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COMPARATIVE STUDY ON THE ANTIFUNGAL ACTIVITY OF *ALOE VERA* LEAF AND GEL IN DIFFERENT EXTRACTS

L.TAMILARASI*, R.PONNULAKSHMI AND S.EZHILARASI BALASUBRAMANIAN

Post Graduate and Research Department of Zoology, Ethiraj College for Women, Chennai – 600 008.

ABSTRACT

Screening of antifungal activity of *Aloe vera* leaf and gel in three different solvents (aqueous, chloroform and ethanol) were carried out against selected fungal pathogens by agar diffusion method. They were tested against four fungal organisms like *Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus* and *Candida albicans*. The susceptibility of the fungi to these extracts in different concentrations were compared. The highest *in vitro* inhibitory activity was observed with *Candida albicans*. Of all the three different extracts, ethanol extract of *A. vera* gel exhibited significant antifungal activity than the *A. vera* leaf extract. Hence, *A. vera* gel extract with ethanol can be used as an antifungal agent. The results of the present study provide a information about the role of *A. vera* gel for its antifungal properties.

KEYWORDS: ***Aloe vera*, antifungal activity, *Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus* and *Candida albicans*.**

L.TAMILARASI
Post Graduate and Research Department of Zoology,
Ethiraj College for Women, Chennai – 600 008.

INTRODUCTION

Diseases due to the pathogenic bacteria and fungi represent a critical problem in human health and they are one of the main causes of morbidity and mortality worldwide[1]. Fungi are eukaryotic organisms including yeast and moulds as well as the more familiar mushrooms. It causes serious infectious human diseases like aspergillosis and candidiasis. Aspergillosis is caused by the fungus *Aspergillus*. Although most people are often exposed to *Aspergillus*, infection occurs in people with less immunity. Rare infections caused by *Aspergillus* include pneumonia and aspergilloma (fungus ball)[2]. Candidiasis can affect various regions of the body like mouth, oesophagus, skin and vagina. Invasive candidiasis leads to death in 15 to 25% of affected adults and 10 to 15% of affected infants and children[3]. In the recent years, infections have increased to a great extent and antibiotic resistance has become an ever-increasing therapeutic problems[4]. Natural products of higher plants may possess a new source of antimicrobial agents with possibly novel mechanisms of action[5and6]. Traditionally used medicinal plants produce a variety of compounds known for their therapeutic properties[7and8]. Nearly all the identified bioactive compounds of the plants can either inhibit the growth of pathogens or kill them and the host will not have any toxic effect. In recent years, the antimicrobial properties of the medicinal plants are being increasingly reported from different parts of the world[9–16]. The genus *Aloe* is scientifically named as "*Aloe barbadensis* Miller" which is a member of the Lilliaceae or Asphodelaceae family[17]. *A. barbadensis* is also known as *A. vera*[18]. *Aloes* are one of the oldest medicinal plants known to mankind. It has been most popular in both folk and traditional medicine[19–21]. The extraordinary healing power of the *A. vera* plant has been passed down through the centuries and there is an increasing amount of evidence that *A. vera* can help numerous ailments, both internal and external. *Aloes* are primarily used as an herbal remedy in alternate medicines and home first aid[22]. *A. vera* leaf and gel extracts possess compounds with antimicrobial properties which could be used as an antimicrobial agents[23]. As most of the antimicrobial properties of *A. vera* were depended upon the amount of the phytochemical compounds present in it, the phytochemical compounds of *A. vera* were analysed and reported[24]. *A. vera* is rich in tannins, alkaloids, triterpenoids, flavonoids, saponins, acids and phenol. Each compound has its own amazing effects in humans and other organisms. Most probably these bioactive compounds in *A. vera* might be responsible for the inhibition of microorganisms, which could represent a new source of potent non toxic, less expensive antimicrobial agent than the allopathic drugs. *A. vera* also possesses antifungal, antiviral, antibacterial and acaricidal activities against skin infections such as acne, herpes and scabies[25and26]. Hence, the present study is to evaluate the efficacy of the aqueous, chloroform and ethanolic extracts of *A. vera* leaf and gel against selected fungal species.

MATERIALS AND METHODS

1. Collection of A. vera leaf

A. vera fresh leaves were collected in Thiruvallur District and authenticated by Dr. A. Manoharan, Associate Professor and Head of the Department of Plant Biology and Biotechnology, Presidency College, Chennai – 5.

2. Microorganisms

Four species of fungi *Aspergillus niger* (ATCC NO 9029), *Aspergillus fumigatus* (ATCC NO 46645), *Aspergillus flavus* (ATCC NO 16870) and *Candida albicans* (ATCC NO 2091) were obtained from authorized laboratories and maintained in sterile growth medium.

ATCC NO - represents the American type culture collection

3. Extract preparation

(i) Preparation of aqueous extract

A. vera aqueous extract was prepared by soaking 100gms of the crude paste of *A. vera* leaf and gel separately in a conical flask containing 300ml of distilled water. With frequent mixing, this preparation was allowed to stand for 24hours. It was filtered using Whatman filter paper and concentrated at

100º C in a water bath. The extract yield was weighed and stored in the refrigerator till used for further experimental purposes.

(ii) Preparation of chloroform and ethanol extracts

For chloroform and ethanol extraction 100gms of the crude paste of *A. vera* leaf and gel were taken separately, to which 300ml of chloroform and ethanol were added. With frequent mixing, the preparation was retained for 14days and filtered. The filtrates were concentrated at 60ºC in a water bath. The extracts were then weighed and stored in the refrigerator for further experimental purposes.

4. Assay of antifungal activity

The disc diffusion method[27] was used to evaluate the antifungal activity of *A. vera* leaf and gel extracts against *Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus* and *Candida albicans*. A suspension of fungal organisms were separately added to sterile growth medium at 45°C and poured into petridishes to give a depth of 5mm. Various concentrations of *A. vera* leaf (100, 150 and 200 µg/ml) and *A. vera* gel (50,100 and 150µg/ml) in different extractions were prepared separately. Sterile disc (made from Whatman filter paper previously sterilized in UV lamp) dipped in specific concentrations of *A. vera* leaf and gel extracts and standard (ketoconazole, 50µg/ml) and were placed on the surface of agar plates after drying. The plates were left for 1hour at room temperature and incubated at 37°C for 24 hour. The diameter of the zone of inhibition by the extracts and standard were measured and recorded. Ketoconazole an antifungal agent was preferred as the standard for the present study.

RESULTS AND DISCUSSIONS

The present investigation records the antifungal activities of *A. vera* leaf and gel extracts against few selected fungal pathogens. However, the degree of inhibition varied depending upon the concentration of the extracts. Different concentrations of *A. vera* leaf (100, 150, 200 µl) and *A. vera* gel (50, 100, 150 µl) were analysed with three different extracts (aqueous, chloroform and ethanol) in the present study. *A. vera* gel extract exhibited maximum zone of inhibition against microorganisms, while *A. vera* leaf extract showed minimum growth suppression in all the concentrations tested. Hence, in the present investigation a common concentration of *A. vera* leaf and gel (150 µl) were selected and their efficacy against four fungal organisms were discussed. In the present investigation, by using agar disc diffusion method different extracts of *A. vera* leaf and gel (150µl) were shown to inhibit the growth of fungi. The results of the antifungal assay of *A. vera* leaf extract revealed the chloroform extract to possess maximal growth inhibition than the aqueous extract followed by ethanol extract. *A. vera* acts as an antiseptic agent against a number of bacteria and fungi, like *Staphylococci, Streptococci* and *Candida*[28] and [29]. *C. albicans* registered its higher sensitivity to the aqueous extract followed by the chloroform and ethanol extract of *A. vera* leaf, in the present investigation (Table 1 and Plate 1).

Table 1
Antifungal screening of Aloe vera leaf aqueous extract

No	Organisms	Zone of inhibition in mm			
		STD	•100 µl	•150 µl	•200 µl
	Fungi				
1	*Aspergillus niger*	38	11(29)••	14(37)	18(47)
2	*Aspergillus fumigatus*	36	13(36)	15(42)	19(53)
3	*Aspergillus flavus*	38	12(32)	16(42)	20(53)
4	*Candida albicans*	38	16(42)	20(53)	23(61)

- ***Concentrations of extract***

•• Values in parentheses indicates the percentage of inhibition

However, *A. vera* leaf chloroform extract exhibited the greatest zone of inhibition followed by the aqueous and ethanol extracts against *A. flavus*. *A. flavus*, which is known to produce toxins, was found to be the most resistant microorganism (Table 2 and Plate 1).

Table 2
Antifungal screening of Aloe vera leaf chloroform extract

No	Organisms	Zone of inhibition in mm			
		STD	•100 µl	•150 µl	•200 µl
	Fungi				
1	*Aspergillus niger*	38	16(42)••	20(53)	23(61)
2	*Aspergillus fumigatus*	39	18(46)	20(51)	24(62)
3	*Aspergillus flavus*	38	14(37)	21(55)	25(66)
4	*Candida albicans*	39	12(31)	16(41)	19(49)

• ***Concentrations of extract***
•• ***Values in parentheses indicates the percentage of inhibition***

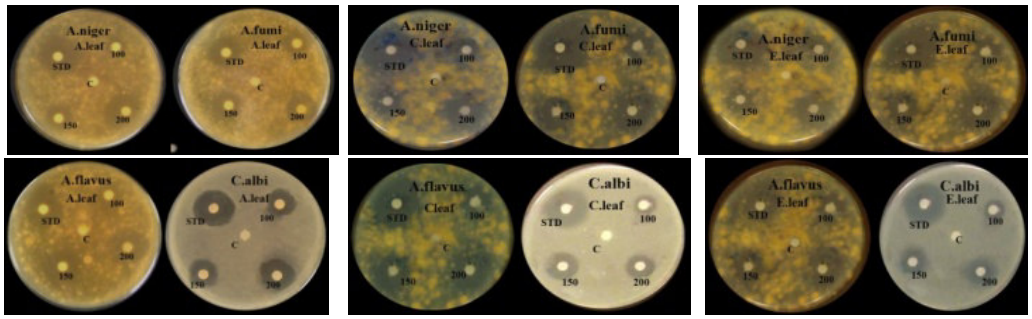
A. vera leaf extract possessed the maximum inhibitory effect on *A. niger* in the ethanol extract followed by chloroform and aqueous extract (Table 3 and Plate 1).

Table 3
Antifungal screening of Aloe vera leaf ethanol extract

No	Organisms	Zone of inhibition in mm			
		STD	•100 µl	•150 µl	•200 µl
	Fungi				
1	*Aspergillus niger*	38	16(42)••	18(48)	22(58)
2	*Aspergillus fumigatus*	39	14(36)	18(46)	21(53)
3	*Aspergillus flavus*	39	11(29)	13(33)	17(44)
4	*Candida albicans*	39	12(31)	14(36)	18(46)

• ***Concentrations of extract***
•• ***Values in parentheses indicates the percentage of inhibition***

Plate 1
Fungal organisms exhibiting sensitivity to different extracts of Aloe vera leaf



In the present investigation, *A. vera* gel aqueous extract (150µl) was also analyzed against selected fungal organisms. The maximum growth suppression was found against *A. flavus* (72%) and the minimum growth suppression against *C. albicans* (62%). The other two fungal organisms were *A. niger* (68%) and *A. fumigatus* (69%) which were moderately inhibited by the aqueous extract of *A. vera* gel (Table 4 and Plate 2)

Table 4
Antifungal screening of Aloe vera gel aqueous extract

No	Organisms	Zone of inhibition in mm			
		STD	•50 µl	•100 µl	•150 µl
	Fungi				
1	*Aspergillus niger*	38	19(50)••	22(58)	26(68)
2	*Aspergillus fumigatus*	36	20(51)	24(62)	27(69)
3	*Aspergillus flavus*	38	18(46)	22(56)	28(72)
4	*Candida albicans*	38	16(41)	20(51)	24(62)

• ***Concentrations of extract***
•• ***Values in parentheses indicates the percentage of inhibition***

The chloroform extract of *A. vera* gel exhibited maximum zone of inhibition against *A. niger* (69%) and *C. albicans* (69%) and the minimum zone of inhibition against *A. fumigatus* (62%). *A. flavus* (67%) was moderately inhibited by the chloroform extract of *A. vera* gel (Table 5 and Plate 2).

Table 5
Antifungal screening of Aloe vera gel chloroform extract

No	Organisms	Zone of inhibition in mm			
		STD	•50 µl	•100 µl	•150 µl
	Fungi				
1	*Aspergillus niger*	38	19(49)••	24(62)	27(69)
2	*Aspergillus fumigatus*	39	18(46)	21(54)	24(62)
3	*Aspergillus flavus*	39	14(37)	21(55)	25(66)
4	*Candida albicans*	39	17(44)	21(54)	27(69)

• ***Concentrations of extract***
•• ***Values in parentheses indicates the percentage of inhibition***

C. albicans was more sensitive to ethanol extract of *A. vera* gel followed by the chloroform and aqueous extract. In the present study, the ethanol extract of *A. vera* gel posses strong inhibitory activity against *C. albicans* (79%) (Table 6 and Plate 2).

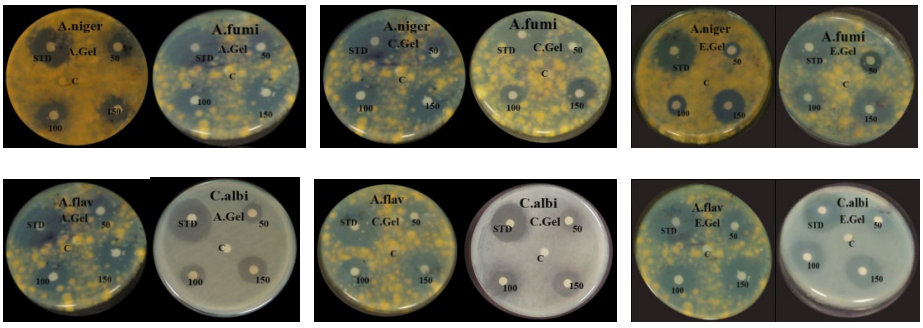
Table 6
Antifungal screening of Aloe vera gel ethanol extract

No	Organisms	Zone of inhibition in mm			
		STD	•50 µl	•100 µl	•150 µl
	Fungi				
1	*Aspergillus niger*	39	14(36)••	16(41)	23(59)
2	*Aspergillus fumigatus*	39	18(46)	22(56)	29(74)
3	*Aspergillus flavus*	39	19(49)	24(62)	30(77)
4	*Candida albicans*	38	18(47)	25(66)	30(79)

• ***Concentrations of extract***
•• ***Values in parentheses indicates the percentage of inhibition***

A. fumigatus was found to be moderately inhibited in all the three different extracts of *A. vera* leaf and gel in the present investigation.

Plate 2
Fungal organisms exhibiting sensitivity to different extracts of Aloe vera gel



This organism is well known to play an important role in the mucous surface (thrush gastrointestinal or urogenital tract) and deep-seated infections such as candidaemia or meningitis. Candida vaginitis is a common infection during pregnancy[30]. The inhibition of *C. albicans* by the *A. vera* leaf and gel extracts were distinct from one another even though they share certain common phytochemical components. These results are in accordance with the earlier report[31]. Therefore, according to the results of the present investigation, the fungal organisms are more sensitive to *A. vera* gel extracts. The results of the antifungal activity revealed that the ethanol extract of *A. vera* gel to possess

maximum growth inhibition followed by aqueous and chloroform extracts than the *A. vera* leaf extract. *A. vera* is used based on its historic and traditional use and analysis of modern pharmacologic and toxicologic research works[32]. Another study suggests that *A. vera* could inhibit infectious diseases by stimulating the host defence mechanism, especially the phagocytic and killing activities of macrophages[33]. Previous reports were the evidences for the usage of *A. vera* as an antimicrobial agent and still it is used for the research in toxicology and pharmacology. It is evident from the present investigation that *A. vera* leaf and gel possess antifungal effects in all the three extracts. Among them, *A. vera* gel ethanol extract was more potent than the other two extracts. Hence, in the comparative studies of *A. vera* leaf and gel, gel act as an antifungal agent.

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