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Antifungal activity of aloevera extracts on the soil borne fungi

Nupur Bahatnagar, Ruby Grover
Department of Biotechnology, MJRP University, Jaipur, Rajasthan, India

Abstract
The ethylacetate and methanol extracts of *Aloevera* gel were used to analyze for their antifungal activity over soil borne fungi by using colony counting procedure. The study suggests that antifungal activity of *Aloevera* gel extract showed effective results to reduce fungal growth and it could be useful in future for fungal infected disease. The biochemical components starch, cellulose, lipase hydrolysis and protein content analysis test of the isolated fungi species was also determined. The results showed that to starch hydrolysis test *R. mucilaginosa* observed no halo zone while *A. nidulans* observed halo zone around the fungi. In cellulose hydrolysis test *A.nidulans* showed halo zone and *R.mucilaginosa* showed no halo zone. In lipase hydrolysis tests both *A.nidulans* and *R. mucilaginosa* showed positive result and formed halo zone. The protein content analyses were determined by Lowry method. In the present results *A.nidulans* showed maximum range of protein content (0.190) as compare to *R.mucilaginosa* (0.151).

Keywords: *Aloevera*, antifungal activity, biochemical components, fungi, halo zone

Introduction
A large group of eukaryotic organisms includes microorganisms such as moulds, yeast and broadly it consists of fungus. These microorganisms distributed as a kingdom Fungi, which differed from protists, plants, bacteria and animals.

The fungi are heterotrophs, best decomposers and the presence of chitin in their cell wall placed the fungi in a separate kingdom, i.e. Eumycota (Eumycetes). The genetic studies have shown that fungi are more closely related to animals rather than plants. They are not able to ingest their food like animals and plants as they get their food by secreting digestive enzymes around their surroundings. The filament-like structures known as hyphae are produced which releases the enzymes and helps to break down the substrate which makes it easier for fungus to absorb the nutrients from the substrate.

Based on the rate of evolution it was estimated the identification of fungal organism about 760-1060 million years ago (Lucking *et al.*, 2009) [1]. Fungi were showing similar features to aquatic organism, which consists of chytrids, and were having flagellum like spores. 10% to 50% of the world's harvested fruit are lost each year due to fungal attack.

The hyphae are having high surface areas are adapted for the efficient extraction of nutrients in solid substrates or single cells in aquatic environments (Moss, 1986) [2]. It is fact that hyphae are exclusively modified to growth on solid surfaces, or to attack substrates and tissues. They can apply large penetrative mechanical forces; for example, the plant pathogen *Magnaporthegrisea* forms a organization called an appressorium that evolve to prick plant tissues. In this regards *Aloevera* plant is the best example to lessen fungal dent.

The mucilaginous gel, which has been used for the treatment of wounds, burning and moreover, it helps increase the rate of healing and reduces the risk of infection (Joseph and Raj, 2010) [3]. The aloe gel consists of various minerals like barium, strontium, aluminium, boron, sodium, calcium, magnesium, iron, phosphorus, silicon and iron. The *Aloe vera* leaf contains over 75 nutrients and 200 bioactive compounds including 20 minerals, 18 amino acids and 12 vitamins (Park *et al.*, 2006) [4]. It contains all essential amino acids and secondary amino acids beneficial for human health. In the present study the antifungal activity of *Aloevera* gel were determined on isolated soil borne fungi.

Material and methods
Collection of soil sample
The soil samples were collected from Jaipur district of Rajasthan including Brahmpuri and Agra road. The samples were collected from 8-10 cm depth using a sterile spatula and transferred into the sterilized polybags and were carried out in the laboratory for future experiments.

Media preparation
Potato dextrose agar was used to isolate fungi. 150ml of distilled water was added into 5.85 gram potato dextrose agar powder final the ph at 7.0 and allowed to autoclaved for 15 min. at 121^0C after autoclaved, media was cooled down and allowed to pour into sterilized petriplates under the laminar air flow hood.

Isolation of fungi from soil sample
In this method soil dilutions were made by suspending 1 gram soil of each sample in 10ml of sterilized water. Dilution 10^{-3}, was carried for isolation of fungi from soil sample. 1ml of microbial suspension of each concentration was added to sterile petriplates in triplicates of each dilution containing autoclaved Potato dextrose agar media. For preventing bacterial growth 1% streptomycin solution was added before pouring. The plates were allowed to incubate at 28^0C for 5 to 7 seven days.

Morphological characterization of fungal isolates by lactophenol staining technique

A fungal colony was first grown on the potato dextrose agar medium and its morphology was studied using standard cover-slip technique and lactophenol cotton blue staining procedure. The fungal morphology was deliberate macroscopically by observed the colony features (shape, color, hyphae size), and microscopically by a compound microscope with a digital camera using a lactophenol cotton blue-stained slide mounted with a small portion of the mycelium (Gaddeyya *et al*., 2012) [5].

Antifungal activity of *Aloe vera*

150 ml distilled water added into the conical flask and add 5.85 gram potato dextrose agar. Autoclaved media and poured into sterilized petriplates under aseptic condition for control treatment. The antifungal activity of the *Aloe vera* gel extracts were measured at 1.0 mg/ml, 0.50, 0.25 and 0.0625 concentration of ethylacetate and methanol extracts in colony forming unit. The growth rate of fungus was recorded after 7 days by incubation.

Preparation of the Extracts

Fresh leaf gel of Aloevera were allowed to dry in the oven at 80^0C for 48 hour and then powdered. 20grams of this powder was soaked in 100ml of each solvent of ethylacetate and methanol for 24 hour whatman filter paper no.1 used to filtrated contents. The dried extract was powdered and allowed to dissolve in distilled water.

Statistical Analysis

Colony forming unit (CFU) analysis was use to expressed population density of fungal growth for each gram soil dilutions. The percentage involvement of every one isolated were measured by given following method.

$$\% \text{ Contribution} = \frac{\text{Total no. of cfu of an individual sps}}{\text{Total no. of cfu of all sps.}} \times 100$$

CFU – Colony forming unit

Starch hydrolysis test

For starch agar medium (starch 20.0 g/l, peptone 5.0g/l, yeast extract 3.0 g/l, agar 15.0 g/l; pH 7.0) was inoculated with isolated fungal cultures. The plates were kept in inverted position for 5 to 7 days after incubation at 25^0C. Iodine solution was flooded over the surface of the plates for 30 sec. examined the disappearance of starch from the starch agar media plates by observing the disappearance of clear zones around the fungal growth. (Madigan *et al*, 2012) [6].

Cellulose hydrolysis test

The Czapek-mineral salt agar medium consisted of KCl 0.5 (g/l), K2HPO4 1.0 (g/l), NaNO3 2.0 (g/l), MgSO4.7H2O 0.5 (g/l), peptone 2.0 (g/l), carboxymethyl cellulose (CMC) 5.0 (g/l). After this the medium was complemented with agar 2% and autoclaved for 15 min. at 15 psi. (Reddi pradeep and Narasimha, 2011) [7] Under laminar flow hood autoclaved medium was poured in sterilize petridish and permitted to solidify. The plates were allowed to inoculate with isolated fungal cultures. After whole process the plates were incubated at 35^0C for 5 days in inverted position. Pates surface was flooded with 1 % aqueous solution of hexadecyltrimethyl ammonium bromide for 30 sec. Formation of the clear zone is the observation for result. Positive result showed an opaque zone around the fungal growth.

Lipase hydrolysis test

Tributyrin was used for lipase hydrolysis test of lipase producing fungi as a substrate on agar plates (Thomas and Kavitha, 2015) [8]. The following composition was use for tributrin agar. For this purpose tributyrin agar used of following composition:

Peptone: 5.0gram
Yeast extract: 3.0 gram
Agar-Agar: 1.50gram
Tributyrin (Glycerol tributyrate): 10.0ml
D.W: 990ml
Ph: 7.5

Clear zone formation around the fungal colonies indicates lipase production. Isolated fungal culture were inoculated on the TBA plates and incubated for 15 days at 27^0 C. Lipase producing fungi showed halo zones around the fungal colonies.

Protein content Determination by Lowry's method

In microfuge tube 500ml of fungal culture and protein contents were equally precipitated with volume ice cold 20%. trichloroacetic acid (TCA) and allowed to refrigerated overnight at 4°C. The pellet were centrifuged to recovered at 12,000 rpm for 5 min at standard temperature and decant the supernatant. Ice-cold of 1ml and 10% TCA and ice-cold acetone was used to wash pellet. Pellet was dissolved in0.5-1.0 ml of 0.1 N NaOH according to their size. Heat the solution for 5min under in boiling water bath vortexes dynamically. The Lowry method was used to determine protein content. (Lowry *et al.,* 1951) [9]. In this method 0.5 ml of protein solution was full in glass test tube and add alkaline solution 2.5ml [prepared by mixing 2 % Na2CO3 solution (in NaOH), 2 % sodium potassium tartrate plus 1 % CuSO4.5H2O in 100:1:1. was added to determine protein content.

Mixed well all the contents and tubes were permitted for incubation for 10 min at room temperature follow by the addition of 0.25ml of 1.0 N Folin' s reagent. *(Everette et al.,* 2010) [10]. Thoroughly mixed the tube of contents after 10 min, absorbance at 660 nm next to reagent blank was indomitable spectrophotometrically use bovine serum albumin fraction V as standard.

Results

Isolation of the fungi

Isolated fungal strain from each soil sample was selected at random for further experiments. The subcultures were maintained on PDA broth. The selected concentration was 10^{-3} for further antifungal activity or biochemical test of fungi.

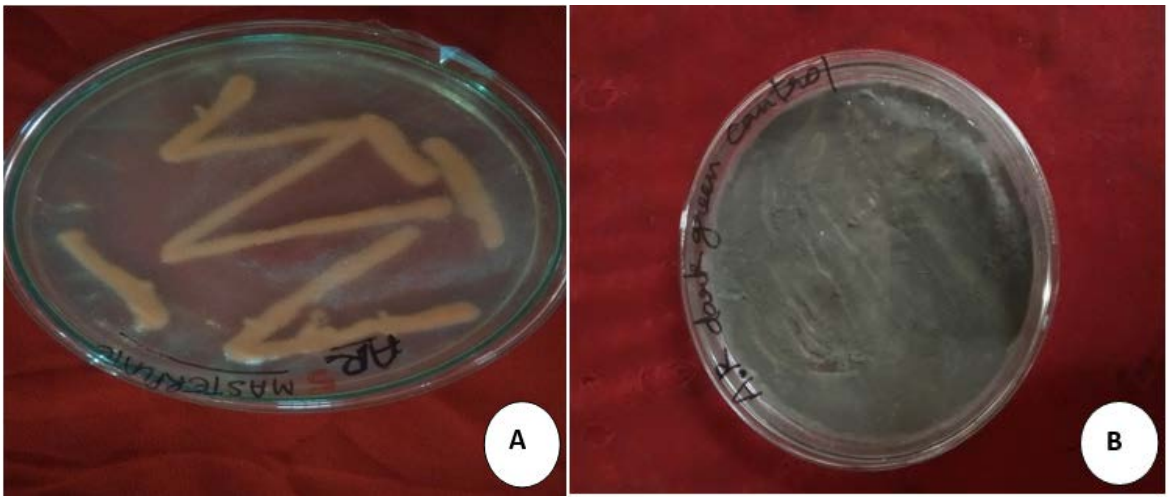
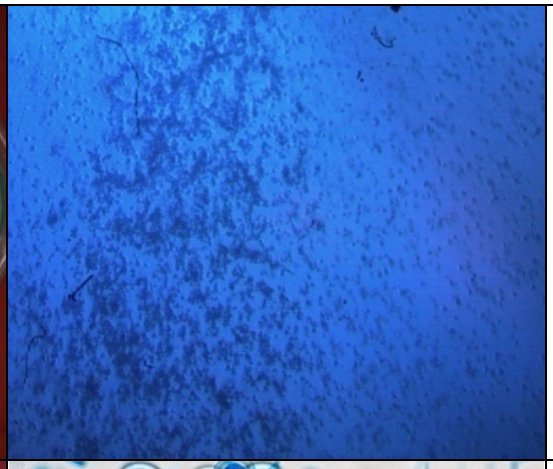
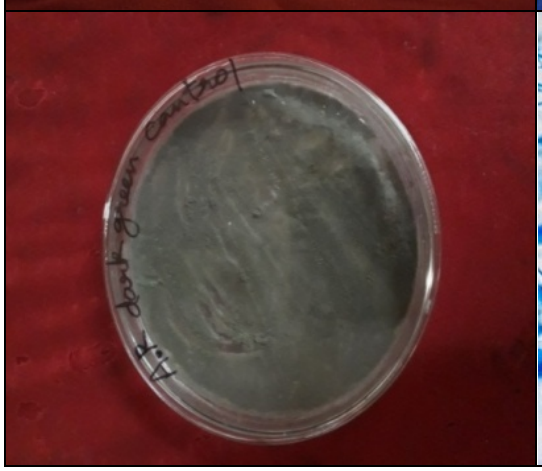
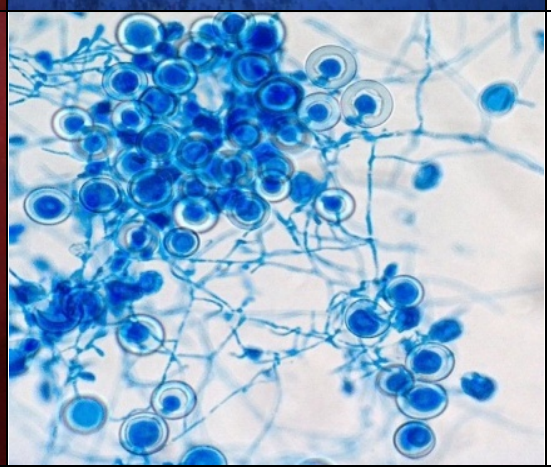


Fig 1: showed isolated fungi (A) *R.mucilaginosa* and (B) *A.nidulans*

Morphological identification of isolated fungi: Isolated fungi were identified by their morphologically characterization according to their color, shape, types of the fungal species, mycelium and by spores.

Table 1: Morphological characterization of isolated fungi by microscopic examination

		*Rhodotorula mucilaginosa*
		*Aspergillus nidulans*

Antifungal activity of *Aloe vera* on soil borne fungi

Fresh leaf gel of *Aloe vera* was dried in the oven at 80^0C for 48hour and powdered prepared ethyl acetate and methanol extracts. Twenty grams of this powder was soaked in 100ml of each of the solvents that was ethyl acetate and methanol for 24hour. After soaked the contents filtered by Whatman filter paper no.1 and filtrate was evaporated to dryness. The dried extracts was powdered to approach and dissolved in distilled water.

Table 2: Percentage Yield of Crude Extracts of *Aloe Vera* Gel

Species Name	Type of extract	Gel weight	Extracts weight	% yield of extracts
Aloevera	Ethylacetate	20gram	7gram	35
Aloevera	Methanol	20gram	7.9gram	39.5

Table 3: Colony counting of *Aspergillus nidulans* from soil sample of Jaipur district

S. No.	Fungal flora of soil sample1	Fungal cfu % in PDA (Control)	Fungal cfu % in medium containing ethylacetate *Aloe vera* extraction	Fungal cfu% in medium containing methanol *Aloe vera* extraction
2	*Aspergillus nidulans*	5.85	5.79	5.67

Fig 2: Colony forming unit of *Aspergillus nidulans* of control, ethyl acetate and methanol extraction by plate streak method

Table 4: Colony counting of *Rhodotorula mucilaginosa* from Agra road soil sample of Jaipur district

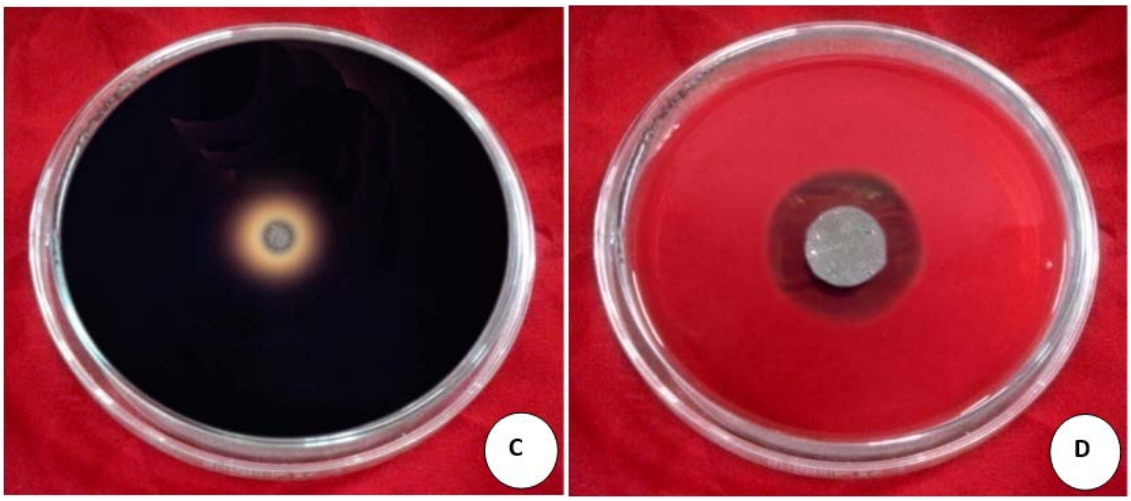
S. No.	Fungal flora of soil sample1	Fungal cfu % in PDA (Control)	Fungal cfu % in medium containing ethylacetatae *Aloe vera* extraction	Fungal cfu% in medium containing methanol *Aloe vera* extraction
1	*Rhodotorula mucilaginosa*	5.70	5.66	5.63

Fig 3: Colony forming unit of *Rhodotorula mucilaginosa* of control, ethylacetatae and methanol extraction by plate streak method.

Biochemical analysis of isolated fungi

Table 5: Analysis of starch, cellulose and lipase hydrolysis test of fungal species

S. No	Area	Fungal species	Starch hydrolysis test	Cellulose hydrolysis test	Lipase hydrolysis test
1	Agra road	*Rhodotorula muciliginosa*	_	_	+
2	Brahmpuri road	*Aspergillus nidulans*	+	+	+



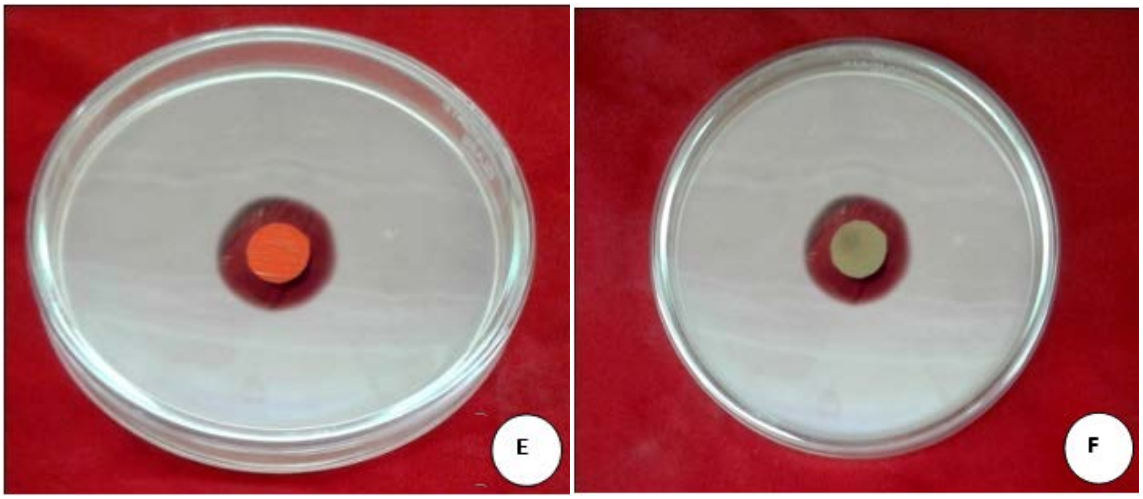


Fig 4: showed (C) starch hydrolysis test of *A.nidulans*. (D) Cellulose hydrolysis test of *A. nidulans.* (E) Lipase hydrolysis test of *R. muciliginosa* and (F) lipase hydrolysis test of *A.nidulans.*

Table 6: Determination of protein content

S. No.	Fungal species	Protein content analysis
1.	*Rhodotorula muciliginosa*	0.151
2.	*Aspergillus nidulans*	0.190

Discussion

The antifungal activity of *Aloe vera* gel extracts were assayed in vitro by colony counting procedure against the two pathogenic fungi including *Rhodotorula muciliginosa, Aspergillus nidulans* respectively shown in fig. No: 1. Twenty gram of the aloe gel was scrapped out from the selected *Aloe vera* plant. The gel was colorless, smoothing after grinding. The two isolated fungi were isolate from the soil sample by serial dilution method.

The fungal growth inhibition of ethylacetate and methanol of *Aloe vera* gel was summarized in Table: 2and 3. In the present study methanol extract had the highest potential to reduce fungal colonies on *Rhodotorula muciliginosa* and *Aspergillus nidulans.*

The results showed that *Aloe vera* gel effectively reduce the growth rate of fungi. In between of ethylacetate extract and methanol extract the methanol has recorded most powerful solvent to reduce fungal growth. This results showed that in *Aloe vera* there active compounds are present (*Yebpella et al*., 2011) [11].

In the countenance of yet rising microbial antibiotic resistance, it is suitable more crucial for studies which look for to recognize natural antifungal compounds and the future development of this compound. The results showed that methanol extract of *Aloe vera* gel had appreciable antimicrobial activity against *Rhodotorula muciliginosa* and *Aspergillus nidulans.*

Methanol had a better potential to reduce CFU rate as compared to ethylacetate. It was analyzed that *Aloe vera* gel had inhibitory effects against fungal causing diseases in human, animals and plants. *Aloe vera* can used as alternative for cosmetic, industry, medicine besides of chemicals. The study proved that *Aloe vera* plant lead to establishment of some compounds that could be used to formulate new and more potent antifungal drugs of natural origin. The biochemical test including starch, cellulose, lipase hydrolysis and protein content determination was also assayed the results showed that *Rhodotorula muciliginosa* failed to show hydrolysis test for starch and cellulose hydrolysis test while *Aspergillus nidulans* showed positive result for all starch, cellulose and lipase hydrolysis test. The maximum content of protein was observed by *Aspergillus nidulans* (0.190) and lowest by *Rhodotorula muciliginosa* (0.151).

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Refrences

1. Lucking R, Huhndorf S, Pfister DH, Plata ER, Lumbsch HT. Fungi evolved right on track. *Mycologia*, 2009:101(6):810–822.
2. Moss ST. The Biology of Marine Fungi. Cambridge University Press, Sydney, 1986.
3. Joseph B, Raj S Justin. Pharmacognostic and phytochemical properties of *Aloe Vera* Linn – an overview. International Journal of Pharmaceutical Sciences Review and Research,2010:4(2):106-110.
4. Park JH, Cox-Ganser J, Rao C, Kreiss K. Fungal and endotoxin measurements in dust associated with respiratory symptoms in a water-damaged office building. *Indoor Air*,2006:16(3):192–203
5. Gaddeyya G, Niharika PS, Bharathi P, Kumar PKR. Isolation and identification of soil mycoflora in different crop fields at Salur Mandal. Adv Appl Sci Res,2012:3(4):2020-2026.
6. Madigan MT, Martinko JM, Stahl DA, Clark DP. Brock biology of microorganisms, 13th ed. Benjamin Cummings, San Francisco, CA, 2012.
7. M Reddi Pradeep, G Narasimha. Production of cellulose enzyme by mutant fungal strain *A.niger* In submerged fermentation. Journal of Biotechnol-ogy. 2011:5(3):148-152.
8. Thomas SM, Kavitha S. Isolation and screening of lipase producing microorganism from soil and comparative study of enzyme activity with different

substrates Int. J. Sci. Eng. Technol. Res, 2015:4:5778-5781.
9. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent, J. Biol chem.,1951:193(1):265-75.
10. Everette JD, Bryant QM, Green AM, Abbey YA, Wangila GW, Walker RB. "Thorough Study of Reactivity of Various Compound Classes toward the Folin−Ciocalteu Reagent". Journal of Agricultural and Food Chemistry,2010:58(14):8139–8144.
11. Yebspella GG, Adeyimi HM, Ham Ri, Aie C, Magomya AM, Agbaji AS, *et al*. Phytochemical Screening and Comparative Study of Anti-microbial activity of *Aloe vera* Various extracts. Africans journal of Microbiology,2011:510:1182-1187.