**A RESEARCH PROPOSAL TO BE SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELORS DEGREE OF SCIENCE TECHNOLOGY BIOLOGY OF KYAMBOGO UNIVERSITY**

**FACULTY OF SCIENCE**

**DEPARTMENT OF BIOLOGICAL SCIENCES**

**ANTIMICROBIAL ACTIVITY OF *DATURA STRAMONIUM* PLANT LEAF EXTRACT AGAINST *TINEA CORPORIS***.

**BY**

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**SUPERVISOR:** MR. KIIZA HILLARY.

JANUARY 2023

# DECLARATION

I TUMUSINGIZE DICKSON declare that, the matter presented in this research project proposal has not been submitted by me elsewhere. It is only prepared for my academic requirement and not for any other purpose. It should not be used with the interest of opposite party of the corporation.

**Signature of Student**

**………………………**

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# APPROVAL

This is to certify that this research proposal of Tumusingize Dickson and was carried out under my supervision and is now ready for submission to research committee and board of examiners with my approval

Sign: ………………………………

Mr. KIIZA HILLARY.

SUPERVISOR.

Date: ………………………………

# DEDICATION

I dedicate this research project proposal to my beloved parents, friends and relatives for their encouragement, unconditional love and support that has made me the person am today. Thank you for believing in me and may the Almighty God reward you abundantly.

Also, to my sisters and brothers whose love, support and company was very important to keep moving me forward. I wish you the best in all your future endeavours and plans.

# ACKNOWLEDGEMENTS

I thank God for the gift of life and ability towards my studies. Great appreciation goes to my parent for the financial, spiritual, emotional and social support rendered to me up to this time. I am greatly indebted to Mr. Kiiza Hillary my Lecturer who tirelessly guided me through this research proposal. His maximum cooperation, honesty, constant parental care, guidance and encouragement gave me intellectual strength and courage to easily take me though this research project proposal. I also owe much gratitude to Mr. Kigozi Steven and Tushabomwe Bedda of Department of biological sciences who supported me when I developed interest in “medicinal plant’’.

May the good Lord reward them abundantly.

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# LIST OF ABBREVIATIONS

**MIC-Minimum Inhibitory Concentration**

**MFC-Minimum Fungal Concentration**

**AMR – Antimicrobial Resistance**

**eg-Forexample**

**i.e.- That Is**

***D.Metel* - *Datura Stramonium Metel Species***

***D. Stramonium*-*Datura Stramonium***

# CHAPTER ONE

## 1.0 INTRODUCTION

Dermatophyte infections are a global health problem but neglected in Worldwide. In fact, it was estimated that 20-25% of the world population is infected by dermatophytes infections.(Coulibaly et al., 2018)

Tinea corporis, also known as ‘ringworm,’ is a superficial dermatophyte infection of the skin, other than on the hands (tinea manuum), feet (tinea pedis), scalp (tinea capitis), bearded areas (tinea barbae), face (tinea faciei), groin (tinea cruris), and nails (onychomycosis or tinea unguium).(Leung et al., 2020a)

Tinea corporis is most commonly caused by dermatophytes belonging to one of the three genera, namely, Trichophyton (which causes infections on skin, hair, and nails), Microsporum (which causes infections on skin and hair), and Epidermophyton (which causes infections on skin and nails). Dermatophytes are grouped as either anthropophilic, zoophilic, or geophilic, depending on whether their primary source is human, animal, or soil, respectively. Because tinea corporis is common and many other annular lesions can mimic this fungal infection, physicians must familiarize themselves with its etiology and its treatment. Tinea corporis is the most common dermatophytosis. While tinea corporis occurs worldwide, it is most commonly observed in tropical regions. The lifetime risk of acquiring tinea corporis is estimated to be 10–20%. Tinea corporis occurs most frequently in post-pubertal children and young adults(Agarwal et al., 2019)

While an earlier study showed that dermatophytosis occurs in between 10% and 70% of children throughout Africa, with *Tinea capitis* being the most common presentation, an accurate estimate of the true burden of *Tinea corporis* in Africa remains unknown. An accurate quantification of the burden of this neglected tropical disease is required to inform clinical and public health intervention strategies. However, Literature has shown that the global burden of dermatophytes infection was estimated to be 20-25%, there was scarcity of data on dermatophyte infection in East Africa, Uganda inclusive especially from western part of the country. Research has revealed that *T. capitis* affects 10% of all Kenyan school going children. Wiegand et al, reported 82.6%prevalence of dermatophytosis in children attending Mbarara Regional Referral Hospital in south western Uganda.(Tsamiya et al., 2015a)

This work is aimed at determining antifungal activity of ethanolic crude leaf extract of *Datura stramonium* against dermatophytes (*Tinea corporis*)*. Datura stramonium* is a common plant in most bushy areas and it has stingy thorny fruits and broad leaves. It has common local names like Rweziringa in Runyankole-Rukiga.

It grows both in terrestrial and wetland areas and it is widely used as a local medicinal plant.

Dermatophytes are a group of fungi that invade keratinized tissues of the hair, skin(epidermis), and nails, and some cause conditions commonly referred to as Tinea-meaning group of diseases. They grow best in humid and warm environment hence common in the subtropical and humid regions.(Kakande et al., 2019a)

Recent classifications of dermatophytes using multilocus phylogenetic study regrouped the organisms into seven genera; Arthroderma, Epidermophyton, Lophophyton, Microsporum, Nannizzia, Paraphyton, and Trichophyton

The most causative organisms of *Tinea capitis* fall in three genera of, Trichophyton, Microsporum and Nannizzia.

Tinea infection can spread from person to person or indirectly from fomites e.g., clothes, hair brush and huts, through soil or contact with animals. They infect areas of the body such as the groin, hands and legs, full body (*Tinea corporis*), hips, waist, scalp, (*Tinea capitis* or scalp ringworm) (Kovitwanichkanont and Chong, 2019)

These infections are more common among rural than urban population; the infections are more pronounced in males as compared to females and high in children especially *Tinea capitis.* The predominant infecting species is *Tinea tonsurans*. Disease manifestations range from small scaling patches to involvement of the entire scalp with extreme hair loss. Although *T capitis* infections are not life threatening, they have significant social and health related economic impacts due to the reoccurrences from exposure to the sources of the causative organisms such as sharing towels, clothes or hair accessories with infected individuals, long duration of treatment regimens, poor adherence, and cases of reoccurrences.(Leung et al., 2020b)

Although many effective antifungal drugs are available, most of them are relatively expensive and others with marked toxic effects such as hepatoxicity. Herbal extracts have been used in dentistry for reducing inflammation, for inhibition the growth of pathogens, for preventing the release of histamine and 6as antiseptics, antioxidants and analgesics.

Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive compounds or substances are called phytochemicals.

These compounds are synthesized by primary or secondary metabolism. They are naturally occurring in the plant leaves, roots, bark. The phytochemicals are of two categories; primary and secondary. Primary constituents of chlorophyll, proteins, sugars and amino acids.(Kaur, 2020)

Secondary constituents terpenoids, alkaloids, saponins, tannins etc. Plants used in herbal treatment as antifungal agents such as *Datura stramonium* could provide an alternative, relatively cheap and improved accessibility to treatment for such opportunistic infections especially in HIV/AIDS patients. In Uganda *Datura stramonium* was reported to be used in treatment of both bacterial and fungal infections. Therefore, this study will be aimed at investigation of antifungal activity of ethanolic crude leaf extract of *Datura stramonium* against *Tinea corporis*(Vega-Ceja et al., 2022)*.*

## 1.1 Problem statement

Fungal diseases continue to be a major health problem worldwide. Dermatophytes are the major organism implicated with dermatophytosis estimated at 20-25% worldwide. Ringworms and diseases related to keratinized body tissues are prevalent in lower social-economic status groups in populations where ringworms (*Tinea corporis*) are largely untreated. It affects the sleep, work performance and productivity. If not treated well can lead to loss of body hair. In children, the pain can affect school attendance, and then impair with growth and development. The prevalence of dermatophytosis in school aged children is up to 20-25% in many parts of the world and the adults are also affected. Access to dermatophytosis health care is limited in most developing countries.

Due to the increasing resistance of pathogens to conventional antifungal drugs, plant extracts and compounds are of interest as antiseptics and alternative antifungal substances. Therefore, this study will be aimed at investigation of antifungal activity of ethanolic crude leaf extract of *Datura stramonium* against dermatophytes (*Tinea corporis*).(öz arık, 2017a)

## 1.2 Objectives

### 1.2.1General objective

To investigateantimicrobial effect of *Datura stramonium* leaf extract on *Tinea corporis*

### 1.2.2 Specific objectives

1. To determine minimum inhibition concentration (MIC) of *Datura stramonium* leaf extract on *Tinea corporis*
2. To determine minimum fungicidal concentration (MFC) susceptible to *Datura stramonium* leaf extract.

## 1.3 Research hypotheses

There is no significant difference in the antifungal effect of *Datura stramonium* leaf extract against *Tinea corporis.*

There is no significant difference in the minimum inhibition concentration (MIC) of *Datura stramonium* leaf extract against *Tinea corporis*.

There is no significant difference in the minimum fungicidal concentration (MFC) susceptible to *Datura stramonium* leaf extract.

## 1.4 Scope of the study

The plant will be collected from the bushes around Kyambogo university. The plant leaves will be pricked off from the main plant and kept in a sample bag and the sample bag will be labelled with a masking tape showing the date of collection and the place where it is obtained. The authenticity of the plant will be confirmed with the biological sciences department.

All dirt particles will be removed from the leaves by gentle brushing and washing under moving cold water and also to remove water soluble contaminants. Fresh weight of the leaves will be obtained by measuring on the analytical balance and recorded.

The fresh leaves will be dried under a shade and powdered with a mechanical grinder or with a mortar and pestle. The powder will then be passed through a sieve and then weighed on an analytical balance and weight recorded then stored in an airtight dark container at room temperature till extraction. This will be labelled properly with the initials of the student and sample name.

Reference fungal strains will be obtained from the department of biological sciences or purchased with guidance from the technicians and supervisor.

Analysis will be conducted both in the chemistry and biological sciences laboratory of Kyambogo university.

## 1.5 Significance

The plant kingdom is a potential of drugs. Drugs from the plants are easily available, less expensive, safe and efficient and rarely have side effects. Medicinal plants would be the best source to obtain variety of drugs. (Ali Boutlelis et al., 2019)

Antimicrobial resistance (AMR) represents one of the most concerning threats to global health and new anti-infectives are needed to overcome it.

AMR occurs when micro-organisms are able to survive in the presence of drugs that would normally inhibit their growth. It is estimated that 700,000 people currently die each year from AMR infections and this number is projected to reach 10 million annually by the year 2050.(Gul et al., 2012)

Plants represent a promising source of natural products in efforts to identify bio-active compounds. Plants used in traditional medicinal practices against infections have been found to inhibit growth and virulence of various microbes. Since *Datura stramonium* has been reported to be used in treatment of both bacterial and fungal infections. Therefore, this study will help in the investigation of antifungal activity of ethanolic crude leaf extract of *Datura* *stramonium* against *Tinea corporis*.(S et al., 2015)

# CHAPTER TWO

## 2.0 LITERATURE REVIEW.

## 2.1 Antimicrobial activity *Datura stramonium* plant leaf extract

*Datura stramonium* is also known as thorn apple, Jamestown weed or devil’s trumpet. It belongs to Kingdom: Plantae, Division: Magnoliophyta, Class: Magnoliopsida, Order: Solanales, Family: Solanaceae, Genus: Datura, Species: *Datura stramonium*. (Kaur, 2020)This weed produces flowers of white to creamy or violet colour. Generally, flower is of 2.5 to 3.5 in. long and rarely opens completely. (Kakande et al., 2019b) In Uganda this plant can be found anywhere from roadside to farmlands as well. Plants have provided a source of inspiration for novel drug compounds as plant have antimicrobial substances which have made significant contribution towards human health (Huda et al., 2015).

Continuing with the antimicrobial activities, *Datura stramonium* leaf extracts have been tested for antifungal activity and findings indicated a high significant inhibition of many harmful fungi. As shown in many experiments, the methanol extract of *Datura stramonium* has been the most efficacious, with the highest percentage of inhibition seen against fungi with radial growth (83.3%), though Similarly, Bawazeer and Rauf (2021) discovered that the chloroform fractions exhibited a promising antifungal activity against different fungi but *Candida albicans*, on the other hand, was resistant to all extracts tested. Furthermore, many study data have supported the use of *Datura stramonium* in traditional medicine to treat dermatitis of the hair, nails, and skin. The results of the ethanol extract of leaf of *D. metel* showed that at 100 % concentration there was significant (P ≤ 0.05) difference in inhibition in the fungal mycelia growth. The leaf extract at 100% ethanol concentration inhibited the fungal mycelial (2.20 ± 0.20). Akharaiyi (2011) had earlier reported that *D. metel* is a natural source of antioxidants and phytochemical having antimicrobial activities. This report agreed with the present findings whereby the leaf extracts significantly inhibited the mycelial growth of L. theobromae. This is consonance with the present result findings. Gachande and Khillare (2013) reported that water and ethanolic extracts of the *D. metel* showed good antimicrobial activities. They stated further those extracts of leaves had a better efficacy than stem and root. This is consistent with the present research findings where the ethanol leaf extracts of *D.* *metel* inhibited the mycelial growth of L. theobromae more than the ethanolic extract of the seeds. In another study the results of zone of inhibition of L. theobromae by ethanolic leaf extracts of *D. metel* compared with standard antibiotic (Ciprofloxacin) indicated that 100 % concentration of leaf extracts of *D. metel* exhibited high inhibitory effects on the test fungus with the standard antibiotic having diameter inhibition zone of 0.070 ± 0.08 cm and -0.50 ± 0.06 cm*. Datura* leaf extracts has provided mycelial growth inhibition of the fungi as studied, with the ethanolic extract being more effective in the control. Its antifungal potential was probably related to the plant’s chemical constitution, which has an abundance of alkaloids and phenolic compounds, related to the plant’s defence system against invasive organisms, that negatively affected the development of the vegetative mycelium.(öz arık, 2017b)

## 2.2 Minimum Inhibitory concentration.

Inhibition of fungi has been tested using aqueous extract of *Datura stramonium*. Many plants show antifungal activity against many pathogenic fungi. These plant contents alkaloids, phenols, steroids, tannins etc. as a chemical compound. The experiments carried out to check the effect of *Datura stramonium* against inhibition of fungi using aqueous extracts of leaf, stem, root and flower, has been tested under laboratory condition against fungi by hanging drop technique. Hexaconazole (0.05%) used as a standard check and distilled water as a control. Aqueous leaf extract (2% and 3%) showed superior inhibition of fungi than the extracts of root, stem and flower. Maximum inhibition was recorded 86.89 and 82.27% % over control in 2% and 3% leaf extract. Rest of the treatments showed better inhibition than the control. The *Datura stramonium* is a possible source of fungicide to manage many pathogenic fungi.(“ringworm\_11\_594k,” n.d.)

By agar Dilution method the Minimum Inhibitory concentration (MIC) of the fungal strains (“Ringworm,” n.d.)has been determined. The Minimum Inhibitory concentration (MIC) of chloroform and ethanol extracts against some fungi was 3.12mg/ml while of benzene extract was found as 6.25mg/ml. The Minimum Inhibitory concentration (MIC) of both benzene and ethanol extracts against fungi was 6.25mg/ml while of chloroform extract against the fungi was 12.5mg/ml. Comparative result of Minimum Inhibitory concentration (MIC) of *D.* *stramonium* leaf extracts against different fungi described that minimum concentration of 12.5mg/ml of all the three extracts is quite effective in the inhibition of fungal growth

# CHAPTER THREE

## 3.0 MATERIALS AND METHODS

## 3.1. Study Area and Research Design.

This research is going to be a laboratory experimental study involving isolation and identification of dermatophytosis causing fungus, Tinea *corporis*. Isolation and identification of dermatophytosis causing fungi will be done at biosciences Laboratory, Department of biological sciences. Plant samples (leaves) will be collected from bushes around Kyambogo university.

Extraction of leaf extract and antidermatophytic activity testing will be carried out at the biological science Laboratories at Department of biological sciences.

## 3.2 Sample Collection.

### 3.2.1 Fungal strains.

Reference fungal strains (*Tinea corporis*) will be obtained from the department of Biology or purchased with guidance from the technicians and supervisor.

Analysis will be conducted both in the chemistry and biology laboratories of Kyambogo university.

### 3.2.2 Plant Samples Collection.

Plant sample collection, identification, and drying will be done according to the method described by Esazah. The fresh leaves of *Datura stramonium* will be collected from bushes around Kyambogo university. The leaves will be dried under shade to avoid decomposition of volatile chemical constituents. The dried leaves were powdered using mortar and pestle, then sieved into fine powder, and stored in airtight jar(Tsamiya et al., 2015b)

### 3.2.3 Extraction of Ethanolic Crude Extract.

Extraction of the ethanolic crude extract will be carried out according the method described by Esazah with some modification. Two hundred grams (200 g) of powder will be soaked in ethanol (95% v/v) in a two-litre (2 l) conical flask, with periodic shaking for 24 hours. The extract will be filtered using Whatman No. 1 filter paper and concentrated (ethanol left to evaporate of) in hot air oven at 45∘ C. The concentrated crude extracts will be kept at 4∘ C in a refrigerator in a container and labelled appropriately. The yield (%, w/w) from dried leaves crude extract will calculated using the following formula: yield (%) = (W1 x 100)/W2, where W2 is the weight of powder before drying and W1 is the weight of dry extract.(Cornelius et al., n.d.)

## 3.3 Sampling Methods.

### 3.3.1 Screening for antimicrobial activity

Antimicrobial activity will be determined following agar well diffusion method. Pure isolates of microorganisms will be sub cultured on the medium recommended by the technician at 370C for 24 hours. An inoculum of the fungus will be spread onto petri dish.(Durgeshlal et al., 2019)

Three wells will be bored with a sterilized borer in the inoculated agar plate.

An equal volume of the extract, sterile distilled water and a positive control will be put each into the wells. The plates will be allowed to stand for 10 minutes for diffusion of the extract to take place and incubated at 370C for 24 hours.

Sterile distilled water will be used as a negative control.(Maheshwari et al., 2013)

The antifungal activity will be indicated by the formation of an inhibition zone (zone of clearance) surrounding the well containing the extract and a positive control. This will be measured in millimeters using a suitable measuring device.

### 3.3.2 Determination of Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration (MIC) fungal suspension concentration will be adjusted to an optical density of 0.014 equivalent to 1.0x105 spores/ml using a spectrophotometer at a wave length of 530 nm. MIC of *D. stramonium* leaves ethanolic crude extract will be determined using tube dilution method. One millilitre (1ml) of Brain Heart infusion Broth + 0.5% Agar will be placed in 10 tubes. (Bawazeer and Rauf, 2021)One millilitre (1 ml) of stock solution 2 g/ml will be transferred in the first test tube of organism concentration mixture and 2-fold serial dilution will be done till concentrations below 0.0156 g/ml will be attained. One millilitre (1ml) of positive control terbinafine (2 mg/ml) will be transferred in different sets of test tubes and also serially diluted (2-fold) using the same procedure as above.(Gul et al., 2012) Then twenty microlitres of inoculum preparation of the fungi will be placed in the above serially diluted tubes. One millilitre (1 ml) of 10% DMSO will be used as negative control. The test tubes will be then incubated at 28 to 30∘ C for 4-7days. The test tubes will be observed for the lowest concentration with no visible growth (no visual turbidity) and will be considered as the MIC.

### 3.3.3 Determination of Minimum Fungicidal Concentration (MFC).

Following the MIC determination using tube dilution method, MFC will be determined by subculturing twenty microlitres of the culture from each negative well(Kakande et al., 2019c) (with no visual turbidity) of both the extract and the positive control. MFC will be defined as the lowest concentration resulting in negative subcultures (no growth) after incubation of the plates at 28 to 30∘ C for 3-5 days.

## 3.4 Ethical Consideration.

Permission to carry out the study will be sought from Kyambogo university department of biological Sciences. Participation in the study will be on voluntary basis.

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N.d.

N.d.

# APPENDICES.

## Work Plan.

|  |  |  |
| --- | --- | --- |
| **SN** | **ACTIVITY** | **PERIOD** |
| 1. | Writing Research proposal and editing | January – February 2023 |
| 2. | Selection of research topic and presentation to departmental research committee | February 2023 |
| 3. | Liaise with a supervisor provided by research committee | February 2023 |
| 4. | Submission of research proposal to department of biological sciences | February 2023 |
| 5. | Liaise with local authorities of study area (Kyambogo) | February 2023 |
| 6. | Commencement of sampling and laboratory analysis | February-March 2023 |
| 7. | End of sampling and laboratory analysis | March – April 2023 |
| 8. | Editing of draft report helped by the supervisor | April 2023 |
| 10. | Binding edited report and submission to Department of Biological Sciences | April 2023 |

## ii) Budget

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Item** | **Unit cost** | **Total cost** |
| 1. | Laboratory consumables. | 200,000 | 200,000 |
| 2. | Sampling cost per lot. | 48000 | 48000 |
| 3. | Transport | 50,000 | 50,000. |
| 4 | Secretarial work | 80,000 | 80,000 |
| 5 | Communication (phone and internet) | 20,000 | 20,000 |
| 6. | Technical assistance | 100,000 | 100,000 |
| 7 | Miscellaneous | 100,000 | 100,000 |
|  | **GRAND TOTAL** |  | **526,000** |