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## Research Article

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### Mercy Itohan Aboh

a) Department of Microbiology and Biotechnology, National Institute for Pharmaceutical Research and Development, Idu, P.M.B 21 Garki Abuja, Nigeria

b) Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Kaduna State, Nigeria

### Solomon Fidelis

Department of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and Development, Idu, P.M.B 21 Garki Abuja, Nigeria

### Oluwale Peters Oladosu

Department of Microbiology and Biotechnology, National Institute for Pharmaceutical Research and Development, Idu, P.M.B 21 Garki Abuja, Nigeria

### Gbonjubola Olusesan Adeshina

Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Kaduna State, Nigeria

### Busayo Olalekan Olayinka

Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Kaduna State, Nigeria

### Stephen Olayeni Olonitola

Department of Microbiology, Faculty of Life Science, Ahmadu Bello University, Zaria, Kaduna State, Nigeria

### Correspondence:

#### Mercy Itohan Aboh

c) Department of Microbiology and Biotechnology, National Institute for Pharmaceutical Research and Development, Idu, P.M.B 21 Garki Abuja, Nigeria

d) Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Kaduna State, Nigeria

Email: mercybenaboh[at]gmail.com

## Antifungal potentials of *Luffa cylindrica* (Roem) ethyl acetate leaf extract

Mercy Itohan Aboh\*, Solomon Fidelis, Oluwale Peters Oladosu, Gbonjubola Olusesan Adeshina, Busayo Olalekan Olayinka, Stephen Olayeni Olonitola

### ABSTRACT

**Background:** Plants remain as an untapped reservoir of potentially useful chemical compounds. **Objectives:** The objectives of this study were to evaluate the *in vivo* antifungal activities, bioautographic profile and *in vitro* mechanism of action. **Methods:** The *in vivo* antifungal activity was investigated against *Candida albicans* and *Trichophyton rubrum* in Wistar rats. Thin layer bioautographic profile of the ethyl acetate extract was assessed using agar overlay method. The possible *in vitro* mechanism of action of the crude ethyl acetate extract was assessed by the sorbitol protection assay and release of cellular materials assay. **Results:** The bioautographic profile showed the presence of three active spots having retardation factors of 0.4, 0.6 and 0.92 and diameter zone of inhibition, 15.0 – 30.0 mm. The *in vivo* antifungal (dermal) activity of the crude ethyl acetate extract, revealed a gradual healing of the infected rats skin upon treatment with the extract formulation, with no visible clinical signs of toxicity to the skin. *In vivo* systemic antifungal evaluation of the crude extract at a dose of 1500 mg/kg produced the strongest systemic antifungal activity with a total kill of all the fungal isolates at day 3 of treatment in rats however, histopathological examination after oral administration revealed signs of toxicity to vital organs (kidney, liver and lungs). **Conclusion:** The good *in vivo* activity supports its ethno-botanical use in the treatment of skin diseases.

**Keywords:** Antifungal, Extraction, Phytochemical screening, Minimum Inhibitory concentration.

### INTRODUCTION

Dermatophytosis (syn. ringworm, *Tinea*), a zoonotic skin infection of keratinized tissues caused by a specialized group of fungi named dermatophytes, has worldwide distribution and is a public health problem all over the world. They utilize keratin as the major source of growth causing cutaneous mycosis [1, 2]. They are grouped into three Genera *Trichophyton*, *Epidermophyton* and *Microsporum* [3, 4, 5]. *Candida albicans* is the most predominant pathogens in systemic fungal infection. They also infect the skin and oral cavities. Superficial candidiasis is a common infection of the skin, oral cavity, esophagus and vagina in immuno compromised patients [6].

The incidence of fungal infections is rising with an alarming rate thus the growing need for novel antifungal agents highly efficacious and with better safety and toxicity profile compared to the current antifungal agents [7, 8]. An increase in the resistance of some pathogenic fungi of clinical importance to available antifungal agents worldwide is fast becoming a global health problem especially among people infected with HIV in which drug resistance lead to persistent infections like oropharyngeal candidiasis [9]. Despite the large number of antifungal agents that have been developed over the past few decades only few classes have been shown to be active due to their high toxicities [6, 10]. A study by Pierce and Lopez-Ribot, (2013) revealed that the currently available classes of antifungals were very few with high impact on drug resistance and little expectations of new entities to the market in the nearest future hence the discoveries of novel antifungal agents have become necessary.

Nigeria a West African country located in the tropical Sub Saharan region is endowed with a favourable climate for the flourishing and growth of a vast number of plants with strong antimicrobial activities and several medicinal benefits which has been used for decades because of its medicinal potentials, however most of these studies did not give reference to their phytochemical constituents. Smooth luffa syn. (*Luffa cylindrica* L. syn. *Luffa aegyptica*) belongs to the family of Curcubitaceae. Studies on *Luffa cylindrica* have recorded its medicinal and nutritional potentials [11]. Some of its application is the use of its seeds in the management of asthma, febrile conditions and sinusitis [12]. It has been reported that abortifacient

proteins such as luffaculin which acts on the ribosomes of HIV infected cells like lymphocyte and phagocyte preventing its replication, the reason it is used in management of AIDS [13]. In a study by Abirami *et al.* (2011) stem juice was shown to treat respiratory diseases while extracts from the seeds has been used to stimulate emesis [14].

The study was aimed at of determining the antifungal activities of the *Luffa cylindrica* leaf extract. The specific objectives of this study were to evaluate bioautographic profile of the ethanol extract; *in vitro* antifungal mechanism of action; investigate the *in vivo* antifungal activities and toxicity profile of the ethanol extract of the leaves of *Luffa cylindrica* in rats.

## MATERIALS

### Chemicals

Saboraud dextrose agar - SDA (Oxoid, England), Saboraud dextrose broth- SDB (Oxoid, England), Amphotericin B powder (Sigma-Aldrich), Fluconazole powder (Sigma- Aldrich), Terbinafine powder (Sigma- Aldrich), Terbinafine cream (TydisilXtra®). All other reagents were of analytical grade.

### Plant

Leaves of *L. cylindrica* were collected fresh from the botanical garden of the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria, given specimen number (NIPRD/H/6643) and deposited in its herbarium. The leaves were sorted, dried under shade and pulverized to powder with a mortar and pestle.

### Test Organisms

The fungal strains include clinical strains of *Candida albicans*, *Candida*

*tropicalis*, *Microsporium canis* and *Epidermophyton floccosum* and typed cultures of *Candida albicans* ATCC 2876, *Candida tropicalis* ATCC 19092 and *Trichophyton rubrum* ATCC 28188. They were acquired from the National Institute for Pharmaceutical Research and Development, Abuja, Nigeria. Fungi culture were maintained on Sabouraud dextrose agar slant at 4 °C until when needed.

## METHODS

### Extract preparation

Five hundred grams of the pulverized leaves was steeped in ethyl acetate for 72 hours at 25 °C and filtered with Whatman filter paper. The filtrate was concentrated in vacuum, dried at 60 °C on a hot water bath and weight taken.

### Preparation of herbal ointment

The herbal ointment was prepared by fusion method according to the formula in Table 1 by incorporation of different concentrations (1, 2 and 3 % w/v) of the ethyl acetate extracts of *L cylindrica* leaves to the ointment base [15]. Simply, the contents of the base were transferred to a melting pan and melted at 70 °C, followed by gentle stirring at the same temperature for about 5 min before allowing to cool with uninterrupted stirring. The extract was then incorporated into the base by mixing in a mortar obtaining 100g of herbal ointments. The prepared herbal ointments were put in ointment jars, labeled and were stored at room temperature. Terbinafine hydrochloride cream (TydisilXtra®) was used as control for the *in vivo* studies, while Niprifan® ointment (herbal antifungal ointment of *M. villosus*) was used as control in evaluating the physical characteristics of the formulated ointments.

**Table 1:** Formulation of *L. cylindrica* ointment from crude ethyl acetate extractn

| Ingredients         | 1 % w/w ointment | 2 % w/w ointment | 3 % w/w ointment | Niprifan®    |
|---------------------|------------------|------------------|------------------|--------------|
| Extract (g)         | 1.0              | 2.0              | 3.0              | 1.5          |
| Hard paraffin (g)   | 9.0              | 9.0              | 9.0              | 9.0          |
| Soft paraffin (g)   | 59.0             | 59.0             | 58.0             | 59.0         |
| Liquid paraffin (g) | 31.0             | 30.0             | 30.0             | 30.5         |
| <b>Total (g)</b>    | <b>100.0</b>     | <b>100.0</b>     | <b>100.0</b>     | <b>100.0</b> |

### Thin Layer Chromatography (TLC) Bio-autography

The agar overlay method was adopted to establish the antifungal activity of the crude extract [16]. Plant extracts (10 µL) were spotted equidistantly unto the origin, 2.5 cm from the base of a normal phase silica gel plate (Merck Silica Gel 60 F254). After drying, the plates were developed in a chromatographic tank containing 50 mL of mobile phase, hexane – ethyl acetate (3:1). After the mobile phase reached the solvent front, the TLC plates were removed and allowed to air dry for 24 h or until any residual solvent have evaporated. Standardization of suspension of *C. albicans* and spores of dermatophytes was done with a haemocytometer [17] by centrifugation at 3000 rpm for 10 min, suspended in sterilized normal saline and seeded in molten SDA containing 1% glycerol (v/v). The overlay (molten media seed with fungi) was poured on the developed TLC plate placed in a sterile petri-dish, allowed to gel and the cover of petri dish replaced. The petri-dish was incubated at 30 °C for 48 h. After incubation the plates were sprayed with tetrazolium dye (2,5- Diphenyl 3- p-iodophenyl tratrazolium) and incubated 30 °C for 1 h. The presence of a white zone within the purple background represent an active compound. The retardation factor (Rf) values of the active phyto-compounds were recorded and the zone of inhibition was measured.

### *In vitro* determination of mechanism of action of most active extract

*Determination of effects of ethyl acetate crude extract and fractions on fungal cell membrane permeability and integrity- Release of cellular material assay*

Loss of materials absorbing at 260 nm from *Candida albicans* cells was analyzed by method of Khan *et al.* (2013). Simply, *C. albicans* (2.5 x10<sup>6</sup> CFU/mL) cells were suspended in SDB and incubated at 37 °C for 48 h and cell suspension was then treated with a fixed concentration (4x MIC) of the ethyl acetate extract at time intervals of 30, 60, 90 and 120 min. Untreated sample and amphotericin B served as negative and positive controls respectively. After centrifugation of the resultant sample at 10,000 rpm for 10 min, of the supernatant the absorbance was taken at 260 nm with a UV-Vis spectrophotometer (Jenway, United Kingdom).

### *In vivo* Antifungal Study

#### Animals

Hundred healthy Wistar rats of either sex weighing 150-200 g, age ranging between 8 – 12 weeks old were allowed to acclimatize for at

least 5 days in the animal facility center of NIPRD, Nigeria. The rats were housed in separate plastic cages in a well ventilated room at a temperature of 25 °C ( $\pm$  2 °C). They were fed with standard rodent chow and had free access to portable water when needed. The test was done according to OECD guidelines [19] and NIPRD's standard operating procedures.

#### Induction of fungal infections (dermal)

*Trichophyton rubrum* was grown on SDA at 30 °C for 5-7 days. Subsequently, the fungal spores were scraped off aseptically, and suspended in 30 ml of sterile water and mixed. Hundred microliters of the spore suspension were spread onto the back of the rat after removal of the hair (depilation) with slight abrasion, then allowed to incubate for 3 days. On the fourth day post infection, the presence of pathogens cultured from skin scaled on SDA plates, confirms the successful infection. On days 8- 10 post infection, the animals were assessed visually to confirm that infection with the fungi was successful, those infected were subjected to treatment on day 20 until total recovery was accomplished.

#### Administration of doses and observation

The formulated ointment from crude extracts of the plants was used for the study. A commercial fungicidal cream terbinafine (TydisilXtra®) served as positive control. The animals received treatment daily and were assessed for presence inflammation of fungal scales by a visual scoring method. Modified scores (0-4) for presence of clinical lesions were adopted as follows: 0- no obvious lesion; 1- skin showing few slightly erythematous lesions; 2 - clear vesicles; 3- large zones of marked redness coating, scaling, blade patches, wounds; score 4, well defined mycotic foci with wounds, in addition to a score 3 lesion [5]. Skin scrapings from infected loci were cultured on SDA plates containing chloramphenicol each day.

#### Induction of fungal infections (Systemic)

Candidiasis infection was induced according to method by Dzoyem *et al.*, (2014). Simply, intravenous injection of 0.2 mL of standardized *Candida albicans* culture ( $10^6$  cfu/mL) from a 48 h culture was prepared in sterile saline and administered to the rats. The fungal load in the kidneys and blood were assessed after twenty-four hours of infection by sacrificing the animals [20].

#### Antifungal treatment

The infected animals were placed in five groups of 5 five animals each and kept in plastic enclosures having liberty to food and water when needed. Extracts at the doses of 0.5, 1.0 and 2.0 g/kg of body weight, were given by mouth for 3 days consecutively from 24 h post infection. The animals serving as negative controls were administered distilled water while the positive control group received fluconazole (Sigma) at 10 mg/kg of body weight.

#### Quantification of viable yeasts in organ samples

Quantification of the viable yeast present in organ samples were carried out on three consecutive days (at days 2, 3 and 4 of infection) post infection. Simply 0.5 mL of blood were collected into EDTA tubes and transferred to the laboratory. Hundred microliters of serial dilutions of the blood samples were cultured on SDA plates with chloramphenicol and incubated for 24- 48 h at 37°C. The total fungal count (expressed as log of the mean number of cfu/mL).

#### Acute dermal toxicity test

This experiment was done in rats (weighing 150–200 g) according to the Organization of Economic Co-operation and Development (OECD) guideline number 404 [19]. Approximately 10 % of the total surface area the rats' sides were shaved, leaving the animals undisturbed for 24 h. Afterwards, a single dose of 1500 mg/kg of the extract and 1, 2 and 3 % ointment formulated from the crude extract, was evenly applied on the shaved area of the skin and the animals observed for signs of toxicity. Effects of toxicity to the fur, eyes, respiratory system such as salivation, diarrhoea and urination; and central nervous system like tremors and convulsion, changes in the level of activity, movement and balance, level of alertness or distorted strength were assessed. The Draize scoring criteria for erythema and oedema was used as follows:

No erythema /oedema - 0

Very slight erythema/ oedema (barely perceptible) 1

Well developed erythema/ oedema- 2

Moderate to severe oedema 3

Severe erythema/ oedema (beef redness) to eschar formation preventing grading of erythema 4.

#### Acute oral toxicity test

This test was performed on rats based on Organization of Economic Co-operation and Development (OECD) guideline 420 for testing of chemicals [21]. Male and female animals 8–12 weeks' old were employed. The ethyl acetate extract of *L. cylindrica* was suspended in 10 % Tween 80 and given via the oral route in divided doses of 10, 100, 1000, 1600, 2900 and 5000 mg/kg at a proportion of 20 mL/kg to the animals (5 males and 5 females per group), whereas the control group received only 10 % Tween 80 as a vehicle. The animals were observed daily for a period of 14 day with close attention given to the first 4h of observation. The rats were monitored by visually for effects of toxicity to the fur, eyes, respiratory system such as salivation, diarrhoea and urination; and central nervous system like tremors and convulsion, changes in the level of activity, movement and balance, level of alertness or distorted strength, physical changes, pain or injury. At the completion of the test, the rats were euthanized by chloroform inhalation and blood samples taken via cardiac rupture into plain tubes without coagulant for biochemical analysis and EDTA tubes for haematological analysis. The organs were removed and the macroscopic features examined. Vital organs (liver, kidney, lung, heart, and spleen) were well-preserved in 10% solution of buffered formalin for histopathological study.

#### Statistical analysis

Results gotten were expressed as mean  $\pm$  standard deviation and analyzed for significance using one way ANOVA (GraphPad Prism 5) at  $p < 0.05$ .

## RESULTS

#### Thin Layer Chromatography (TLC) Bio-autography

The TLC chromatogram of the crude ethyl acetate extract of *L. cylindrica* after development revealed the presence of six distinct spots with Rf values of 0.05, 0.19, 0.4, 0.6, 0.85 and 0.92. However after in situ antifungal testing the bioautogram revealed the presence of three bioactive spot having retention factor (Rf) values of 0.4, 0.6 and 0.92. Their diameters zone of inhibition against the test fungi ranged from 15.0 – 30.0 mm (Table 2). The pictorial representation of the bioautograms on exposure to different fungi is shown in plates I – III.

**Table 2:** Bioautographic profile of the crude ethyl acetate extract of *L. cylindrica* against test fungi.

| Organisms                       | Number of spots | Refractive index | Diameter zone of inhibition (mm) |
|---------------------------------|-----------------|------------------|----------------------------------|
| <i>C. albicans</i> ATCC 2876    | 2               | 0.4              | 20.0                             |
|                                 |                 | 0.92             | 16.0                             |
| <i>C. tropicalis</i> ATCC 19092 | 1               | 0.92             | 15.0                             |
| <i>M. canis</i>                 | 3               | 0.4              | 20.0                             |
|                                 |                 | 0.6              | 15.0                             |
|                                 |                 | 0.92             | 18.0                             |
| <i>T. rubrum</i> ATCC 28188     | 2               | 0.4              | 20.0                             |
|                                 |                 | 0.92             | 30.0                             |
| <i>E. floccosum</i>             | 2               | 0.6              | 25.0                             |
|                                 |                 | 0.92             | 19.0                             |



Plates I

Plate II

Plate III

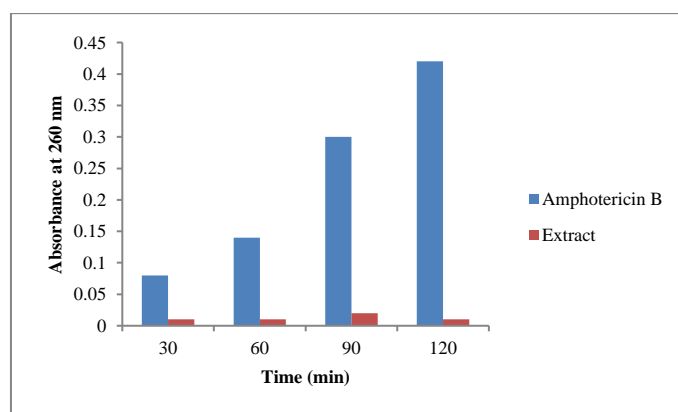
Bioautogram of ethyl acetate extract of *L. cylindrica* against *T. rubrum* ATCC 28188 (I), *E. floccosum* (II) and *C. tropicalis* (III).

**In vitro Mechanism of Action**

**Release of cellular materials**

The release of cellular material from the fungal cells on exposure to the ethyl acetate extract and amphotericin B at a wave length of 260 nm is

represented in Figure 4.1. The crude extracts of *L. cylindrica* did not show any appreciable absorption at 260 nm unlike the positive control amphotericin B which showed a gradient increase in absorbance (0.08 to 0.42) as the exposure time increased.



**Figure 1:** Effects of ethyl acetate extracts of *L. cylindrica* and amphotericin B on release of materials absorbing at 260 nm.

**In vivo Antifungal Studies**

**Formulation of antifungal ointment of ethyl acetate extract of *L. cylindrica***

Formulation of an antifungal ointment from the ethyl acetate extract of

*L. cylindrica* produced a stable ointment, smooth to touch and dark greenish in colour. The concentration of the extract in the ointment ranged from 1 % to 3 %. Table 3 shows the physical properties of the herbal ointment.

**Table 3:** Physical properties of herbal ointment from ethyl acetate extract of *L. cylindrica*

| Characteristics | 1 % w/w ointment | 2 % w/w ointment | 3 % w/w ointment | Niprifan®       |
|-----------------|------------------|------------------|------------------|-----------------|
| Colour          | Dark green       | Dark green       | Dark green       | Dark green      |
| Viscosity       | Paste like       | Paste like       | Paste like       | Paste like      |
| Consistency     | Smooth to touch  | Smooth to touch  | Smooth to touch  | Smooth to touch |

**In vivo dermal antifungal activity of ethyl acetate extract of *L. cylindrica***

The results of the effect of the ointments formulated with different concentrations (1, 2 and 3%) of ethyl acetate extract on the skin of rats infected with *Trichophyton rubrum* is shown in Table 4. The results revealed a gradual healing of the infected skin with treatment with the

extract formulation. The formulated extract containing 3 % of *L. cylindrica* was found to be most effective producing complete healing by day 5 of application. The 1 % extract formulation had the least antifungal activity. Although, complete healing was not achieved after 14 days of application, the level of infection decreased compared to the control. The schematic diagram of the *in vivo* application of the dermal ointment on rat skin infected with *T. rubrum* is represented in Plate IV.

**Table 4:** *In vivo* dermal antifungal activity of ointment formulated from crude ethyl acetate extract *L. cylindrica*

| Extracts (%) | Score |       |       |       |       |       |       |       |        |
|--------------|-------|-------|-------|-------|-------|-------|-------|-------|--------|
|              | Day 0 | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 | Day 14 |
| 0            | 4     | 4     | 4     | 4     | 4     | 4     | 4     | 3     | 2      |
| 1            | 4     | 4     | 4     | 4     | 4     | 3     | 3     | 2     | 1      |
| 2            | 4     | 4     | 3     | 3     | 2     | 2     | 1     | 1     | 0      |
| 3            | 4     | 4     | 3     | 2     | 2     | 1     | 1     | 0     | 0      |
| Control      | 4     | 4     | 2     | 2     | 1     | 0     | 0     | 0     | 0      |

Score  
 4-mycotic foci well developed with ulceration in addition to a score 3 lesion  
 3-large areas of marked redness incrustation, scaling, blade patches, ulcerated in places  
 2-well-developed vesicles  
 1- skin showing few slightly erythematous lesions  
 0-no obvious lesion

**In vivo Antifungal Assay**



**Plate IV:** Systematic *in vivo* dermal antifungal screening (flow chart) of *L. cylindrica* extract in rats

**In vivo systemic antifungal activity of ethyl acetate extract of *L. cylindrica***

The results on the effect of different concentrations of the ethyl acetate extract on the rats infected with *Candida albicans* is shown in Figure

2. Treatment with a dose of 1500 mg/kg produced the strongest systemic antifungal activity with a total kill of all the fungal isolates at day 3 of treatment. There was minimal antifungal effect at a dose of 500 mg/kg.

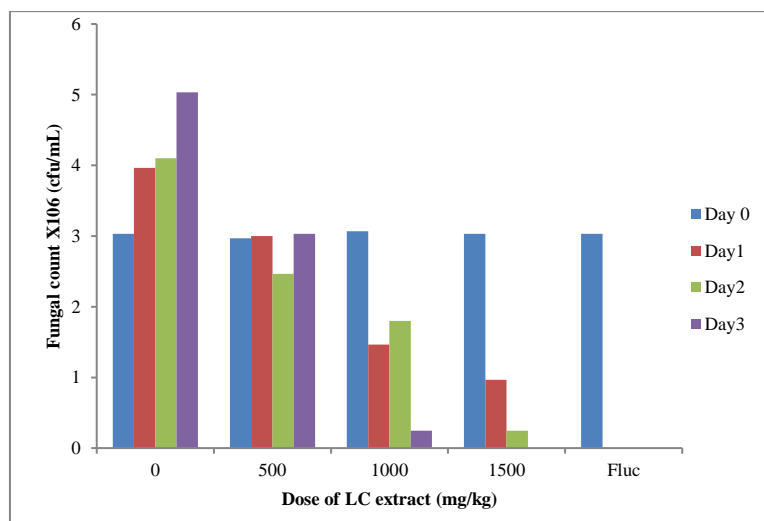


Figure 2: In vivo systemic antifungal activity of ethyl acetate extract of *L. cylindrica*

**Acute dermal toxicity test**

*Effect of dermal application of extracts L. cylindrica on the skin in rats*

The summary on the clinical signs due to dermal application of the extracts on the skin of rat is represented in Table 5. The ethyl acetate

extract and formulated ointments of *L. cylindrica* showed no visible clinical signs of irritation as no reddening or oedema of skin was observed. There were no deaths from dermal application of the different concentration of the ointments formulated from the extracts. Figure V represent a schematic diagram of acute dermal toxicity test of the ethyl acetate extracts of *L. cylindrica*.

Table 5: Summary of clinical signs of dermal toxicity of *L. cylindrica* in rats

| Sex    | Group           | Dose (mg/kg) | No. of animals | Test site           |        |           |
|--------|-----------------|--------------|----------------|---------------------|--------|-----------|
|        |                 |              |                | Erythema and eschar | Oedema | Mortality |
| Male   | Vehicle control | 0            | 5              | 0                   | 0      | 0         |
|        | 1% Ointment     | 50           | 5              | 0                   | 0      | 0         |
|        | 2% Ointment     | 100          | 5              | 0                   | 0      | 0         |
|        | 3% Ointment     | 150          | 5              | 0                   | 0      | 0         |
|        | Crude Extract   | 2500         | 5              | 0                   | 0      | 0         |
| Female | Vehicle control | 0            | 5              | 0                   | 0      | 0         |
|        | 1% Ointment     | 50           | 5              | 0                   | 0      | 0         |
|        | 2% Ointment     | 100          | 5              | 0                   | 0hh    | 0         |
|        | 3% Ointment     | 150          | 5              | 0                   | 0      | 0         |
|        | Crude Extract   | 2500         | 5              | 0                   | 0      | 0         |

4- Severe erythema/ oedema (beef redness) to eschar formation preventing grading of erythema  
 3- Moderate to severe oedema  
 2- Well developed erythema/ oedema  
 1- Very slight erythema/ oedema (barely perceptible)  
 0-No erythema /oedema



Housing

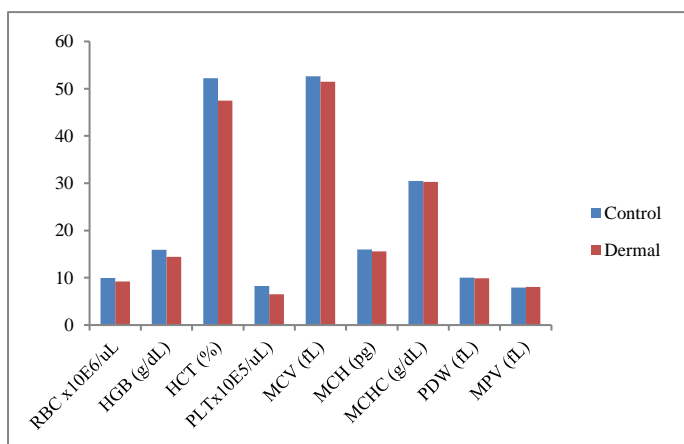
Depilation

Treatment

Observation

Plate V: Picture slides depicting flow chart of dermal toxicity testing in rat

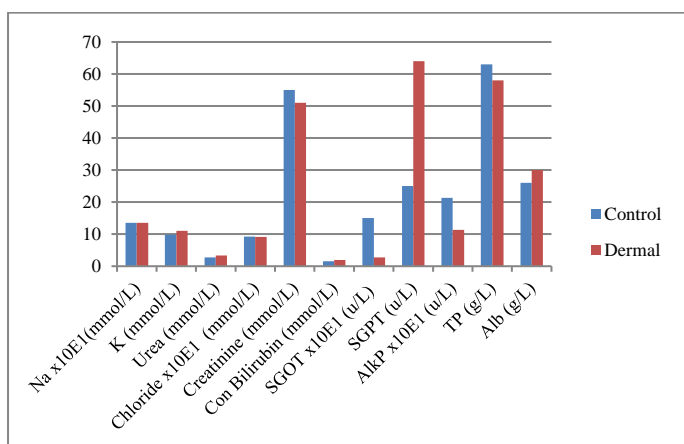
**Effect of *L. cylindrica* ethyl acetate extracts on haematological parameters from plasma in rats:** The effect of the crude ethyl acetate extract (2500 mg/kg) of *L. cylindrica* on the haematological parameters of the rats tested is represented in Figure 3. All the haematological parameters were found to be within normal range. There was no marked difference between the control skin and the skin to which the extract was applied after 14 days of application.



Key: RBC- red blood cell  
HCT- haematocrit  
MCV- mean corpuscular volume  
MCH- mean corpuscular Haemoglobin  
MCHC- mean corpuscular haemoglobin concentration  
PWD- platelet distribution width  
MPV- mean platelet volume  
HGB- haemoglobin  
PLT- platelet

**Figure 3:** Effect of *L. cylindrica* ethyl acetate extracts on haematological parameters from plasma in rats after dermal application.

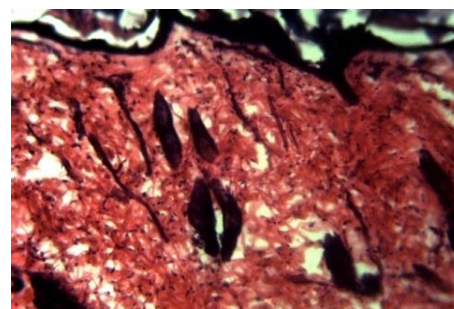
**Effect of *L. cylindrica* ethyl acetate extracts after dermal application on biochemical parameters from serum in rats:** The effect of the crude ethyl acetate extract (2500 mg/kg) of *L. cylindrica* on the biochemical parameters of the rats tested is represented in Figure 4.5. All the haematological parameters were found to be within normal range. There was no marked difference between the control skin and the skin to which the extract was applied after 14 days of application.



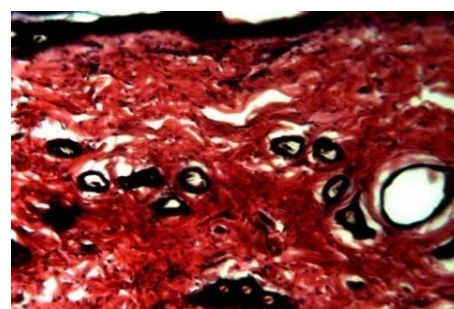
Key: K- potassium  
SGPT- Alanine Aminotransferase  
SGOT- Aspartate Aminotransferase  
Alk P- alkaline phosphatase  
Na- sodium  
Alb- albumin  
TP- total protein

**Figure 4:** Effect of *L. cylindrica* ethyl acetate extracts on biochemical parameters from plasma in rats after dermal application.

**Effect of *L. cylindrica* ethyl acetate extracts on the skin in rats:** The histopathological section of the skin after dermal application of 2500 mg/kg of the crude ethyl acetate extract of *L. cylindrica* revealed a normal structural features (Plate VI).



Control



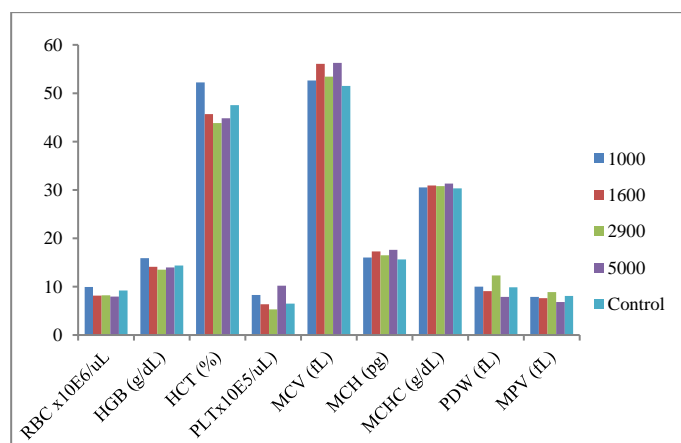
After dermal application (2500 mg/kg) Magnification x100

**Plate VI:** Histopathological section of the skin after dermal application of 2500 mg/kg of the crude ethyl acetate extract of *L. cylindrica*

**Acute oral toxicity test**

**Clinical signs and mortality from oral administration (acute toxicity) of different doses of extracts of *L. cylindrica* in rats:** Physical observations of the rats after oral administration of *L. cylindrica* extracts showed no clinical signs of toxicity changes in the skin, fur, eyes mucous membrane. The rats' compartment patterns were normal with absence of tremors, salivation or diarrhoea.

**Effect of *L. cylindrica* ethyl acetate extracts on Haematological parameters in rats:** The haematological parameters of treated and control rats is represented in Figure 4.7. Analysis of the The results haematological parameters revealed no marked difference between treatment group and the control group.

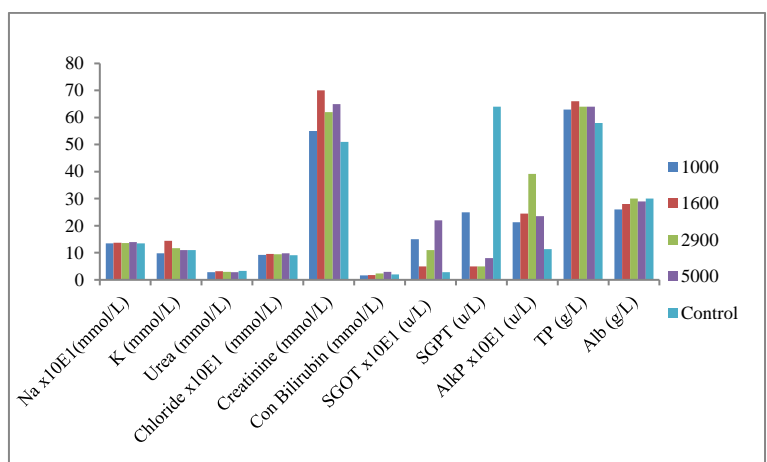


Key: RBC- red blood cell  
HCT- haematocrit  
MCV- mean corpuscular volume  
MCH- mean corpuscular Haemoglobin  
MCHC- mean corpuscular haemoglobin concentration  
PWD- platelet distribution width  
MPV- mean platelet volume  
HGB- haemoglobin  
PLT- platelet

**Figure 5:** Effect of *L. cylindrica* ethyl acetate extracts on haematological parameters in rats after oral administration.

*Effect of L. cylindrica ethyl acetate extracts on biochemical parameters in rats:* The biochemical parameters of treatment group and control group is represented in Figure 4.8. The results revealed no marked difference in most of the biochemical parameters of the treated rats to

the control group. However, there was marked difference in the liver function parameters (serum alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase) levels.

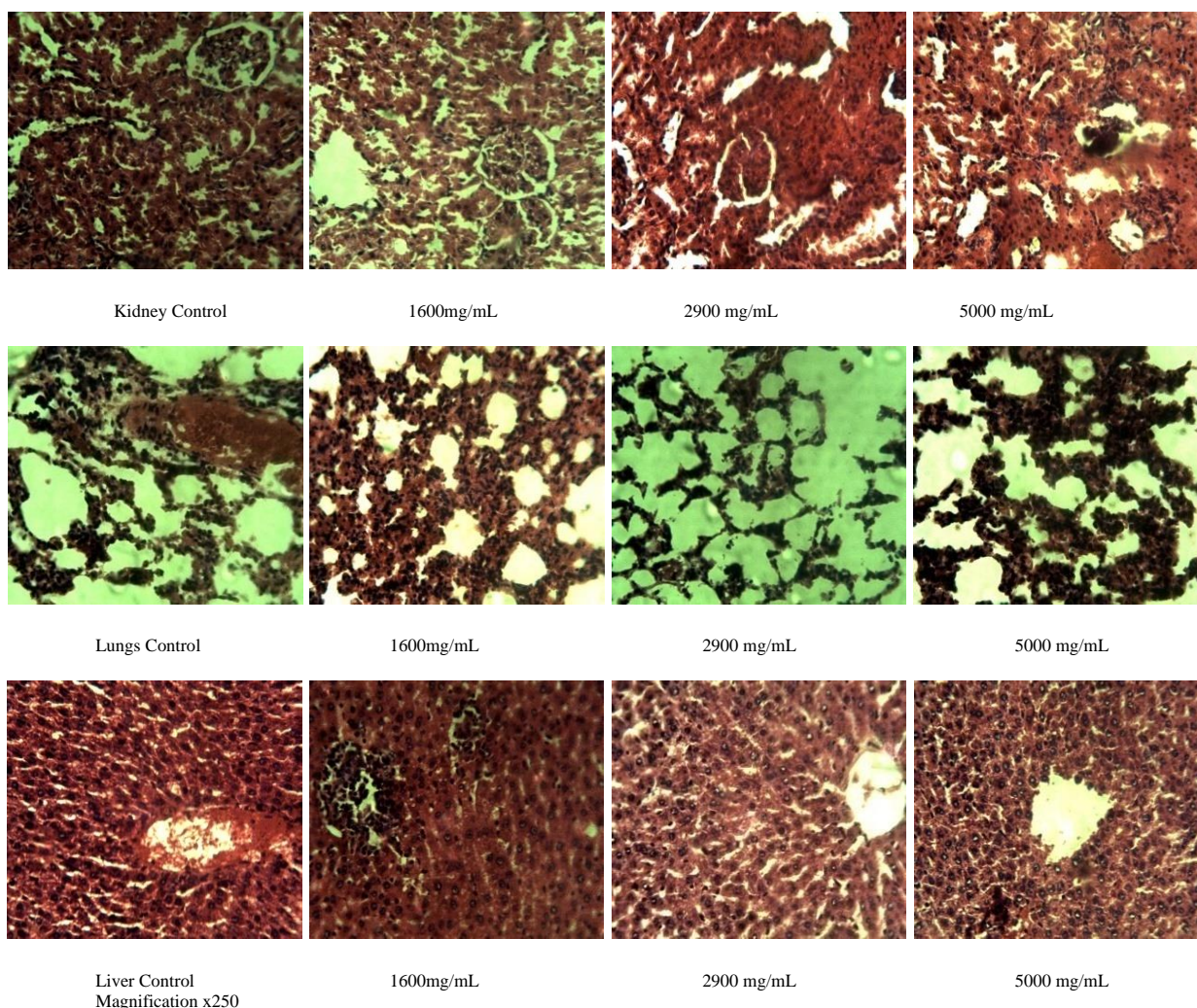


Key: K- potassium Na- sodium SGPT- Alanine Aminotransferase Alb - albumin  
 SGOT- Aspartate Aminotransferase Alk P- alkaline phosphatase TP- total protein

**Figure 6:** Effect of *L. cylindrica* ethyl acetate extracts on biochemical parameters in rats after oral administration.

*Effect of L. cylindrica ethyl acetate extracts on vital organs in rats:* The histopathological sections from oral administration of the different doses of the extracts is shown in Plate VI. The histopathological

examinations of the various organs stained with haematoxylin and eosin revealed signs of toxicity with increase in the dose from 1600 – 5000 mg/kg.



**Plate VI:** Histological sections of vital organs in rat from the control, 1600, 2900 and 5000 mg/kg of treated groups.



## DISCUSSION

The release of intracellular components (which absorbs at 260 nm) from the cell membrane of *Candida albicans* into the medium has been used as a measure of the integrity of the cell membrane. Thus, the mechanism of action of substances that interfere with the cell membrane integrity can be assessed by their ability to cause leakage of the cell membrane resulting in the release of intracellular components like nucleotides [18]. Nucleotides have been reported to exhibit strong absorbance at 260 nm and hence are good markers in assessing the effect of novel antifungal compounds on cell membrane integrity [22]. The inability of the test medium to absorb at 260 nm indicates that the extracts and fractions of *L. cylindrica* do not produce its antifungal activity via other pathways other than interference with the integrity of the cell membrane of *C. albicans*. There is little or no documentation on the *in vitro* mechanism of action of *L. cylindrica* against *C. albicans*.

The *in vivo* antifungal efficacy determination of the ethyl acetate extract of *L. cylindrica* leaves in albino wistar rats infected with *T. rubrum* revealed a marked healing effect upon administration of the herbal ointment to the animal's infected skin. The magnitude of the extract antifungal activity on the treated rats increased with an increase in the dose and progressed positively with time until there was complete healing. Treatment with the extract led to healing of the skin of rats with disappearance of the lesions, scaly crust and red like colouration. Upon healing the rats regained their furs with no scar left behind. This result agrees with a study by Abirami *et al.* (2011), who reported that the ointments formulated from the chloroform extracts of the whole plant of *L. cylindrica* possessed a strong anti-inflammatory and wound healing effect in rats.

The evaluation of synthetic antifungal agents using animal models (pre-clinical studies) remains a useful tool in the development of new and novel antifungal agents. Pre-clinical studies are more frequently applied in development of orthodox drugs than herbal medicines especially in the development of herbal products with potential to cure fungal infections [4]. Although studies on the *in vitro* antifungal activities have been reported [23], there is limited documentation on the *in vivo* systemic antifungal efficacy of the leaves of *L. cylindrica* in rats. *Candida albicans* the causative organism of candidiasis are among the common fungi that infect rats.

The results from this study revealed that the administration of 1500 mg/kg of the ethyl acetate extract of *L. cylindrica* was most effective producing reduction in the number of cfu/mL of *C. albicans* load in blood to levels below detection after three days of oral administration. Its effect however, was not as strong as oral administration of the control drug fluconazole with complete elimination of the *C. albicans* in the blood of the rats within 24 h of oral administration. The presence of triterpenoids like luffalin has been shown to be responsible for its antifungal activities.

The application of the crude ethyl acetate extract of *L. cylindrica* ointment to the rats' skin revealed no clinical signs of dermal toxicity during the 15 days period of observation and there was no mortality seen. The experiment on acute dermal toxicity revealed absence of toxicity to the skin with no erythema, eschar, oedema seen to either intact or abraded sites all animals in the treatment or control groups. There was no significant change in body weight of the rats from crude ethyl acetate extract of *L. cylindrica* application during the observation period. A single dermal dose of the crude ethyl acetate extract of *L. cylindrica* had no toxic effects on mortality, clinical signs, body weight changes and gross findings in both the male and female rats at dose of 1500 mg/kg body weight.

There are little or no documentation on the dermal toxicity profile of *L. cylindrica* leaves extract. However, Sutharshana *et al.* (2013), reported the protective role of the leaves in inflammation, oedema and abscesses [24]. The histopathological findings revealed no marked difference in the

structural features between the skin of the control rats and the treated rats. This result explains the application of exudates from the crushed fresh leaves on the skin for treatment of skin infection [25]. The biochemical and haematological parameters were within normal ranges and did not show any sign of toxicity from absorption through the skin. Thus, the extract from the leaves of *L. cylindrica* may be formulated as ointment for application to the skin in the treatment of skin diseases.

The use of herbal medicines as the first point of call for primary health care among majority of the population in developing countries has been documented, however there is limited scientific documentation on the safety profiles of these medicine [26]. In most cases, the studies on the safety of these remedies are carried out during the process of commercialization- as a requirement for registration of these products by the appropriate regulatory bodies. However it is a known fact that most of these traditional medicine practitioners never reach the stage of commercialization or registration, hence the safety profile of the medicines remain unknown. The assertion that these herbal medicines are safe for human consumption should be taken only after the plant product passes through toxicity testing using established and validated scientific method [27].

The effects of treatment with the extracts on the haematological parameters from the plasma in rats revealed a slight reduction in the red blood cell count ( $7.96 \times 10^6 / \mu\text{L}$ ) of treated rat at a dose of 5000 mg/kg as compared to control ( $9.23 \times 10^6 / \mu\text{L}$ ) rats administered water. The serum haematocrit levels also slightly decreased from 47.5 % in control to 44.8 % in treated (5000 mg/kg) rats. The effects of *L. cylindrica* extracts on the haematological parameters have not been documented. The effects of treatment with the extracts on the biochemical parameters from the plasma in rats revealed an increase in the levels of serum alkaline phosphatase and aspartate amino transferase but a decrease in the levels of alanine amino transferase. This indicates toxicity to the liver.

The histopathological (post mortem) examination of the vital organs – liver, kidney and lungs (Plate VI) showed morphological changes in the structure of the cells of the organs which indicate signs of toxicity as a result of prolonged exposure of the organs to the ethyl acetate extract especially after administration of 5000 mg/kg. The mostly affected organs were the kidney and liver. The kidney showed signs of deterioration and necrosis in the tubular epithelia of the kidney with cellular infiltration. This effect agrees with the principle of target organ toxicity [28]. The liver on the other hand, showed moderate portal to portal inflammation, vasculations and slight necrosis and lymphocyte hyperplasia. These effects tally with the biochemical tests, which revealed a marked change in the liver function parameters – AST, ALT and amino transferase.

Although several studies have been done on this plant, and the ethno-botanical use of the plant dates back to several years, there however limited information on its safety for systemic consumption in humans. There is therefore need for caution in the ingestion of this plant orally as it might be toxic to the organs of the body.

## CONCLUSION

The plant show good *in vivo* antifungal properties with no sign of toxicity when applied externally on the skin of rats but showing toxicity to some vital organs after oral administration. The good *in vivo* activity supports its ethno-botanical use in the treatment of skin diseases.

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