Aeruginosa*. Additionally, *Socotrine aloe* displayed heightened antifungal activity, particularly against *Candida* species.

In conclusion, this research underscores the antimicrobial potential of *Aloe vera gel*, with *Socotrine aloe* showing greater efficacy in certain cases. These findings indicate the promise of incorporating *Socotrine aloe* into new antimicrobial formulations, further expanding the utility of herbal remedies in combating infectious diseases. The study provides valuable insights into the growing role of herbal medicines in modern healthcare, where conventional antibiotics face mounting challenges from drug-resistant pathogens.

**Keywords:** Antimicrobial, *Aloe barbadensis miller*, *Socotrine aloe*, agar well diffusion method

### 1. Introduction

Herbal medicines encompass orally administered compounds that consist of vitamins, minerals, herbs, or various combinations thereof. They are essentially "plants or plant components used for their fragrance, taste, or therapeutic properties." A notable surge in the global usage of herbal remedies has been observed in many countries, with a growing number of individuals turning to these natural products for addressing a wide array of health concerns. An estimated 75 to 80 percent of the world's population, primarily in underdeveloped regions, relies predominantly on herbal medicine for their primary healthcare needs [1]. This preference stems from the commonly held belief that herbal treatments are economical, easily accessible, and generally free from adverse side effects. According to the World Health Organization (WHO), the utilization of herbal remedies surpasses that of conventional medications by a factor of two to three globally [2].

Throughout history, nature has consistently demonstrated the remarkable concept of symbiosis. The foundation for treating human ailments has consistently revolved around natural substances derived from plants, animals, and minerals. In the present day, approximately 80% of the population in developing countries still places its trust in traditional medicine, which heavily leans on plant and animal resources, as the cornerstone of their basic healthcare [3].

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Various indigenous medical systems, including Siddha, Ayurveda, Unani, and allopathy, draw upon numerous plant species to address a variety of health issues. The escalating concerns over the toxicity and side effects associated with allopathic remedies have fuelled the growing popularity of herbal medicine. Consequently, this surge has given rise to a significant expansion in the number of companies involved in the production of natural drugs. The time-tested practice of herbal remedies continues to be relevant today due to their inherent biological advantages, their deep-rooted significance in diverse global cultures, and their substantial contributions to the preservation of human health [4].

In the context of combating microbial infections, particularly of bacterial and fungal origin, antibiotics have long served as the cornerstone of treatment. The medical community had initially held the optimistic belief that the discovery and use of antibiotics as chemotherapeutic agents would ultimately lead to the eradication of infectious diseases. However, the excessive use of antibiotics has emerged as the primary catalyst behind the development and dissemination of multi-drug resistant strains within various bacterial families. Notably, pathogens such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Haemophilus*, producing  $\beta$ -lactamases, have achieved a global spread, posing a formidable challenge for effective treatment. Hospitals are grappling with the presence of multi-drug resistant *E. coli* and *K. pneumoniae* strains, which are increasingly linked to community-acquired infections. An example of a nosocomial pathogen, *Candida albicans*, is responsible for a significant percentage (50-70%) of invasive candidiasis cases. Alarming, the incidence of nosocomial candidemia has witnessed a substantial rise over the past decade, with consequential impacts such as soaring drug prices and adverse patient outcomes [5].

Traditional medicine has relied on natural remedies for centuries, well before the advent of antibiotics and modern medications. Some astonishing assertions have been made regarding the antibacterial properties of numerous plants in addressing ailments. While only around 1% of flowering plants have been scientifically identified, it is believed that local communities have utilized approximately 10% of these plants to manage various illnesses. The search for plants containing antimicrobial compounds is a common endeavor due to their extensive use in treating various infectious diseases [5].

Many plant-derived secondary metabolites, such as tannins, alkaloids, and flavonoids, have demonstrated in vitro antibacterial properties. Because of their ready availability, minimal side effects, and low toxicity, numerous medicinal plants have been advocated in numerous phytotherapy references for managing infectious ailments. A multitude of reports document the antibacterial attributes of diverse plant extracts. Several plant extracts have demonstrated antibacterial properties, and a multitude of plants have been found effective in addressing skin infections, respiratory ailments, gastrointestinal problems, and urinary tract infections [6]. Numerous plant extracts are known for their antibacterial characteristics, with a wide range of plants found effective in addressing skin infections, respiratory conditions, gastrointestinal problems, and urinary tract infections [7].

Diseases caused by pathogenic bacteria and fungi pose a significant risk to human health, ranking among the primary contributors to global morbidity and mortality. The rise of drug-resistant human pathogenic bacteria has instigated a search for new reservoirs of antimicrobial substances, with a particular focus on plant metabolites [8].

The Aloe genus consists of approximately 400 species of flowering succulent plants within the Liliaceae family. Among these, *Aloe vera* stands out as a typical xerophyte with thick, fleshy, uniquely structured, and spiky leaves. *Aloe vera* has gained popularity in contemporary folk remedies and is promoted for a broad spectrum of ailments. The specific plant is scientifically known as *Aloe barbadensis* Miller and boasts a composition of about 99% water and 0.1% to 0.05% solids at a pH of 4.5 [8].

*Aloe vera* gel is derived from the parenchyma cells of its smooth-leaved peel and is characterized as a mucilaginous jelly. The gel is transparent and has a watery consistency. It contains several bioactive components, including mucopolysaccharides, prostaglandins, gamma-linolenic acid, glycoprotein, anthraquinone glycosides, and glycoprotein, which primarily contribute to its antibacterial and antifungal properties [8].

*Aloe vera* is the desiccated sap obtained by cutting the base of the leaves from several aloe species within the Liliaceae family. These species include *Aloe ferox* Miller, *Aloe vera* Linn, *Aloe barbadensis* Mil, and *Aloe perryi* Baker. *Aloe perryi* Baker is unique to the islands of Socotra and Zanzibar and their adjacent areas, so aloe derived from this species is often referred to as *Socotrine* or *Zanzibar aloe* [9].

Distinguishing it primarily by its distinct aroma from *Curacao aloes*, *Socotrine aloe* exhibits characteristics such as small cavities on the dried substance's uneven surface, which has an opaque appearance and a colour ranging from yellow-brown to dark brown. Both the taste and the texture of the fractured substance are bitter [9].

The most crucial elements of aloes are the three Aloin isomers: barbaloin,  $\beta$ -barbaloin, and Isobarbaloin, collectively known as 'crystalline' Aloin, found in concentrations of 10% to 30% in the substance. Additional components present in aloes include aloin, resin, emodin, and aloe-emodin. Barbaloin, a water-soluble, bitter, and somewhat yellow crystalline glycoside, is common in all varieties. *Socotrine* and *Zanzibar aloes* predominantly consist of barbaloin, while they lack a crystalline component called Isobarbaloin, which is present in *Curacao aloe* and in smaller amounts in *cape aloe*. Barbaloin and  $\beta$ -barbaloin are the primary constituents of *Socotrine* and *Zanzibar aloe* [9].

#### **Aloe exhibits therapeutic attributes that encompass [10]**

1. Healing burns and wounds.
2. Providing moisture and anti-aging benefits for the skin.
3. Demonstrating anti-inflammatory properties.
4. Aiding in the restoration of the immune system.
5. Exerting anti-diabetic effects.
6. Displaying anti-mutagenic and antioxidant effects.
7. Offering immunomodulatory effects.
8. Delivering anti-bacterial, anti-fungal, and anti-viral actions.
9. Easing symptoms related to arthritis, joint issues, and muscle pain.

#### **2. Materials and Methods**

High-quality analytical chemicals, including hexane, methanol, ethyl acetate, and nutrient agar, were acquired from Thermo Fisher Scientific India Pvt Ltd. Additionally, Beef extract powder and Nutrient agar were sourced from HiMedia Laboratories Pvt. Ltd. Fine Chemicals. Furthermore, in the experiments, Chloroform and concentrated Sulphuric acid were employed.

**Table 1:** Apparatus/instruments

Sr. No.	Equipment	Manufacturer
1.	Autoclave	Quality Instruments & Equipments Kudal, Maharashtra
2.	Hot air oven	Electrolab Industries
3.	Incubator	Electrolab Industries
4.	Weighing balance	Eagle

## 2.1. Collection, identification, and authentication of plant materials

The plant material, specifically *Aloe vera* gel from Socotrine aloe, was gathered during the months of August and September in 2022 on Socotra Island in Yemen. This material received formal authentication from the Republic of Yemen Ministry of Water and Environment, Environment Protection Authority.

In the case of *Aloe barbadensis miller*, the plant material was collected during the months of April and May in 2023. The plant specimen was carefully preserved on a herbarium sheet and subsequently submitted to the Department of Botany at Dr. Babasaheb Ambedkar Marathwada University for the purpose of identification and authentication, with the reference number being BOT/2022-2023.

The *Socotrine aloe* gel was imported from Socotra Island in Yemen, acquired during August and September 2022. It has been diligently stored and maintained at optimal temperature conditions.

On the other hand, fresh *Aloe vera* gel, derived from *Aloe barbadensis miller*, was collected in April and May 2023 from the botany garden of Y.B. Chavan College of Pharmacy in Aurangabad.

## 2.2. Extraction method

*Aloe vera* gel is derived from the leaves of *Aloe barbadensis Miller*. To create the gel, undesired components such as leaf bases, leaf tips, conical dots, and the outer edges of the leaves are routinely removed. The fillets are then meticulously cleaned, ensuring the purity of the gel. The gel is further processed by filtration, resulting in the extraction of pure gel. To preserve the bioactivity of delicate molecules within the gel, it is carefully maintained at low temperatures.

## 2.3. Physicochemical tests

The identification tests for aloe gel can be divided into several specific procedures:

### 2.3.1. Solubility Test

1 gram of aloe gel is taken and dissolved in 100ml of water by boiling, followed by stirring and filtration. It is worth noting that Socotrine aloe does not fully dissolve in water, and therefore it is dissolved in alcohol [11].

### 2.3.2. Bromine Test

For the bromine test, 2 ml of the aloe gel solution is mixed with a freshly prepared bromine solution, resulting in the observation of a pale-yellow colour [11].

### 2.3.3. Nitric Acid Test

In the nitric acid test, a 5ml solution of aloe gel is combined with 2ml of nitric acid, leading to the observation of a colour change from yellow to brown [11].

### 2.3.4. Tannins Test

For the tannins test, 2 ml of the test solution is added to a 5% FeCl<sub>3</sub> (iron chloride) solution, which produces a green-black colour [11].

### 2.3.5. Saponins Test (Foam Test)

In the foam test, the extract or dry powder is agitated with water, resulting in the formation of persistent foam [11].

### 2.3.6. Flavonoids Test (Shinoda Test)

To conduct the flavonoids test, a few drops of concentrated HCl (hydrochloric acid) and 0.5gm of magnesium turnings are added to 5ml of 95% ethanol and the resulting solution yields a red colour [11].

### 2.3.7. Cardiac Glycoside Test (Killer Killani Test)

To perform the cardiac glycoside test, take the chloroform extract and dry it. Following this, add glacial acetic acid (0.4 ml) along with traces of ferric chloride. This process will yield a brownish-red colour, indicating the presence of cardiac glycosides [11].

**Table 2:** Preliminary test

Phytochemicals	Observation	Result
Tannin	Green or brownish colour	Positive
Saponin	Foam formation	Positive
Flavonoid	Pink or Red colour	Positive
Anthraquinones	Red or Pink or Violet	Positive
Cardiac glycosides	Brownish	Positive
Steroid	Violet to Blue	Negative

## 2.4 Antimicrobial activity studies

### 2.4.1 Microorganisms

Clinical strains of microorganisms utilized in the study were sourced from the Microbiology Department at Government Medical College in Aurangabad. Bacterial strains were subjected to sub-culturing on nutrient agar, while fungal strains underwent sub-culturing on Sabouraud dextrose agar. Subsequently, these cultures were incubated for 24 hours to assess their growth.

**Table 3:** Bacterial strains

Bacterial strains	Code number
<i>Escherichia coli</i>	ATCC/25922
<i>Staphylococcus aureus</i>	ATCC/25923
<i>Salmonella Typhi</i>	ATCC/ 14028
<i>Pseudomonas aeruginosa</i>	ATCC/27853
<i>Bacillus Subtilis</i>	NCTC/8236

**Table 4:** Fungal strains

Fungal strains	Code number
<i>Candida albicans</i>	ATCC 90028
<i>Candida Glabrata</i>	ATCC 64677
<i>Candida Krusei</i>	ATCC 14243
<i>Candida tropicalis</i>	ATCC 750
<i>Candida Parapsilosis</i>	ATCC 2209

### 2.4.2 Media preparation

#### 2.4.2.1 Preparation of nutrient broth

The preparation for Nutrient broth involves combining 10 grams of Beef extract, 10 grams of Peptone, and 5 grams of Sodium chloride with 1000 ml of distilled water to maintain a pH of 7. This Nutrient broth is then dispensed into test tubes,

and bacterial strains are inoculated into them under aseptic conditions. The test tubes are subsequently kept at room temperature for 24 hours to facilitate bacterial growth [12].

#### 2.4.2.2 Preparation of Nutrient agar

For Nutrient Agar preparation, mix 2.8 grams of Nutrient Agar with 100 ml of distilled water, ensuring a pH level of 7 [12].

**2.4.2.3 Preparation of Sabouraud Dextrose broth:** For the creation of Sabouraud Dextrose broth, combine 4 grams of Dextrose and 1 gram of Peptone with 100 ml of distilled water while maintaining a pH of 5.4. This Sabouraud Dextrose broth is dispensed into test tubes, and fungal strains are inoculated into it under aseptic conditions. Subsequently, the test tubes are placed at room temperature for 48 hours to facilitate fungal growth [12].

#### 2.4.2.4 Preparation of Sabouraud Dextrose Agar

To create this mixture, combine 4 grams of Dextrose, 1 gram of Peptone, and 2 grams of Agar with 100 ml of distilled water while maintaining a pH of 5.4 [12].

#### 2.4.3 Method used

The Agar well diffusion method, commonly employed for assessing the antimicrobial activity of plant or microbial extracts, involves the following procedure:

According to the guidelines of the National Committee for Clinical Laboratory Standard (NCCLS), the agar well diffusion technique was utilized to evaluate the antifungal activity of aqueous and solvent extracts. To begin, an agar plate was swabbed with a sterile swab that had been dipped into the fungal culture under investigation. Subsequently, the plant extract was introduced into wells within the agar medium, each having an 8mm diameter. These plates were allowed to incubate for 2 hours at room temperature. For fungal strains, further incubation occurred for 48 hours at 37 °C, while for microbial strains, it lasted for 24 hours at 37 °C, both in an upright position [12].

In these wells, the same volume of *Socotrine aloe* gel, water for injection, *Aloe barbadensis miller* gel, and the standard

antifungal drug clotrimazole at 10ug/ml concentration were placed. Clotrimazole served as the typical antifungal reference. Similarly, identical volumes of *Socotrine aloe* gel, water for injection, *Aloe barbadensis miller* gel, and the standard antibacterial drug streptomycin at 150ug/ml concentration were introduced into separate wells. Streptomycin acted as the typical antibiotic. Following incubation, the diameters of the growth inhibition zones were measured in millimetres [12].

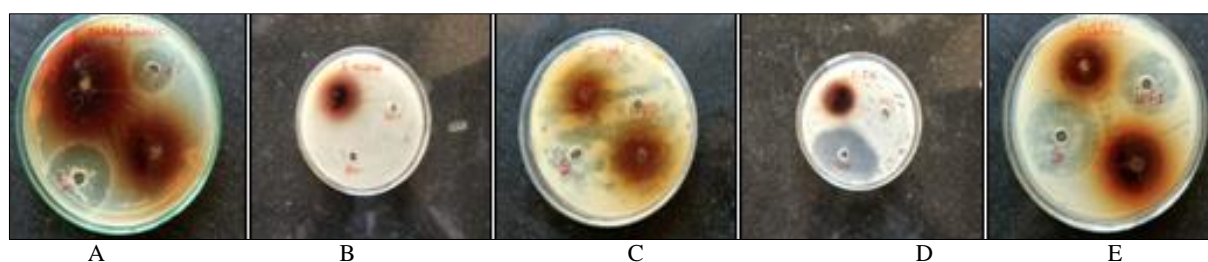
### 3. Results and Discussion

The result obtained confirms that the traditionally used *Aloe vera* gel is a potent and effective antimicrobial agent. Moreover, the result establishes a good antibacterial property. The comparative evaluation of antimicrobial activity of crude extract of *Socotrine aloe* and *Aloe Barbadensis Miller* was determined against 5 bacterial and 5 fungal strains respectively. Antibacterial activity was observed, which is evidence that it can be used to treat different bacterial illnesses. Furthermore, the research demonstrates that the *Socotrine aloe* gel has effective antifungal properties against *Candida tropicalis*.

In the preliminary antibacterial study, the crude extract of *Socotrine aloe* shows higher activity against *Pseudomonas aeruginosa* with 34.6mm and lowest against *Bacillus Subtilis* with 25.6mm zone of inhibition. While the preliminary antifungal study, shows highest activity against *Candida albicans* with 38.3mm and lowest activity against *Candida Krusei* with 32.3mm zone of inhibition respectively.

The comparative antimicrobial study of the crude extract of aloe species were evaluated using agar well diffusion method. For crude extract of *Socotrine aloe*, *Staphylococcus aureus* shows the highest antibacterial activity among the selected strains with the average zone of inhibition of  $30.6 \pm 0.577$  mm, while *Pseudomonas aeruginosa* shows highest inhibition with  $39.6 \pm 0.839$  mm for *Aloe barbadensis miller*.

Similarly, *C. tropicalis* and *C. Krusei* ( $26.6 \pm 0.577$  mm) and *C. glabrata* ( $10.6 \pm 0.115$  mm) shows maximum antifungal activity among the selected strains for *Socotrine aloe* and *Aloe barbadensis miller* respectively.



(a = *Pseudomonas aeruginosa*, b = *Staphylococcus aureus*, c = *Salmonella Typhi*, d = *Escherichia coli*, e = *Bacillus Subtilis*)

**Fig 1:** Preliminary antibacterial activity of *Socotrine aloe*

**Table 5:** Zone of inhibition Preliminary antibacterial activity of *Socotrine aloe*

Sr. No.	Strains	Zone of Inhibition (mm)		
		Socotrine Aloe	WFI	Streptomycin
1	<i>E. coli</i>	28.3	10	51
2	Subtilis	25.6	12	51.6
3	Aureus	27	11.6	10
4	Typhi	29.3	20	10
5	<i>P. Aeruginosa</i>	34.6	22	40

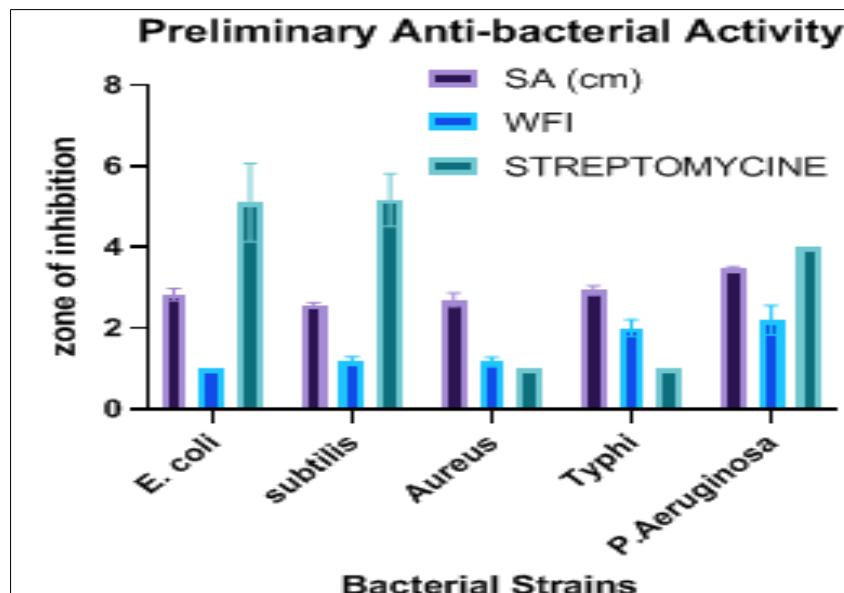
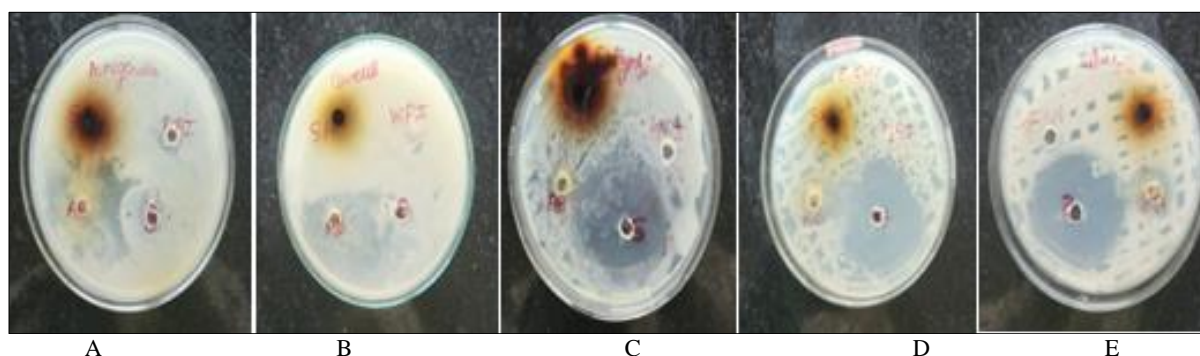


Fig 2: Graphical representation of the zone of inhibition achieved by agar well diffusion method



a = *Pseudomonas aeruginosa*, b = *Staphylococcus aureus*, c = *Salmonella Typhi*, d = *Escherichia coli*, e = *Bacillus Subtilis*

Fig 3: Antibacterial activity of *Socotrine aloe* and *Aloe barbadensis miller*

Table 6: Zone of inhibition Antibacterial activity of *Socotrine aloe* and *Aloe barbadensis miller*

Zone of Inhibition (mm)					
Sr. No.	Strains	Socotrine Aloe	Aloe Barbadensis	WFI	Streptomycin
1	<i>E. coli</i>	24.3	20	10	34.6
2	<i>Subtilis</i>	25	20.6	10	34.6
3	<i>Aureus</i>	30.6	32.6	10	11.3
4	<i>Typhi</i>	30	17	20	21.6

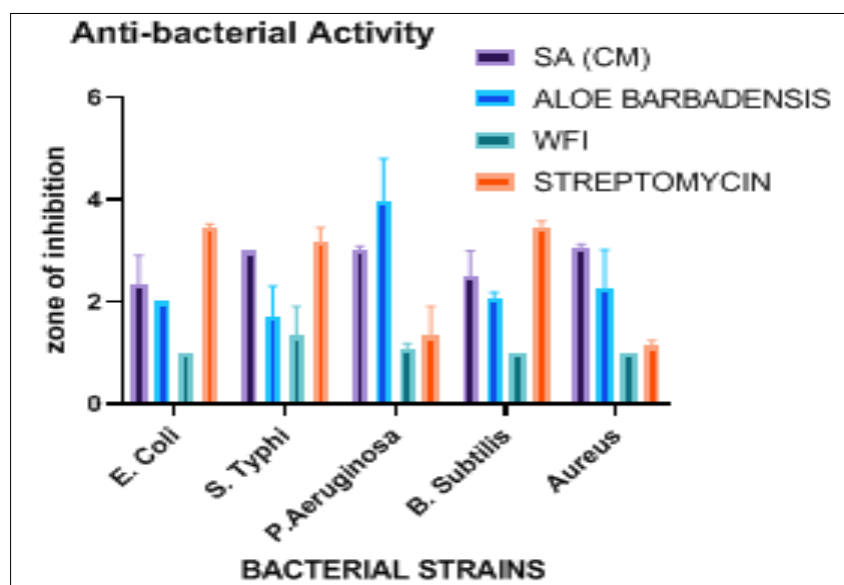


Fig 4: Graphical representation of the zone of inhibition achieved by Agar well diffusion method

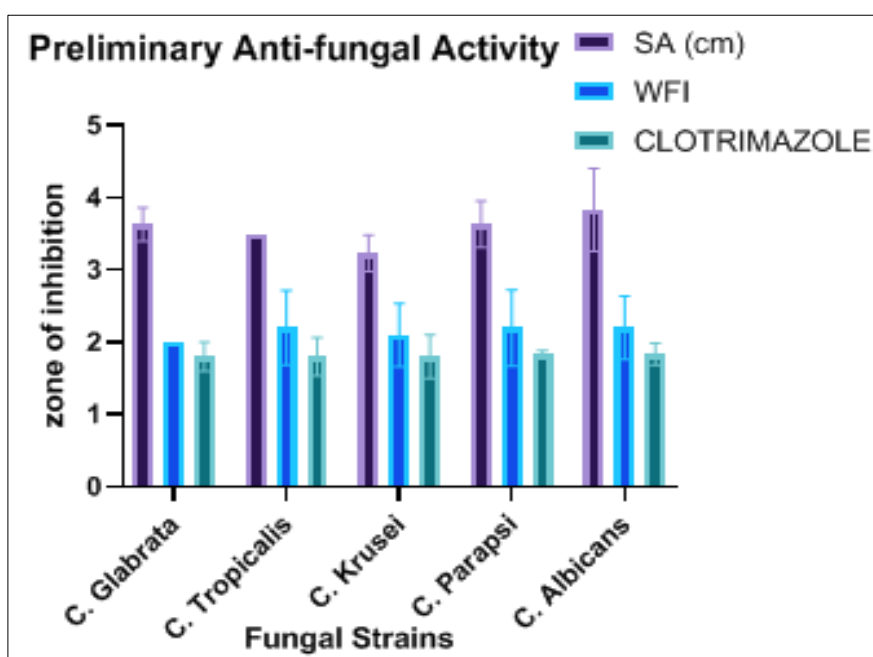


A = *Candida tropicalis*, B = *Candida Krusei*, C = *Candida Parapsilosos*, D = *Candida Glabra*, e = *Candida albicans*

**Fig 5:** Preliminary antifungal activity of *Socotrine aloe*

**Table 7:** Zone of Inhibition: Preliminary antifungal activity of *Socotrine aloe*

Zones of Inhibition (mm)				
Sr. No	Strains	Socotrine aloe	Clotrimazole	WFI
1	<i>Candida albicans</i>	38.3	18.3	22
2	<i>Candida Glabrata</i>	36.3	18	20
3	<i>Candida Parapsilosis</i>	36.3	18.3	22
4	<i>Candida Krusei</i>	32.3	18	21
5	<i>Candida tropicalis</i>	35	18	22



**Fig 6:** Graphical representation of the zone of inhibition achieved by Agar well diffusion method

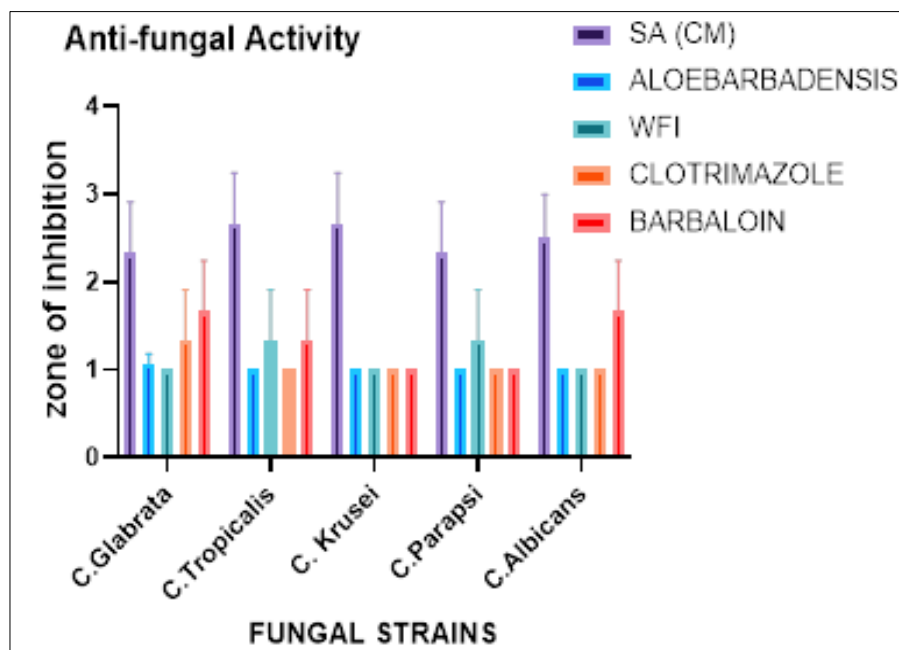


A = *Candida tropicalis*, B = *Candida Krusei*, C = *Candida Parapsilosos*, D = *Candida Glabra*, E = *Candida albicans*

**Fig 7:** Antifungal activity *Socotrine aloe* and *Aloe barbadensis miller*

**Table 8:** Zone of inhibition: Antifungal activity *Socotrine aloe* and *Aloe barbadensis miller*

Zone of Inhibition (mm)					
Sr. No.	Strains	Socotrine Aloe	Aloe Barbadensis	WFI	Clotrimazole
1	<i>C. albicans</i>	25	10	10	10
2	<i>C. glabrata</i>	23.3	10.6	10	13.3
3	<i>C. parapsilopsis</i>	23.3	10	13.3	10
4	<i>C. krusei</i>	26.6	10	10	10
5	<i>C. tropicalis</i>	26.6	10	13.3	10



**Fig 8:** Graphical representation of the zone of inhibition achieved by Agar well diffusion method

#### 4. Conclusion

The study concluded that the plant gel, *Aloe barbadensis miller* and *Socotrine aloe* possess excellent biological property. The Antimicrobial activity of both with standard antimicrobial and anti-fungal drug were performed. The study performed in the comparison between *Aloe barbadensis miller* and *Socotrine aloe* gel. This study indicate that *Socotrine aloe* possess greater antimicrobial activity than *Aloe barbadensis miller* against *E. coli*, *B. subtilis*, *S. typhi* strains and *Aloe barbadensis miller* against *P. Aeruginosa*. Hence, we can use *Socotrine aloe* gel to make new antimicrobial formulation. The antibacterial study with *Aloe barbadensis miller*, shows highest activity against *Staphylococcus aureus* i.e., 30.6 mm, and lowest activity against *Escherichia coli* i.e., 24.3 mm. While *Aloe barbadensis miller*, shows highest antifungal activity against *Candida tropicalis* i.e., 26.6 mm and lowest antifungal activity against *Candida Glabrata* i.e., 23.3 mm.

*Socotrine aloe* shows greater antimicrobial activity against *E. coli* by 4.4 mm, against *B. subtilis* by 4.4 mm, against *S. typhi* by 13 mm and shows grater antifungal activity against *C. albicans* by 15 mm, against *C. glabrata* by 12.7 mm, against *C. Parapsilosis* by 13.3 mm, against *C. Krusei* by 16.6 mm and against *C. tropicalis* by 16.6 mm compared with *Aloe barbadensis miller*.

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