

# The Antifungal Activities of *Plumbago Zeylanica* Extract against *Aspergillus flavus*, *Lasiodiplodia Theobromae* and *Cladosporium Oxysporum* for Potential Human Cream Development

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## ABSTRACT

The experimental study was carried out to evaluate the antifungal activities of *Plumbago zeylanica* Extract against *Lasiodiplodia* (*Oncobasidium*) *theobromae*, *Cladosporium oxysporum* and *Aspergillus flavus* for medical purposes. A standard extraction procedure of the plant leaf and preparation of test microorganisms was carried out. The rate of mycelial growth inhibition of the microorganisms measured at different concentrations of 12.5 mg/ml, 25 mg/ml, 50 mg/ml and 100 mg/ml over a period of 48 - 120 hours showed that the extract has significant antifungal effects on the three fungal species; with indications of positive manifestations of the extract on the test pathogens at different tested hours and all the fungi pathogens were found susceptible at different hours of incubation. Furthermore, the high percentage inhibition for the three fungi mostly at 50 mg/ml and 100 mg/ml concentrations shows that *Plumbago zeylanica* leaf extract contains very active ingredients in the control of fungal diseases. Then, further studies will be carried out for the development of potential antifungal cream that can be applied on human skins.

Keywords: Antifungal, *Aspergillus flavus*, *Cladosporium oxysporum*, *Lasiodiplodia theobromae*, Nigeria, *Plumbago zeylanica*, Pathogens.

## 1 Introduction

The use of the *Plumbago zeylanica* plant known as 'Inabiri' among the rural dwellers in some part of Southwestern Nigeria is common for the treatment of some gastrointestinal and dermatological ailments caused by pathogenic microorganisms. There are lots of work done and reported on the plant on its ethnobotanical uses. For instance, the documented abstracts of studies on the antimicrobial activities of some plants presented during the 99th Indian Science Congress, section of plant science, 2012. Work on Antibacterial Activity of Methanol and Chloroform Extracts of Himalayan Gymnosperms Plant *Cedrus deodara* Roxb carried out [1]. The results indicated the plant had broad-spectrum activity for the management of the most common human and plant bacterial diseases.

Furthermore, the study on Antifungal Activities of Leaf Extract of *Calotropis procera* (Ait) R.Br. (asclepiadaceae) and the antifungal activities of the plant was confirmed [2]. Then, for the issue of antibiotic drugs resistance, development of alternative remedies is being sought where some herbal plants extract was tested against pathogenic microorganisms. The study of [3], the use of medicinal plants rosemary, at concentrations of 400 mg/ml was active against *Pseudomonas aeruginosa*, *Bacillus cereus*, *Staphylococcus aureus* and *Escherichia coli* among others. On the other hand, various pharmacological studies on *Plumbago zeylanica* extracts have indicated antiplasmodial, antimicrobial, antifungal, anti-inflammatory, and antiatherosclerotic activities among others. [4], [5], [6], [7], [8], and [9].

Furthermore, the work of Adegbite, et.al, [9] carried out to investigate the antimicrobial activities/potentials of *Plumbago zeylanica* components (leaf, stem and root) on four bacterial species, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumonia* with the aim of using the active part of the plant for water and laboratory disinfection. At higher concentration, all the plant extracts became effective against the bacteria; with root extract possessing the highest antimicrobial potentials for disinfection and showed significance reduction level of *Escherichia coli* when the root extracted tested on the effluents water.

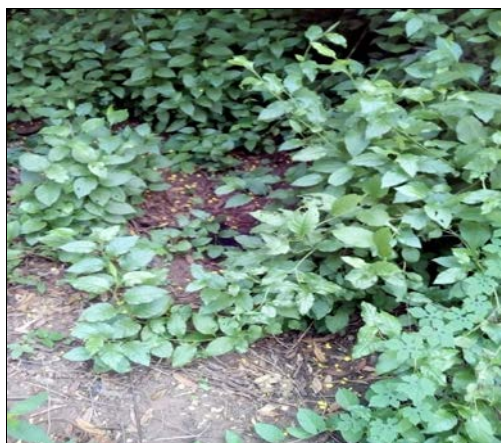


**Figure 1: the picture of the plant Source: (Adegbite et.al, 2015)**



**Figure 2: Shaving of the rat for the application of the cream. Source: Adebajo, et al, (2016)**

Then, Adebajo, et.al, [10], evaluated the Acute Toxicity of *Plumbago zeylanica* extracts on the rat skin for the use of the plants extracts for the potential development of medicated body cream (figure 2). The results indicated no presence of rash or visible spots observed in the rats used for the experiments, no development of redness, swelling, erosion and ulceration of the skin [10].

Consequently, the present study was designed to evaluate the Antifungal Activities of *Plumbago zeylanica* Extract against the following fungi that are of medical and health importance- *Aspergillus flavus*, *Lasiodiplodia theobromae* and *Cladosporium oxysporum*.

## **2 Materials and Methods**

### **2.1 The Collection and Preparation of Plant Material**

The leaves of *Plumbago zeylanica* were obtained from Ipokia Ogun State Nigeria (see figure 1). The leaves were air dried at room temperature of about 27°C for 5 days, after which they were oven dried at temperature of 55°C for 30 minutes before grinded into a lump form. The grinded sample of *Plumbago zeylanica* was macerated using cold extraction method to prepare the extract. 0.4kg (400g)

of leaves of (*Plumbago zeylanica*) the plants materials were macerated (soaked) in an air-tight plastic container containing 1500ml of 96% ethanol at room temperature and stored using a sterile aluminum rod of 50cm long for 5days to allow for maximum extraction of the plants components and to encourage homogeneity of the plants extract and the solvent used. Follow by filtration with cotton wool and filter paper (what-man filter paper), the filtrate was concentrated using steam-water bath. The residue was put into sample bottle as the crude extract of the test plants and stored [9].

## 2.2 Test Microorganisms

The tests microorganisms (*Lasiodiplodia* (*Oncobasidium*) *theobromae*, *Cladosporium oxysporum* and *Aspergillus flavus*) used for this research work were collected from laboratory specimen. The prepared slanting culture of the test microorganisms were collected from the Plant Pathology Research Laboratory of Olabisi Onabanjo University, Ago-Iwoye Ogun Nigeria. Then, the Central Medical Laboratory of the Ogun State College of Health Technology used for the laboratory analysis.

## 2.3 Extraction Procedure

The plant powders of the leaves of *Plumbago zeylanica* was macerated using 98% ethanol for seven days, they were filtered in conical flasks and the filtrates were concentrated in a water bath which took some hours, before crude extracts of the plant were formed in a paste texture form. The extracts were dispensed into clean sterilized sample bottles and stored at room temperature.

## 2.4 Preparation of Media

Potato Dextrose Agar (PDA) was used for the fungal pathogens. PDA was prepared by weighing 19.5g of potato dextrose agar and was dissolved in 500ml of distilled water in a 500ml capacity conical flask and covered with foil paper which was then sterilized by autoclaving at 121°C for 15 minutes.

## 2.5 Preparation of Biological Assay

Potato dextrose agar (PDA) was prepared following the same method of sterilization and allowed to cool to about 45 °C, different concentration of the extracts of the plants were dispensed into the petri dishes (12.5 mg/ml, 25.0 mg/ml, 50.0 mg/ml and 100.0 mg/ml) and the prepared PDA was (introduced) dispensed into the plates that had already containing extracts of different concentrations, the plates were gently mixed to dissolve the contents together using agar diffusion method. Fungi pathogens were introduced onto the plates. The inhibition of the growth was determined by subtraction of the fungal growth on the plates that contain extracts from the control over long period of time, between 48 hours and 120 hours, the reduction therefore is the extent to which the extract can reduce/inhibits the growth of the test fungal pathogens and in the control plates no extract was introduced. Three replicate plates were used for each concentration. The rate of mycelia growth inhibition was measured. The observations made were recorded started from 2 days (48 -120 hours) till day 5. The rate of mycelia growth inhibition (GI %) was calculated by the following formula:  $GI\% = \frac{dc-dt}{dc} \times 100$ , Where dc is mean colony diameter of control sets and dt is mean colony diameter of treatment sets [11].

## 3 Results and Discussion

Due to nature of fungi microorganisms, the results of the study were considered at 96 hours and 120hours intervals. Table 1 shows the antifungal effects of *Plumbago zeylanica* leaf extract on *Lasiodiplodia* (*Oncobasidium*) *theobromae*, *Cladosporium oxysporum* and *Aspergillus flavus* at different concentrations of 12.5 mg/ml, 25 mg/ml, 50 mg/ml and 100 mg/ml over a period of 48 - 120 hours showed that the extract has significant antifungal effects on the three fungal species.

Antifungal effect of *Plumbago zeylanica* on the test fungal pathogens at the said concentrations was very significant. The extracts therefore showed great antimicrobial properties against *Lasiodiplodia* (*Oncobasidium*) *theobromae*, *Cladosporium oxysporum* and *Aspergillus flavus*. The inhibition of the test fungal pathogens could be due to the presence of active secondary metabolites present in *Plumbago zeylanica* which have been reported as antimicrobial agents.

**Table 1: The Growth (mm) of fungi on plates contained *Plumbago zeylanica* Leaf Extract at different concentrations Over a Period of 96 Hours**

| Fungus                          | Average Diameter Mycelial Growth (mm) of the Fungal Species at Different Hours and Concentrations |         |            |            |            |             |
|---------------------------------|---------------------------------------------------------------------------------------------------|---------|------------|------------|------------|-------------|
|                                 | Hours of Interval                                                                                 | Control | 12.5 mg/ml | 25.0 mg/ml | 50.0 mg/ml | 100.0 mg/ml |
| <i>Aspergillus flavus</i>       | 96                                                                                                | 42.0    | 35.0       | 30.0       | 10.0       | 6.0         |
|                                 | 120                                                                                               | 70.0    | 65.0       | 55.0       | 25.0       | 9.0         |
| <i>Lasiodiplodia theobromae</i> | 96                                                                                                | 85.0    | 25.0       | 21.0       | 9.0        | 6.0         |
|                                 | 120                                                                                               | 85.0    | 53.0       | 41.0       | 11.0       | 9.0         |
| <i>Cladosporium oxysporum</i>   | 96                                                                                                | 42.0    | 30.0       | 20.0       | 8.0        | 9.0         |
|                                 | 120                                                                                               | 55.0    | 40.0       | 23.0       | 14.0       | 10.0        |

The above Table 1 presents the average mycelia growth rate (mm) of the three fungi *Aspergillus flavus*, *Lasiodiplodia theobromae* and *Cladosporium oxysporum* on plates impregnated (contained) with *Plumbago zeylanica* leaf extract at different concentrations (mg/ml) over a period of 120 Hours.

**Table 2: The Antifungal Effects of *Plumbago zeylanica* leaf extract on the three fungal species at Different Concentrations over a Period of 120 Hour**

| Fungus                          | Average Mycelia Inhibition (mm) of <i>Plumbago zeylanica</i> leaf extract on the three fungi at Different Hours and Concentrations |         |            |            |            |             |
|---------------------------------|------------------------------------------------------------------------------------------------------------------------------------|---------|------------|------------|------------|-------------|
|                                 | Hours of Interval                                                                                                                  | Control | 12.5 mg/ml | 25.0 mg/ml | 50.0 mg/ml | 100.0 mg/ml |
| <i>Aspergillus flavus</i>       | 96                                                                                                                                 | 42.0    | 7.0        | 12.0       | 32.0       | 36.0        |
|                                 | 120                                                                                                                                | 70.0    | 5.0        | 15.0       | 45.0       | 61.0        |
| <i>Lasiodiplodia theobromae</i> | 96                                                                                                                                 | 85.0    | 55.0       | 65.0       | 73.0       | 76.0        |
|                                 | 120                                                                                                                                | 85.0    | 45.0       | 62.0       | 71.0       | 75.0        |
| <i>Cladosporium oxysporum</i>   | 96                                                                                                                                 | 42.0    | 17.0       | 21.0       | 33.0       | 36.0        |
|                                 | 120                                                                                                                                | 55.0    | 3.0        | 14.0       | 44.0       | 46.0        |

The above Table 2 presents the Average mycelia growth inhibition of the antifungal effects of *Plumbago zeylanica* leaf extract on the three fungi at different concentrations (mg/ml) over a period of 96 Hours. The extract was very potent at 50.0 mg/ml concentration and above.

**Table 3: Average Percentage Mycelia Inhibition (%) of the Three Fungal Species**

| Fungus                          | Percentage Mycelia Inhibition (%) of the Three Fungal Species at Different Hours and Concentrations |         |            |            |            |             |
|---------------------------------|-----------------------------------------------------------------------------------------------------|---------|------------|------------|------------|-------------|
|                                 | Hours of Interval                                                                                   | Control | 12.5 mg/ml | 25.0 mg/ml | 50.0 mg/ml | 100.0 mg/ml |
| <i>Aspergillus flavus</i>       | 96                                                                                                  | 42.0    | 16.7       | 28.6       | 76.2       | 85.7        |
|                                 | 120                                                                                                 | 70.0    | 7.1        | 21.4       | 64.3       | 87.1        |
| <i>Lasiodiplodia theobromae</i> | 96                                                                                                  | 85.0    | 64.7       | 76.5       | 85.6       | 89.4        |
|                                 | 120                                                                                                 | 85.0    | 52.3       | 72.9       | 83.5       | 88.2        |
| <i>Cladosporium oxysporum</i>   | 96                                                                                                  | 42.0    | 40.5       | 50.0       | 78.6       | 85.7        |
|                                 | 120                                                                                                 | 55.0    | 5.5        | 25.5       | 80.0       | 83.4        |

The above Table presents the Average percentage inhibition (%) of the antifungal effects of *Plumbago zeylanica* leaf extract on the three fungi at different concentrations (mg/ml) over a period of 120 Hours. The extract was very potent especially at 100.0 mg/ml concentration.

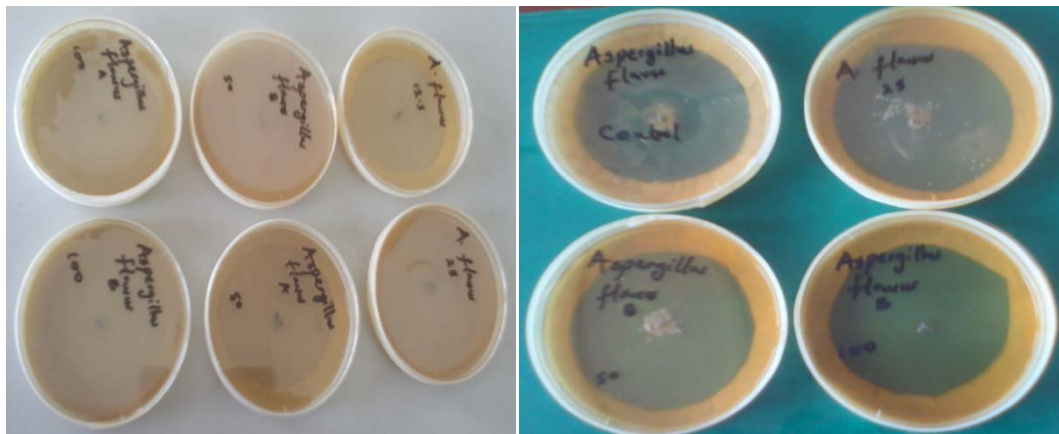


Plate 1: 48 Hours

Plate 2: 72hours



Plate 3: 96 Hours

Plate 4: 120 Hours

Source: (Authors Laboratory results, 2019)

Figure 2: Mycelia Growth Inhibition of *Cladosporium oxysporum* Fungus Pathogen (Plate 1-4)



Plate 1: 48 Hours

Plate 2: 72hours



Plate 3: 96 Hours

Plate 4: 120 Hours

Source: Authors Laboratory results 2019

The plates above and below showed the positive manifestations of antifungal effects of the *Plumbago zeylanica* plant extract on the test pathogens at different tested hours at 48, 72, 96, and 120 hours interval

Figure 3: Mycelia Growth Inhibition of *Lasiodiplodia theobromae* by *Plumbago zeylanica* Fungus



Plate 1: 48 Hours

Plate 2: 72hours



Plate 3: 96 Hours

Plate 4: 120 Hours

Source: Authors Laboratory results 2019

The results of antifungal effects of *Plumbago zeylanica* leaf extract on *Lasiodiplodia (Oncobasidium) theobromae*, *Cladosporium oxysporum* and *Aspergillus flavus* at different concentrations of 12.5 mg/ml, 25.0 mg/ml, 50.0 mg/ml and 100 mg/ml over a period of 48 – 120 hours showed that the extract has significant antifungal effects on the three fungal species. The antifungal effects of *Plumbago zeylanica* leaf extract on *Lasiodiplodia (Oncobasidium) theobromae*, *Cladosporium oxysporum* and *Aspergillus flavus* at different concentrations of 12.5 mg/ml, 25.0 mg/ml, 50.0 mg/ml and 100 mg/ml over a Period of 48 - 120 hours on the three fungi pathogens was found effective as all the fungi pathogens were found susceptible at different hours of incubation (between 48 hours - 120 hours). *Plumbago zeylanica* leaf extract had mild / a very low inhibition against *Aspergillus flavus* having the following results at 48 hours - 4.0 mm, 11.0mm, 15.0 mm and 18.0 mm for the concentrations of 12.5 mg/ml, 25 mg/ml, 50 mg/ml and 100mg/ml and the control was 22.0 mm. At 72 hours the inhibitions were given as follows: 4.0 mm, 10.0 mm, 20.0 mm and 25.0 mm with the control of 30.0 mm. Also, at 96 hour of incubation; the results were 7.0 mm, 12.0 mm, 32.0 mm and 36.0 mm while the control was 42.0 mm. *Aspergillus flavus* was inhibited at 120 hours of incubation having the followings levels of inhibitions; 5.0 mm, 15.0 mm, 45.0 mm and 61.0 mm with the control of 70.0 mm mycelia growth. It was noticed that mild mycelia inhibition occurred at concentrations below 50 mg/ml; and significant mycelia inhibition occurred at 100 mg/ml.

*Plumbago zeylanica* was noticed in this study to have high level of mycelia growth inhibition on *Lasiodiplodia theobromae* during the incubation periods of 48 hours, 72 hours, 96 and 120 hours. *Lasiodiplodia theobromae* was inhibited having the following inhibitory zones; at 48 hours it was 38.0 mm, 39.0 mm, 41.0 mm and 42.5 mm at the concentrations of 12.5 mg/ml, 25.0 mg/ml, 50.0 mg/ml and 100.0 mg/ml but the control growth was 49.0 mm. At 72 hours of incubation, following inhibitory zones were recorded for the five concentrations; 45.0 mm, 49.0 mm, 56.0 mm and 58.0 mm (control was 65.0 mm). Also 55.0 mm, 65.0 mm, 73.0 mm and 76.0 mm were recorded at 96 hours while the control was already had reach the peak of 85.0 mm. however, at 120 hour there was slight little increase in the mycelia growth observed on the plates and the reduced in the mycelia inhibitory growth (45.0 mm, 62.0 mm, 71.0 mm and 75 .0 mm) when compared with that of 96 hour of incubation which is suggested to be as a results of the fact that the control had reach the pick (no room for the expansion in growth length again and the control was used at 85.0 mm) for the concentrations of 12.5 mg/ml, 25.0 mg/ml, 50.0 mg/ml and 100.0 mg/ml.

*Cladosporium oxysporum* was significantly inhibited by the antifungal potency of the *Plumbago zeylanica* as well; *Cladosporium oxysporum* at 48 hours on inoculation it was 6.0 mm, 7.0 mm, 10.0 mm and 13.0 mm at the concentrations of 12.5 mg/ml, 25.0 mg/ml, 50.0 mg/ml and 100.0 mg/ml but the control growth was 18.0 mm. Also, at 72 hours following inhibitory zones were recorded; 17.0 mm, 21.0 mm, 33.0 mm and 36.0 mm; and 3.0 mm, 14.0 mm, 44.0 mm and 46.0 mm for the concentrations of 12.5 mg/ml, 25.0 mg/ml, 50.0 mg/ml and 100.0 mg/ml.

However, their controls were 42.0 and 55.0 mm; and it was at 50.0 mg/ml and 100.0 mg/ml that *Plumbago zeylanica* plant extract appreciable control on *Cladosporium oxysporum*. The low and mild inhibitory concentrations occur at 12.5 mg/ml and 25.0 mg/ml the extract was found to drastically reduce the mycelia growth of the three fungi at 50 mg/ml and 100 mg/ml. It was evident that *Plumbago zeylanica* leaf extract was shown to be fungicidal. At concentration of 50 mg/ml and 100 mg/ml the three fungal were unable to develop to reproduces massive mycelia and the vegetative growth seized. The sporulation of spore was delayed as well. This confirmed that at these concentrations, the medium has become unfavourable for the fungi to survive. The mycelia growth of the three fungi were suppressed from 85.0 mm (control) at 120 hours on *Lasiodiplodia (Oncobasidium) theobromae* to 9.0 mm; from 70.0 mm (control) 9.0 mm for *Aspergillus flavus* and *C. oxysporum* was from 55.0 mm to just 10.0 mm. This attest to the fact that *Plumbago zeylanica* ethanolic leaf extract contains active compounds of potentially therapeutic significance and thus could be a potential agent for drug development; and this justify pharmacological and traditional uses of the plant.

#### 4 Conclusion

The results obtained in this study revealed that *Plumbago zeylanica* plant extract is a potent antifungal plant that could be used to eliminate or significantly reduce the growth of the three fungal pathogens used in this study that are known to be pathogenic to man and plants.

*Aspergillus flavus* have been reported to cause food poisoning (Aspergillosis) and the spores of this pathogen could be transferred by water. It has also been reported in many plant diseases. *Cladosporium oxysporum* is also known as pathogen that could pose health related challenges. *Oncobasidium theobromae* now known as *Lasiodiplodia theobromae* is globally known to be pathogen of many economically plants of which it causes significant loss to cocoa in millions. This shows that *Plumbago. zeylanica* leaf extract contains very active biological agents (secondary metabolites) in the control of fungal diseases of both human plants.

The antifungal effects of *Plumbago zeylanica* extract against *Aspergillus flavus*, *Lasiodiplodia theobromae* and *Cladosporium oxysporum* at different concentrations of 12.5mg/ml, 25.0mg/ml, 50.0 mg/ml and 100.0 mg/ml obtained in this study and its fungicidal effects on the pathogens was very significant and the extract successfully reduced the mycelia growth of the three fungi over a period between 48-120 hours of incubation. This justifies the medicinal claim of the plant as potential plant with antimicrobial properties.

The percentage inhibition of the antifungal effect of *Plumbago zeylanica* against *Cladosporium oxysporum* was at high values at 50 mg/ml and above ranged between 55.5% - 85.7%. Percentage inhibition of *P zeylanica* against the three *Lasiodiplodia theobromae* was very high even at 25.0 mg/ml;

having high percentage of 77.5%, 69.2%, 64.7%, and 52.3% at 12.5 mg/ml concentrations. 79.6% and 72.9 were noticed at 25mg/ml; 83.7%, 85.6% and 83.5% are recorded for 50mg/ml; and at 100mg/ml concentrations, following percentages were observed 86.7%, 89.4% and 88.2% for the whole incubation periods (48 hours, 72 hours 96 hours and 120 hours). High percentage of inhibition was recorded against *Aspergillus flavus* only at concentrations of 50.0 mg/ml and 100 mg/ml having 68.2%, 66.7%, 76.2% and 64.3% (50 mg/ml) and 81.8%, 83.3%, 85.7% and 87.1% (100 mg/ml). At concentration of 50 mg/ml and 100 mg/ml the percentage inhibition of *Plumbago zeylanica* against *Aspergillus flavus*, *Lasiodiplodia theobromae* and *Cladosporium oxysporum* were greatly varied. The high percentage inhibition for the three fungi mostly at 50 mg/ml and 100 mg/ml concentrations shows that *Plumbago zeylanica* leaf extract contains very active biological agents in the control of fungal diseases of plant and human.

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