



# IJRASET

International Journal For Research in  
Applied Science and Engineering Technology



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# INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

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**Volume: 9      Issue: X      Month of publication: October 2021**

**DOI: <https://doi.org/10.22214/ijraset.2021.38404>**

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# Evaluation of Antifungal Activity of Various Plant Latex against Selected Fungal Strains

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**Abstract:** Latex is a natural plant polymer and milky white fluid distributed throughout the plant body like leaves, stems, roots and fruits of all flowering plants and consists of proteins, alkaloids, starches, sugars, oils, tannins, resins and gums that coagulate on-air exposure. It is secreted by specialized plant cells called Laticifers. Plants exude latex in response to physical damage. The present study was carried out to assess the potential antifungal activity of latex of five different plants namely *Plumeria rubra*, *Plumeria alba*, *Aloe vera*, *Calotropis procera* and *Calotropis gigantea* against three different pathogenic strains of fungi. All five latexes were found to show good to moderated activity against all the three fungal strains, namely *Trichoderma viride*, *Aspergillus flavus* and *Aspergillus niger*. The latex of each plant was tested in two volumes (i.e. 10 $\mu$ l & 20 $\mu$ l), and it was found that the antifungal activity was volume-dependent, and a significant difference was also observed in the case of different fungal strains. The antifungal activity of plant latexes was evaluated by Agar well diffusion method; results revealed that among all the five latexes, the fresh latex of *Plumeria rubra* (Vol: 10 $\mu$ l & 20 $\mu$ l) showed excellent antifungal activity against *Aspergillus niger* and *Aspergillus flavus*, whereas fresh latex of *Aloe vera* (Vol: 10 $\mu$ l & 20 $\mu$ l) showed excellent antifungal activity against *Trichoderma viride*. The results of the current research imply that the antifungal activity of latex varies with the species of plants and the fungal strains used. The results therefore demonstrated that, the used five latexes effectively inhibited the growth of three tested fungi. Hence, these plant latexes are natural, ecofriendly and can be used as good candidates for the treatment of various fungal diseases. In further words, they can be used for therapy of antifungal-resistant fungi.

**Keywords:** Antifungal activity, Proteins, Alkaloids, Tannins, Resins, Laticifers, *Plumeria rubra*, *Plumeria alba*, *Aloe vera*, *Calotropis procera*, *Calotropis gigantea*, *Aspergillus flavus*, *Aspergillus niger*, *Trichoderma viride*, Latex, Agar well diffusion.

## I. INTRODUCTION

God gives us a very precious gift of vegetation, where plants are used to cure many diseases. Plants are an essential source of drugs, especially in traditional medicine; in many parts of the world, the plants are used in the form of crude extracts or tincture to treat common and chronic infections (B.Mahesh and S.Satish, 2008; A.Hamidet *al.*, 2010). According to WHO, 80% of individuals in developing countries still use plants as remedies for numerous diseases, utilizing their personal recipes which have been passed through generation to generation. Medicinal plants are a rich source of phytoconstituents like tannins, terpenoids, alkaloids, flavonoids and phenolic compounds. Plants latex has much more attention in the research area because of its excellent plant defense mechanism. Latex is white milky fluid secreted by plants and it is stable dispersion of polymer micro particles in an aqueous medium. It consists of proteins, alkaloids, starches, sugars, oils, tannins, salts, resins and gums that coagulate on exposure to air (Abraham et al., 1979). Plant latex has wider ethno-pharmacological applications as it is used by local tribal communities for wound healing, burns, joint pain and for controlling worm infections. In Senegal, the milky latex is locally functional to treat cutaneous diseases such as ringworm, syphilitic sores and leprosy (Kew 1985). Moreover, preparations from latex with honey are used as anti-rabies and also in the treatment of toothache and cough (Kew 1985). Mixed with vinegar, the latex stimulates healing of abscesses, snakebite and glandular swelling. Several compounds like flavonoids, tannins, coumarins, quinines, terpenoids, essential oils, alkaloids, lectins and peptides are said to possess antimicrobial and biological properties against bacteria, fungi, viruses, protozoans, nematodes, insects and cancer and tumour (Cowan, 1999). Plant latex is made up of various chemical constituents, which play an essential role in protection; it protects the plant from herbivorous insects and animals. It contains chemicals, phytochemicals that show diverse biological activities. It also contains enzymatic proteins such as chitinases and lectins etc., which have insecticidal activity against herbivorous insects. Latex has no side effect on the environment because it is biodegradable, and is observed in 10% of flowering plants (angiosperms). It can be rendered by polymerizing a monomer such as a styrene that has been emulsified with surfactants. In most of the plants, latex is white, but some have yellow, orange, scarlet or even colorless latex. It is a natural plant polymer produced by exceedingly specialized cells called as laticifers. Latex is an emulsion like sticky material that secreted by ducts of laticiferous tissue. It usually oozes out after tissue injury and it is also used to make clothing types, chewing gums, glues, rubber, mattresses and balloons.

The molecular formula of latex is  $C_3H_3N$  (carbon, hydrogen and nitrogen). The Composition of latex sap consists of 30-40% rubber particles, 55-65% water, and small amounts of proteins, sterol glycosides, ash, enzymes, glycolipids, carbohydrates, terpenoids and saponins. Natural latex with a high solids matter is also employed for making moulds for casting plaster, cement, wax and limited run polyester articles. Natural latex can shrink around the object to be reproduced. In most plant species latex is cloudy white liquid, similar in appearance to cow milk. It also has wider applications in the field of medical sciences and is used for preparation of adhesives, polymers, films, gloves and other important diagnostic materials. Synthetic latex is petroleum-based and it contains man-made ingredients. It can be made by polymerizing a variety of chemical substances and suspending them in an emulsion. In most cases, it cost less than natural latex.

#### A. Plant Species

In India, many plants have been reported to possess antifungal activity. *Plumeria rubra*, *Plumeria alba*, *Aloe vera*, *Calotropis procera* and *Calotropis gigantea* are among them, with legal claims for curing pathogenic infections.

- 1) *Plumeria rubra* L. [family; Apocynaceae] is a shrub distributed in many parts of the world. It is commonly known as red frangipani. Its latex is used to treat toothache, rheumatic pains, Piles, and also applied to remove germs from the wound. It is reported to have hepatoprotective (Baghel A et al., 2010), antifungal (Bobbarala V et al., 2009) and antiviral (Tan G., 1991) activities.
- 2) *Plumaria alba* L. [family; Apocynaceae] is a laticiferous shrub known by the vernacular name of white frangipani. Its milky latex mixed with coconut oil is used in itching and also used to treat ulcer, scabies, and herpes. Moreover its bark is buried and applied as plaster over hard tumour. *P. alba* is also reported to have antibacterial and antifungal activities (Radha R et al., 2008).
- 3) *Aloe vera* (L.) Burm.f. [family; Asphodelaceae] is a short-stemmed plant known by the vernacular names of ghee kavara and real aloe. Aloe latex is a resinous compound derived from the pericarp of the leaves. The health benefits of aloe gel and latex include reducing inflammation, promoting hair growth, treating cancer sores, and curing skin cancer. Scientific studies reported its latex, gel and juice as antifungal and antibacterial, in treatment of ulcer, burns, frostbite and skin abrasion.
- 4) *Clotropis procera* (Aiton) W.T.Aiton [family; Asclepiadaceae] is a xerophytic and erect shrub; it is known due to presence of latex in its green parts. Its latex can easily collected when the plant is wounded. The plant is commonly known as Sodom apple and madar. Leaf extracts, slashed leaves and latex of *C. procera* have presented great potential as a nematicide in vitro and in vivo (Khirstova and Tissot, 1995). It is used alone or with other herbs to treat various diseases such as fever, digestion, cold, diarrhea. This plant reported to have many therapeutic activities like anti-inflammatory (N. Alencar et al., 2004), antifungal (Manookar V B et al., 2015), and antimicrobial (Kareem, S et al., 2008) activities.
- 5) *Calotropis gigantea* (L.) Dryand. (family- Asclepiadaceae), commonly known by the vernacular name mudar yercum (milk weed), is a shrub about 6-7m high and is widely distributed in eastern and southern parts of India. The stem and leaves produce a milky sap when cut. Its latex is reported to contain antimicrobial activity (Gaurav Kumar et al., 2010), purgative properties, antifungal activity (Subramanian & Saratha, 2010), procoagulant activity (Rajesh R et al., 2005) and wound healing activity (Saratha V et al., 2009). It also traditionally used to cure earache, sprain, epilepsy, mental disorder and anxiety. Roots are used for the treatment of lupus, tuberculosis, and leprosy etc (Gaurav K et al., 2010).

Hence, the present study was designed to determine the antifungal activity of latexes of five different plants i.e. *Plumeria rubra*, *Plumeria alba*, *Aloe vera*, *Calotropis procera* and *Calotropis gigantea* against three different fungal strains i.e. *Trichoderma viride*, *Aspergillus flavus* and *Aspergillus niger* in vitro. This work is done with some main objectives as below:-

- 1) To collect the latex from different plants.
- 2) To analyse the antifungal activity of all plant latexes against three fungal strains namely *T. Viride*, *A. flavus* and *A. niger*.
- 3) To compare the antifungal activity of various plant latexes.

## II. MATERIALS AND METHODS

#### A. Collection of plant latex

The latex from various plants was collected from Nizam college, Basheerbagh, Abids, Hyderabad district, Telangana (India) during January (2020). Fresh latex was collected as an exudate from the apical part of the plant. It was collected in the early morning by plucking the leaves near the stem, and 4-5 longitudinal incisions were made on the stem of the plant by using a steel knife. The milky white latex was collected into a sterilized test tube and was slightly shaken during collection to avoid coagulation. The test tubes were tightly closed with cotton and stored at 4°C until used. The latex was collected from 5 different plants. The plants used were *Plumeria rubra*, *Plumeria alba*, *Aloe vera*, *Calotropis procera* and *Calotropis gigantea*. The plants were adequately authenticated by Dr. Vijay Bhaskar Reddy (Head of the Botany Department) Nizam College –Hyderabad.



Figure1 : Latexes of various plants

#### B. Maintenance of Test Fungi

Pure cultures of the fungal strains were used for the investigation of anti-fungal analysis. All the three fungal strains were maintained on potato dextrose agar medium at room temperature until required for study. In this study, the tested fungal strains were obtained by soil serial dilution method.

#### C. Preparation of potato-dextrose agar medium (PDA)

In medium preparation, potato, dextrose and Agar are mixed to act as nutrients for fungus. Thus they help in the growth of the fungal colonies. Agar is used for solidification.

##### 1) Ingredients

- a) Potatoes, peeled and sliced - 200g
- b) Dextrose - 20g
- c) Agar-agar - 17g
- d) Distilled water - 1000ml

2) *Procedure:* Potatoes were peeled and about 200g were weighed, finely sliced and boiled to a mash in distilled water. The potato mash was stirred and strained through the muslin cloth into a beaker. The saved effluent, which was potato infusion. About 20g of dextrose, 17g of agar and the saved potato effluent (potato extract) were added into the 1liter conical flask. The volume was made up to 1000ml by adding hot distilled water. The contents were continuously poured and stirred until the consistency was achieved. The conical flask was plugged with cotton wool, over which aluminum foil was tightly wrapped. Then, the flask with the content was sterilized within an autoclave for 15 minutes at 15Lb pressure and at a temperature of 121° C. After sterilization, 50mg of streptomycin was added to the PDA medium, shaken well and 15ml portion was dispensed into each sterilized petri plate. Potato dextrose agar media is used for the cultivation of fungi.

#### D. Soil Serial Dilution

A small amount of soil contains millions of bacteria and fungi. To remove the load of microorganisms, we need to use the serial dilution technique. In this process, we need to dilute our sample serially from one container to another container. The test organisms (fungi) were collected from the soil sample by the dilution method.

1) *Procedure:* About 100ml of distilled water was measured and added into a conical flask. One gram of soil was weighed and added into the conical flask (with 100ml water). Then the flask was closed with the stopper and shaken for about 1 minute to mix the solution thoroughly. The solution was kept aside, for 5 minutes, so that all the heavy particles of soil settle down. It was marked as a stock solution and the pH of this solution was 6.8. The sterile test tubes were labeled with  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$ ,  $10^{-6}$  and  $10^{-7}$ . About 9ml of distilled water was added to each of the test tubes, using sterile pipettes. Then the test tubes were plugged with cotton, over which aluminum foil was wrapped and were placed in an autoclave. 1ml of stock solution was transferred from the conical flask to the test tube labeled  $10^{-1}$ , using a new pipette. Then the tube was plugged and swirled gently to mix the solution. 1ml of diluted solution was transferred from  $10^{-1}$  test tube to the  $10^{-2}$  tube, with a new pipette. Test tube was plugged and swirled to mix the solution. Same procedure was repeated with other test tubes labeled  $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$ ,  $10^{-6}$  and  $10^{-7}$ . About 1ml of diluted solution was transferred from each  $10^{-5}$ ,  $10^{-6}$  and  $10^{-7}$  test tubes to the petri plates containing solidified PDA medium. Then the solution was spread with the sterile glass spreader on the entire surface of medium, known as spread plate method. The petri plates were also marked with their respective dilutions and were sealed with parafilm. These petri plates were incubated at room temperature until the fungal colonies appeared.

**E. Identification of Fungi**

The fungal culture was stained with lactophenol cotton blue stain and observed under microscope to identify the fungus. All three fungal strains used in the present study were identified based on their macroscopic and microscopic characters. Those are as follows:

	T. viride	A. flavus	A. niger
Macroscopic Characters	Green in colour, produces a sweet coconut odour.	Olive to lime-green in appearance, wooly to cottony texture.	Powdery, smooth, dark -brown to black in colour.
Microscopic Characters	Septate hyphae, rough conidia, highly branched conidiophores terminating with phialides.	Septate hyaline hyphae, radiated conidial heads, conidiophores, smooth conidia.	Filamentous fungi producing filamented hyphae, smooth conidiophore, conidia, and phialides.

**F. Sub-culturing of Fungi**

Sub-culturing is a process of transferring microorganisms from one growth container to another by offering them a fresh supply of nutrients either in a tangible medium or in a liquid medium. So the primary aim of this process is to obtain a pure culture of the desired fungus. This must be carried out in the laminar airflow chamber to avoid contamination.

1) *Procedure:* The petri plate containing stock culture of the fungus was placed in the laminar airflow chamber. The tip of the inoculation loop was sterilized by placing it in the Bunsen burner. It was dipped into the alcohol and again placed in the Bunsen burner in order to dry the excess amount of alcohol. The stock culture plate was opened less near the flame and then sterile loop of the needle was allowed to touch the fungal culture. Several spores adhered to the loop, those spores were inoculated on freshly prepared solid PDA medium petri plate. The inoculated plates were incubated at room temperature for 7 days. By this technique, pure cultures of desired fungal strains were obtained and were preserved in refrigerator at 4°C until required.

**G. Test for Antifungal Activity**

The antifungal activity of latexes of five different plants was determined by Agar well diffusion method. The latexes were screened against three fungal strains – *Aspergillus niger*, *Aspergillus flavus* and *Trichoderma viride*. All fungi were grown in the respective growth medium at room temperature till the pure culture was isolated. Pure fungal cultures were used to evaluate antifungal activity. Two different volumes 10µl and 20µl of latex of each plant were used for testing antifungal activity.

1) *Agar Well Diffusion Method:* The potato dextrose agar (PDA) medium was poured into sterilized petri plates and allowed to solidify. After solidification, these plates were inoculated with the respective fungi from an actively growing culture. Seven days old fungal cultures were placed in the center of each petriplate. Three wells, each of 7mm in diameter were made in each of these plates using sterile cork borer and then, agar discs were removed. These wells were filled separately with two different volumes (10µl and 20µl) of latex by using microliter-pipette. Each volume of latex was added into three wells of each petri plate. Then the latex was allowed to diffuse into the medium at room temperature. Same process was repeated with all the five latexes. Some plates were maintained as control without adding any latex to medium. Three replicate plates were used per treatment. All the plates were incubated at ± 25°C for 72 hours. The complete antifungal analysis was carried out under strict aseptic conditions. After incubation period (72 hrs), fungal growth was determined by measuring colony diameter and percentage inhibition calculated according to the following formula as below:

$$\% \text{ Inhibition} = \frac{(C-T)}{C} \times 100$$

Where, C = colony diameter (cm) in control plate

T = colony diameter (cm) in treated plate

**H. Physical Determination**

Various parameters of latexes like, pH, colour, physical state and aroma were evaluated, as shown in **Table 6**.

- 1) *pH:* The pH was determined by using a digital pH meter. 15 ml of plant latex was taken in a beaker, and the bulb of the pH meter was dipped in latex. The obtained pH values were noted.
- 2) *Aroma:* All the plant latexes possessed aroma except *C. procera* latex and *C. gigantea* latex.
- 3) *Time Required To Dry A Drop Of Latex:* A drop of latex was taken on a sterile glass slide. The time taken by the drop of each latex to dry was observed with the help of stopwatch and given in the table 6.

### III. RESULTS AND DISCUSSION

The latex was collected from various plants and was subjected to antifungal activity. The selection of these plants for the present study was based on medicinal properties used in traditional medicinal system. These plants are known for anti-microbial, anti-diarrheal, anti-pyretic and wound healing activities. The antifungal activity of various plant latexes such as *Plumeria rubra*, *Plumeria alba*, *Aloe vera*, *Calotropis procera* and *Calotropis gigantea* was studied with two different volumes 10 $\mu$ l and 20 $\mu$ l against three fungal strains namely *Trichoderma viride*, *Aspergillus flavus* and *Aspergillus niger*.

The fungal strains used in the present study were selected based on their clinical importance-for example *Aspergillus* species are known to produce many secondary metabolites commonly known as mycotoxins. The secondary metabolites are potent carcinogens, hepatotoxins, teratogens, and immunosuppressive compounds. The plant latexes were collected for the evaluation of *in vitro* antifungal activity by agar well diffusion method. According to omneka and osuaba (2000), Agar well diffusion method allows better diffusion of the latex into the medium, thus enhancing contact with the organisms. In the present study, latexes were collected from various plants and applied directly as an antifungal agent. Compared with the control, the petriplates treated with plant latexes (10 $\mu$ l and 20 $\mu$ l) significantly inhibited the growth of all three fungal species. The rate of mycelial growth inhibition was shown to vary with different plant latexes. The results were observed by measuring the fungal growth in both control and treated petri plates after 72 hrs. The results showed a significant variability in efficacy of all the collected plant latexes in inhibiting the growth of tested fungi. The results are depicted in **Tables 1,2,3,4 and 5**. The growth reduction in each Petri plate was taken into consideration and the inhibition effect was evaluated. Formula of percentage inhibition was used to calculate the effect of latex in inhibiting the growth of fungus. Based on the results we can analyze the inhibition effect of latexes against three fungal strains. Where the potency of latexes is exhibited by inhibiting the growth of test-fungi. The inhibitory action of latexes showed volume dependent activity as well as having good inimical response on the basis of fungal strains. Two volumes 10 $\mu$ l and 20 $\mu$ l of each plant latex showed significant inhibition effect on the growth of all three fungi. Among both the volumes 20 $\mu$ l showed remarkable inhibition performance. The efficacies of all latexes were expressed as percentage inhibition of mycelial growth over control.

- 1) Antifungal activity (expressed as % inhibition) of *Plumeria rubra* latex against three different strains of fungi is shown in table 1. From the table, it is evident that 10 $\mu$ l latex of *P. rubra* showed highest reduction (i.e. 62.2%) in the growth of *A. niger*, moderate reduction (i.e. 53.3%) in the growth of *A. flavus* and least reduction (i.e. 51.1%) in the growth of *T. viride*. When latex volume was changed and taken as 20 $\mu$ l, it showed highest reduction (i.e. 71.1%) in the growth of *A. niger*, moderate reduction (i.e. 65.5%) in the growth of *A. flavus* and least reduction (i.e. 64.4%) in the growth of *T. viride*, when compared to control. However, the latex of *P. rubra* showed maximum inhibition effect against the fungus *A. niger* and minimum inhibition effect against *T. viride* with both the volumes used (10 $\mu$ l and 20 $\mu$ l).
- 2) Antifungal activity (expressed as % inhibition) of *Plumeria alba* latex against three different strains of fungi is shown in Table 2. From the results, it is evident that 10 $\mu$ l latex of *P. alba* highly inhibited the growth of *A. niger*, with inhibition percentage of 62.2%, moderately inhibited the growth of *T. viride*, with inhibition percentage of 56.6% and slightly inhibited the growth of *A. flavus*, with inhibition percentage of 52.2%. Similarly, 20 $\mu$ l latex highly inhibited the growth of *A. niger*, with inhibition percentage of 67.7%, moderately inhibited the growth of *T. viride*, with inhibition percentage of 62.2% and slightly inhibited the growth of *A. flavus*, with inhibition percentage of 60% when compared with control. Therefore, both the volumes (10 $\mu$ l and 20 $\mu$ l) of *P. alba* latex showed maximum inhibition effect on the growth of *A. niger* and minimum inhibition effect on the growth of *A. flavus*.
- 3) Antifungal activity (expressed as % inhibition) of *Aloe vera* latex against three different strains of fungi, shown in Table 3. *Aloe vera* latex possessed, significant antifungal activity towards all test fungi. 10 $\mu$ l fresh latex of *Aloe vera* exhibited 61.1% inhibition against the growth of *T. viride*, 57.7% inhibition against the growth of *A. niger* and 51.1% inhibition against the growth of *A. flavus*, whereas 20 $\mu$ l of latex showed 66.6% inhibition against the growth of *T. viride*, 63.3% inhibition against the growth of *A. niger* and least 62.2% inhibition was observed against the growth of *A. flavus*. From the results, it is evident that both the volumes of *Aloe vera* latex showed greater inhibition effect against all three fungal strains. However, more inhibition effect was found on *T. viride* (10 $\mu$ l: 61.1% and 20 $\mu$ l: 66.6%) while less inhibitory effect was found on *A. flavus* (10 $\mu$ l: 51.1% and 20 $\mu$ l: 62.2%).
- 4) Antifungal activity (expressed as % inhibition) of *Calotropis procera* latex against three different strains of fungi is shown in Table 4. From the table, it is evident that the mycelial growth of *A. niger*, *A. flavus* and *T. viride* was inhibited by 54.4%, 46.6% and 45.5% respectively, when treated with 10 $\mu$ l of *C. procera* latex whereas, the growth of these three fungi was inhibited by 64.4%, 60.0% and 55.5% respectively, when treated with 20 $\mu$ l latex. However, *C. procera* latex showed significantly higher inhibitory effect on *A. niger*, whereas significantly low effect on *T. viride*.

5) Antifungal activity (expressed as % inhibition) of *Calotropis gigantea* latex against three different strains of fungi is shown in Table 5. From the table, it is evident that the mycelial growth of *T. viride*, *A. niger* and *A. flavus* was reduced by 53.3%, 50.0% and 50.0% respectively, when treated with 10µl latex of *C. gigantea* whereas, the growth of these three fungi was reduced by 62.2%, 61.1% and 60.0% respectively, when treated with 20µl of latex. However, *C. gigantea* latex (10µl and 20µl) showed maximum inhibition effect against *T. viride* and minimum inhibition effect against *A. flavus*.

Therefore, overall results demonstrated that the growth of *A. niger* and *A. flavus* was strongly inhibited with *P. rubra* latex, whereas *T. viride* was strongly inhibited with latex of *Aloe vera*.

TABLE 1  
Antifungal activity of two different volumes of *Plumeria rubra* latex using Agar well diffusion method:

S.NO	Fungal Strains	Latex volume (10 µl)		Latex volume (20 µl)		Control (Colony diameter cm)
		Colony Diameter (cm)	Percentage Inhibition (%)	Colony Diameter (cm)	Percentage Inhibition (%)	
1.	<i>Trichoderma viride</i>	4.4	51.1%	3.2	64.4%	9.0
2.	<i>Aspergillus flavus</i>	4.2	53.3%	3.1	65.5%	9.0
3.	<i>Aspergillus niger</i>	3.4	62.2%	2.6	71.1%	9.0

TABLE 2  
Antifungal activity of two different volumes of *Plumeria alba* latex using Agar well diffusion method:

S.NO	Fungal Strains	Latex volume (10 µl)		Latex volume (20 µl)		Control (Colony diameter cm)
		Colony Diameter (cm)	Percentage Inhibition (%)	Colony Diameter(cm)	Percentage Inhibition (%)	
1.	<i>Trichoderma viride</i>	3.9	56.6%	3.4	62.2%	9.0
2.	<i>Aspergillus flavus</i>	4.3	52.2%	3.6	60%	9.0
3.	<i>Aspergillus niger</i>	3.4	62.2%	2.9	67.7%	9.0

TABLE 3  
Antifungal activity of two different volumes of *Aloe vera* latex using Agar well diffusion method:

S.NO	Fungal Strains	Latex volume (10 µl)		Latex volume (20 µl)		Control (Colony diameter cm)
		Colony Diameter (cm)	Percentage Inhibition (%)	Colony Diameter(cm)	Percentage Inhibition (%)	
1.	<i>Trichoderma viride</i>	3.5	61.1%	3.0	66.6%	9.0
2.	<i>Aspergillus flavus</i>	4.4	51.1%	3.4	62.2%	9.0
3.	<i>Aspergillus niger</i>	3.8	57.7%	3.3	63.3%	9.0

TABLE 4

Antifungal activity of two different volumes of *Calotropis procera* latex using Agar well diffusion method:

S.NO	Fungal Strains	Latex volume (10 µl)		Latex volume (20 µl )		Control (Colony diameter cm)
		Colony Diameter(cm)	Percentage Inhibition (%)	Colony Diameter(cm)	Percentage Inhibition(%)	
1.	<i>Trichoderma viride</i>	4.9	45.5%	4	55.5%	9.0
2.	<i>Aspergillus flavus</i>	4.8	46.6%	3.6	60%	9.0
3.	<i>Aspergillus niger</i>	4.1	54.4%	3.2	64.4%	9.0

TABLE 5

Antifungal activity of two different volumes of *Calotropis gigantea* latex using Agar well diffusion method:

S.NO	Fungal Strains	Latex volume (10 µl)		Latex volume (20 µl )		Control (Colony diameter cm)
		Colony Diameter (cm)	Percentage Inhibition (%)	Colony Diameter(cm)	Percentage Inhibition (%)	
1.	<i>Trichoderma viride</i>	4.2	53.3%	3.4	62.2%	9.0
2.	<i>Aspergillus flavus</i>	4.5	50%	3.6	60%	9.0
3.	<i>Aspergillus niger</i>	4.5	50%	3.5	61.1%	9.0

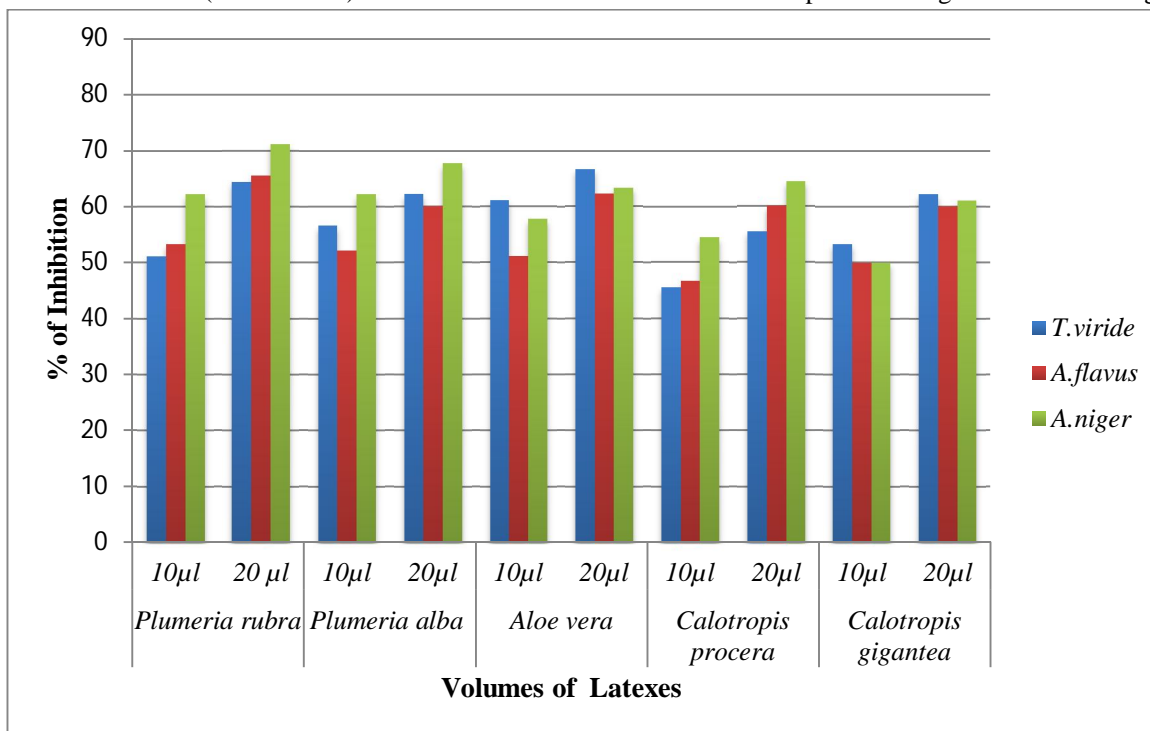
TABLE 6

Physical Properties of Plant Latexes

S.NO	Plant latex	Colour	pH	Physical state	Aroma	Time required to dry (a drop of latex)
1.	<i>Plumeria rubra</i>	White	7.0	Liquid	Mild sweet	6 mins
2.	<i>Plumeria alba</i>	White	6.1	Liquid	Slightly chemical	8 mins
3.	<i>Aloe vera</i>	Yellow	4.8	Greasy liquid	Sour slightly unpleasant	12 mins
4.	<i>Calotropis procera</i>	White	6.8	Liquid	No aroma	9 mins
5.	<i>Calotropis gigantea</i>	White	7.2	Liquid	No aroma	8 mins



Figure 2: Inhibition effect (% Inhibition) of two different volumes of latex of each plant on the growth of three fungal strains



#### IV. CONCLUSION

The results obtained from the current study showed significant antifungal activity (inhibition effect) of various plant latexes against three fungal strains. Among all the five plant latexes, fresh latex of *Plumeria rubra* showed the highest inhibition effect against *A. niger* and *A. flavus*, whereas fresh latex of *Aloe vera* showed the highest inhibition effect against *T. viride*. Hence, the remarkable fungicidal effects of latexes suggest that the latex may be a useful source for the development of antifungal agents against pathogenic fungi. The biologically active compounds found in latex were responsible for the antifungal activity. Moreover these plant latexes can be used to discover natural bioactive products that potentially serve as leads in developing novel pharmaceutical research activities and suggesting for using as potential antifungal agent to cure fungal infections.

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