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Phytochemical analysis and antifungal activity of *Conyza sumatrensis* root extract for management of ringworm infection

BY

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BU/UP/2021/1774

Research dissertation submitted to the department of chemistry in partial fulfillment of the requirements for the award of the degree of Bachelor of Science Education of Busitema University.

May, 2024

DECLARATION

I, WALAKILA FRANCIS WESWA declare that this research dissertation is my original work and it has not been submitted for award of a degree or professional qualification in any other institution of higher learning. Where other people's work was used, this has been acknowledged and cited according to the university policy.

Signature.....**Date**.....13/MAY/2024.....

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APPROVAL

This research dissertation has been submitted for examination with my approval as his university supervisor.

Signature.....**Date** ...13/5/2024

DR OWOR RICHARD ORIKO

DEDICATION

I would like to express my heartfelt gratitude and dedicate this research report to my loving parents, Mr. Weswa David and Mrs. Namono Sarah.

AKNOWLEDGEMENT

I deeply indebted in a special way to my supervisor, Dr Owor Richard Oriko for his tireless academic support and funding my research project. He has been my mentor throughout my course and has motivated me to take my further studies in natural products. Thank you, Doctor, may God bless you abundantly. Mr. Olowo mosses, Ms. Chepijira Mercy, Ms. Amado Mary, Dr Andima Mosses and who have been always on my side during my research project may God bless you and maintain the same heart that you showed to me.

I take this opportunity to thank all my colleagues of natural products research project (Chemusto Dan, Nangoli Cranimer, Akello Norah, Alowo Florence Abbo Margeret, and Wabuteya peter) who have been working hand in hand with me may God bless them abundantly my fellow future herbalist. Not forgetting the entire chemistry department which has imparted both knowledge and skills related to chemistry as a science subject. May God bless it to continue producing competent chemistry teachers for the incoming generation.

I appreciate my parents (WESWA DAVID AND NAMONO SARAH) for the wonderful support they have provided for my wellbeing while pursuing this course. It has been a real struggle but they have always been on my side.

Above all, I would like to thank the almighty God, for his divine knowledge, love, mercy, protection, health and divine direction from the time I was born up to date and how I pray that he remains in me forever amen. And also, to my parents Weswa David and Namono Sarah.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Ringworm causing a red ring rash is a common fungal infection of human skin. It is caused by a group of fungi like *Microsporum canls* and *Aspergillus niger* which affect people of all ages and it is mainly managed by antifungal agents. However, the current antifungal drugs are becoming ineffective because of antifungal resistance and alternatives medications derived from medicinal plants are being considered. *C. Sumantresis*, a plant with a long history of traditional use in the management of ringworm was the focus of this study with the aim of profiling its phytochemical and antifungal properties for management of ringworm. Alcoholic root extract of *C. Sumantresis* was subjected to different phytochemical analysis to investigate active constituents present, quantified with different standards whose calibration curves were used to determine the concentration of specific metabolites in the extract. Fungal culture was performed on *Aspergillus niger* to determine the antifungal activity of the extract. The results indicated that the extract had higher alkaloids, moderate flavonoids and relatively low tannins and phenols. Antifungal activity showed total inhibition of fungal growth at the concentration of 7mg/ml of the extract reflecting therapeutic properties of the extract. The findings of this study suggest that *C. Sumantresis* is a promising natural resource for the development of antifungal medications and warrant further investigation.

ACRONYMS

DCM: dichloromethane

FCS: flavonoid *C.sumatrensis*

PCS: Phenol *C. Sumantresis*

TCS: Tannin *C. Sumantresis*

ACS: Alkaloids *C. Sumantresis*

AE: aqueous extract

OE: organic extract

DMSO: Dimethyl sulfoxide

TA: Total alkaloids

TF: Total flavonoids

TP: Total phenol

TT: Total tannins

PDA: Potato dextrose agar

Chapter 1

INTRODUCTION

BACKGROUND

Ringworm, also known as dermatophytosis, is a common fungal infection of the skin causing loss of hair, and nails. Despite its name, it is not caused by a worm but by a group of fungi called dermatophytes.(Branscomb, 2005). Ringworm can affect people of all ages and can be transmitted through direct contact with an infected person or animal, or by sharing personal items such as clothing, towels, and combs (Sewhunegn Molla, 2005). The infection typically appears as a red, circular rash with raised edges and clear skin in the center(Powell, n.d 2014.), which may be itchy or scaly (Kumar, 2015).

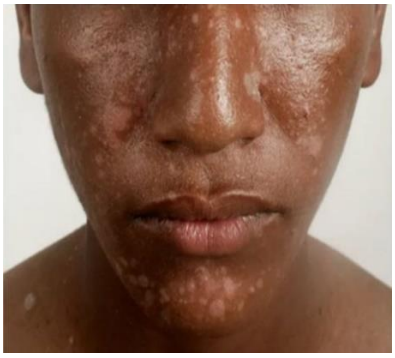


Figure 1:**skin ringworm infection.**

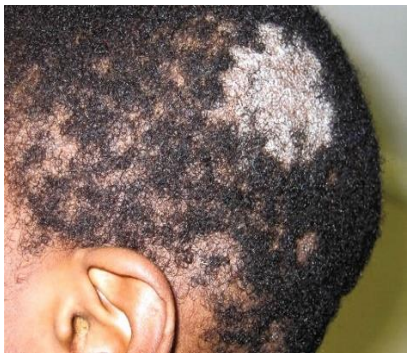


Figure 2:**hair ringworm infection.**

Ringworm can be treated with antifungal medications such as topical creams or oral pills. Proper hygiene practices and avoiding contact with infected individuals or animals can help prevent the spread of ringworm(Odhiambo, 2009).

The World Health Organization estimates that approximately 80 percent of the world's population relies primarily on traditional medicines as sources for their primary health care(Mazid et al., 2012). Medicinal plants are plants which contain in one of its organic substances that can be used for therapeutic or which are precursors for the synthesis of useful drugs (Bell, 2013). Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine (S Rashmi, 2011). Over 100 chemical substances that are considered to be important drugs that are either currently in use or have been widely used in one or more countries in the

world have been derived from several natural plants(David et al., 2015). Approximately 75 percent of these substances were discovered as a direct result of chemical studies focused on the isolation of active substances from plants used in traditional medicine(Njoku et al., 2011).

C. sumatrensis has been traditionally used for healing ringworms by applying its chlorophyll directly to the skin which cures within two to three weeks(Heinrich, 2003).However, its phytochemical profile has not yet been studied. By this approach, we hope to develop an effective but cheap and readily available antifungal product for use.

STATEMENT OF THE PROBLEM

A large number of people especially athletes are suffering from a common skin problem known as ringworm which has affected their beauty and handsomeness leading to a bad smell(Abdul & Al-Janabi, 2014). This is because majority of the people neglect it as not being a serious disease{Formatting Citation}. Once ringworm is left without treatment, it accumulates and affect inside the skin which leads to death. Several drugs like griseofulvin, Terbinafine, itraconazole and fluconazole have been used for treatment of ringworm. However, many of these antifungal drugs are becoming ineffective because of antifungal resistance but many of these drugs are expensive and rear(Mangwani & Vaishya, 2020).The leaves of *C.sumatrensis* have been traditionally used for their medicinal properties such as antifungal, antibacterial, antitoxic, antimicrobial and antioxidant effects (Zami, 2013). However, there is limited or no comprehensive information regarding their phytochemical profile and inadequate investigation more specifically about antifungal activity of the roots of *C. Sumatrensis*. Therefore, this study aims at Identifying and characterizing the specific bioactive compounds present in the roots of *C. sumatrensis*, determining its antifungal properties and formulation of herbal jelly hence management of antifungal infections (ringworm).

OBJECTIVES OF THE STUDY

1.1.1 General Objectives

To investigate the phytochemical compositions, antifungal activity and formulate the herbal jelly from root extract of *C. sumatrensis* management of ringworm

1.1.2

1.1.3 Specific objective

1. To extract and determine the phytochemical compositions of *C. sumatrensis*
2. To analyze the efficacy of antifungal activity in the root extract of *C. sumatrensis*.

JUSTIFICATION

Medicinal plants have been a rich source of therapeutic compounds, leading to the development of effective drugs (Azhar, 2021). For example, Quinine is derived from the bark of the cinchona tree for treatment of malaria, vincristine from the Madagascar periwinkle plant is used to treat childhood leukemia (Mbugua, 2012), Similarly, *C. Sumantresis*, with its diverse phytochemical composition, including alkaloids and flavonoids, is likely to possess therapeutic properties, making it a promising resource for developing new drugs to treat diseases like ringworm and other fungal infections.

SCOPE

This research was purely experimental with explorative studies on bioactive components of *C sumatrensis*. It was majorly focused on comparative analysis of antifungal components from the roots of *C sumatrensis* within Tororo.

Chapter 2 :

LITERATURE REVIEW

2.1.1 Description of *C sumatrensis*

C. sumatrensis commonly known as Singapore daisy or broadleaf fleabane is a dicotyledonous herb of the Asteraceae family occurring widely in Nigeria especially in the Niger Delta region and also in central Kenya. It is an erect, hairy, annual herb up to 120 cm high with sessile and deeply serrated leaves. The most prominent uses of it in Nigeria include treatment of stomach disorder and facial pimples. It also serves as a good source of food for the fowls. It is the success recorded in the treatment of facial pimples with extract from the leaves of *C. sumatrensis* that has prompted the investigation into the phytochemical constituents as well as the antimicrobial activities of the plant. It is a medicinal plant with a long history of traditional use. The leaves and stem bark of *C sumatrensis* have been of particular interest due to their potential bioactive properties (A Hamid, 2016). This literature review aims to provide a comprehensive overview of the phytochemical analysis and bioactive activity of the roots, leaves and stem bark of Conyza sumatrensis, as reported by various researchers.

Figure 3*: C. sumatrensis* plant Figure 4:Roots of *C. sumatrensis*

2.1.2 Phytochemistry of *C. sumatrensis*

Phytochemical analysis of the extracts from the leaves of the plant fleabane (*C sumatrensis*) revealed the presence of some substances such as tannins, flavonoids, saponins, steroids and glycosides. Number of compounds has been isolated from the plant including stigmasterol as illustrated below (Azhar, 2021).

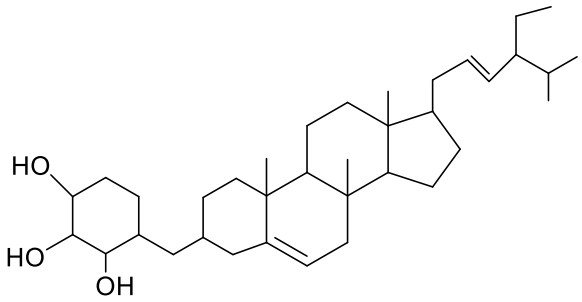
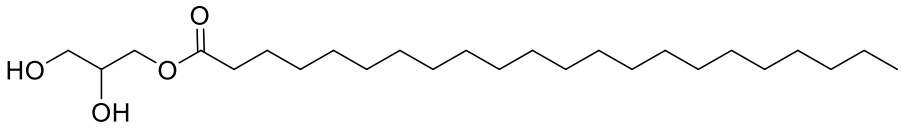


Figure 5: stigmasterol



2,3-dihydroxypropyl docosanoate

Figure 6: **2,3-dihydroxypropyl docosanoate**

2.1.3 Biological activities of cs

The presence of these substances is an indicator of the pharmacological property as well as the nutritive value of the plant leaves. Antimicrobial tests showed that the leaves extract is not sensitive towards the bacteria Pseudomonas aureginosa, staphylococcus aureus, Bacillus spp and Escherichia coli, but inhibited the growth of the fungus *Aspergillus niger* (Mbugua, 2012).

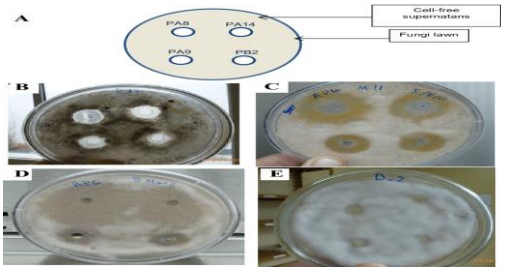


Figure 7: Antifungal Assy description

Preliminary phytochemical investigation of crude n-hexane, ethyl acetate and methanol extracts of the aerial parts of Conyza sumatrensis revealed the presence of anthraquinones, flavonoids, terpenoids, phenolics, tannin, glycosides and carbohydrate. All the crude extracts gave a clear zone of inhibition against the growth of the test bacteria (Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomona aeruginosa, Salmonella typhi, Klebsiella pneumonia at moderate to high concentrations, as well as test fungi (Candida albicans, Aspergillus niger, penicillium notatum and Rhizopus stolonifer) at high concentration.

This work focused on investigating the cytotoxic, phytotoxic and as well as the insecticidal activities of the extract and fractions of *C. sumatrensis.* However, the aqueous fraction was observed to give a higher phytotoxic activity as it gave complete inhibition at 20 and 30 mg/mL as well as 100 and 1000 µg/mL against the radicle of Sorghum bicolor and fronds of Lemna minor respectively. The DCM and aqueous fractions of the plant have expressed higher cytotoxic, phytotoxic and insecticidal activities over the crude extract, which is an indication of potent cytotoxic, phytotoxic and insecticidal compounds which require further investigation.

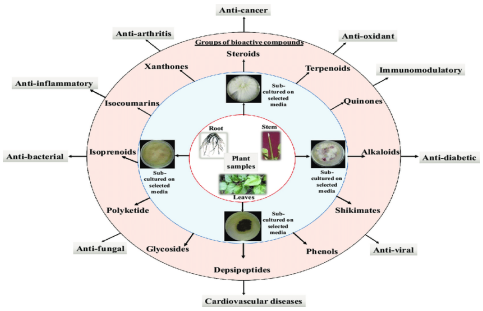


Figure 8:**Bioactive compounds and their activities**

These studies collectively highlight the diverse phytochemical composition and bioactive properties of the roots of Conyza sumatrensis. Further research is warranted to explore the phytochemical analysis and antifungal activity on roots of *C sumatrensis*and formulate herbal jelly for management of ringworm (Ikpefan,E O,UkwubileC.A,Nwankwo L U, 2020, p. 35)

Chapter 3 :

MATERIALS AND METHODS

PLANT MATERIAL

The roots of the plant which was identified by Mrs. CAROL KAWUMA a lecturer from biology department of Busitema university Nagongera campus as *C. sumatrensis* (Erigeron) was collected from its natural habitat from Namwaya Village alongside Nagongera-Tororo road in Tororo district (U). They were thoroughly washed with distilled water and dried under the shade until they were ready to crush. The dried roots were cut into small pieces using the knife and ground using an electric motor to obtain its powder (*C. sumatrensis* powder). The powder was stored in air tight container for further analysis.

EXTRACTION

3.1.1 Aqueous extraction (AE)

Using decoction technique, 25g of *C. sumatrensis* powder was mixed in 200ml of distilled water and the suspension obtained was boiled for about 2 hours.

After boiling, the mixture was cooled by placing container with its contents in cold water.

The mixture was filtered using the sieve to obtain a residue of suspended particles and a filtrate (African extract A)

3.1.2 ORGANIC EXTRACTION

This extract was obtained through adding 10g of *C. sumatrensis* powder to 100ml of 80% methanol in a conical flask and the mixture was shaken occasionally for about 12 hours using an electric shaking machine.

The mixture was filtered through 110 mm Whatman filter paper and then evaporated to yield a crude extract (African extract O)

Photochemical analysis

The phytochemical analysis of the plant extracts was carried out by the standard methods provided(Siddiqui, 2021).

3.1.3 Test for tannins

(a) 1cm3 of freshly prepared 10% KOH was added to 1cm3 of each of the extracts and observed for dirty white precipitate.

(b) 2 drops of 5% Fe Cl3 was added to 1cm3 of each extract and observed for green precipitate.

3.1.4 Test for phenolic compounds

2 ml of the extract of C.S solution was mixed with ferric chloride solution. Formation of dark blue colour shows the presence of phenolic compounds.

3.1.5 Test for saponins

2 cm3 of each extract in a test tube was vigorously shaken for two minutes and observed for persistent foaming.

3.1.6 Test for Flavonoids

To 3cm3 of each extract was added 1cm3 NaOH and observed for yellow colouration.

3.1.7 Salkowski's for test steroids

5 drops of concentrated H2SO4 were added to 1cm3 of each extract and observed for red colouration.

3.1.8 Fehling's test for glycosides

10 cm3 of 50% H2SO4 was added to 1 cm3 of the extract in a test tube. The mixture was heated in a boiling water-bath for 15 minutes. 10 cm3 of Fehling's solution was then added and the mixture was boiled and observed for brick red precipitate.

3.1.9 Test for alkaloids

1 cm3 of HCl was added to 3 cm3 of each extract in a test tube. The mixture was heated for 20 minutes, cooled and filtered. 2 drops of Wagner's reagent were added to 1 cm3 of the filtrate and observed for reddish brown precipitate.

3.1.10 Test for triterpenoids

Salkowski test; 200mg of plant material was added to 2ml of chloroform with few drops of conc.H2SO4. The solution slowly turns to red, indicating the presence of triterpenes.

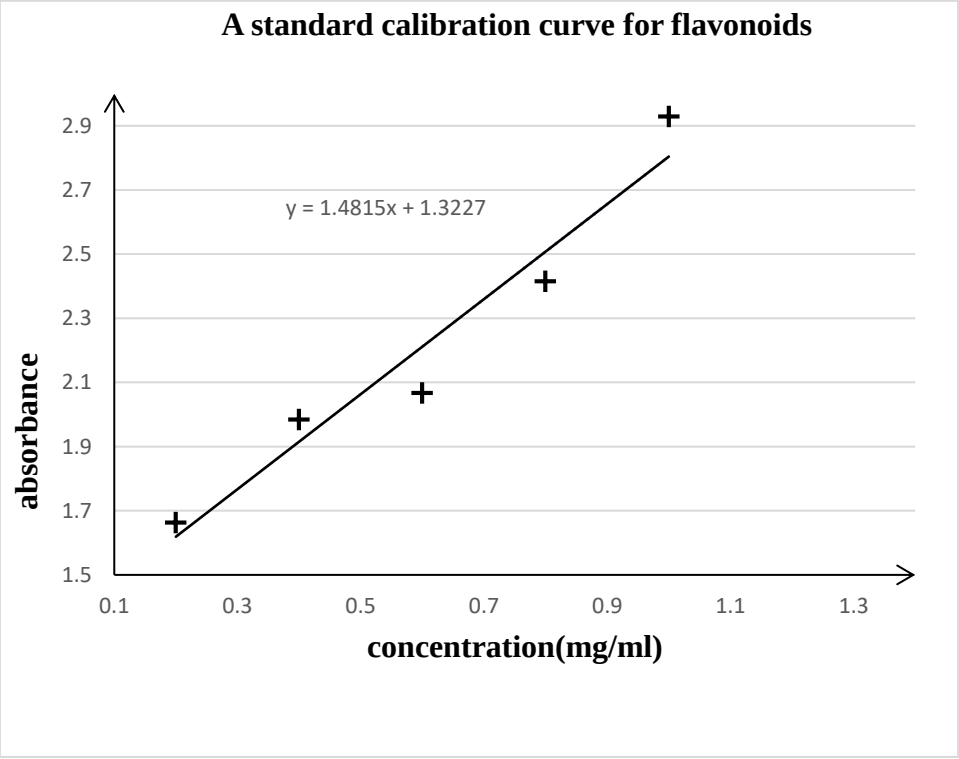
TOTAL QUANTITATIVE ANALYSIS

This was majorly focused on determining the concentration of the compounds in the extract of *C sumatrensis*

3.1.11 Total flavonoid content

The total flavonoid content in the roots of *C sumatrensis* was determined by Aluminium chloride spectrophotometric method. Apigenin was used as a standard and flavonoid content was determined as Apigenin equivalent from the standard Apigenin solution at the concentrations of 0.2 ,0.4 ,0.6 ,0.8 and 1.0 µg/ml that was prepared in methanol.

100 µl of each of the apigenin dilutions was mixed with 1500 µl of distilled water followed by 100µl of 5% sodium nitrate solution and allowed to stand for 6min. Then 150 µl of 10% aluminium chloride solution was added and then allowed to stand for 5min after which 200 µl of 1M sodium hydroxide solution was added. The absorbance of this reaction mixture was measured at λmax 420nm using a single beam UV-VIS spectrophotometer (6705 JENWAY). The same procedure was repeated for petroleum ether extract solution of *C sumatrensis*. All the measurements were performed in triplicate for each analysis. The total flavonoid content was determined from the linear equation of a standard curve prepared with apigenin and expressed as mg/g Apigenin equivalent (AE) of *C sumatrensis*



$$Total\ Flavonoids\ (\text{mg/g of the extract}) = \frac{x * DF * vol\ of\ 80\%\ methanol * volume\ added}{weight\ of\ the\ sample}$$

Where X = concentration of the extract in mg/ml obtained from the calibration curve

3.1.12 Total phenol content

Folin-Ciocalteau method was used to estimate the total phenol content in roots of *C. sumatrensis*. In this method, a petroleum ether solution of the extract (1000 µg/ml) was added to 2500 µg of 10% Folin-Ciocalteau reagent dissolved in water and 2500 µg of 7.5% sodium carbonate. A blank was similarly prepared containing 500 µg methanol, 2500 µg of 10% Folin-Ciocalteau reagent dissolved in water and 2500 µg of 7.5% sodium carbonate. The samples were incubated in a thermostat at 45^0C for 45 minutes and the absorbance was determined using spectrophotometer at λmax 292nm using a single beam UV-VIS spectrophotometer (6705 JENWAY). The same procedure was repeated for the standard solution of propyl gallate. The calibration curve was constructed using standard propyl gallate solution prepared at concentrations of,62.50, 125.00, 250.00 ,500 and 1000 µg/ml. The concentration of phenols was read (µg/ml) from the calibration curve. Thereafter total phenolic content in the plant was expressed in terms of propyl gallate equivalent (mg of PGA/mg of extract). All the measurements were evaluated in triplicate.

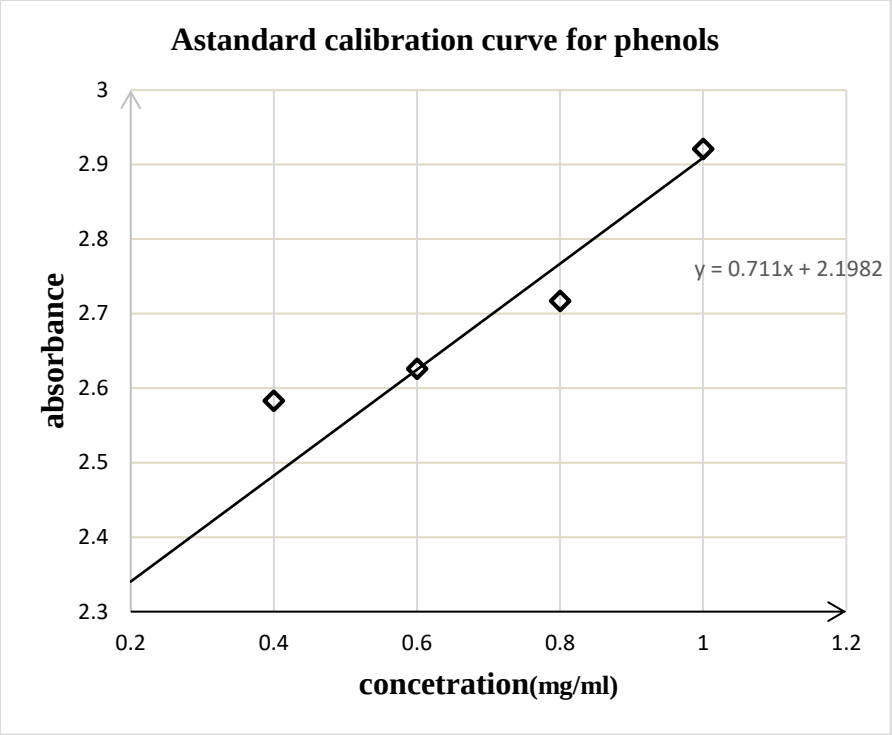


Figure 9: **A standard calibration curve for phenols**

$$Total\ phenols\ (\text{mg/g of the extract}) = \frac{x * DF * vol\ of\ 80\%\ methanol * volume\ added}{weight\ of\ the\ sample}$$

Where X = concentration of the extract in mg/ml obtained from the calibration curve

3.1.13 Total tannins

100 µL of 10 mg/mL extracts was added to a clean test tube containing 7.5mL distilled water. The Folin-Ciocalteau reagent (500 µL) was added to the mixture and vortexed thoroughly. 10mL of 35% solution of sodium carbonate ($NaCO_3$) was added to the mixture. The mixture in the test tube was transferred to 10 mL volumetric flask and the volume of the mixture made up to 10mL with distilled water. The mixture was shaken and kept at ambient temperature for 30 minutes in the dark. Tannic acid was used as a standard and reference standard solutions (31.3- 500mg/ml) was prepared. The absorbance of the solution was measured at 650nm against a blank reagent a single beam UV-VIS spectrophotometer (6705 JENWAY). The calibration curve was constructed using standard tannic acid solution prepared at concentrations of (31.3, 62.5, 125.0, 250.0 and 500mg/ml). The concentration of phenols was read (µg/ml) from the calibration curve. All the measurements were evaluated in triplicate.

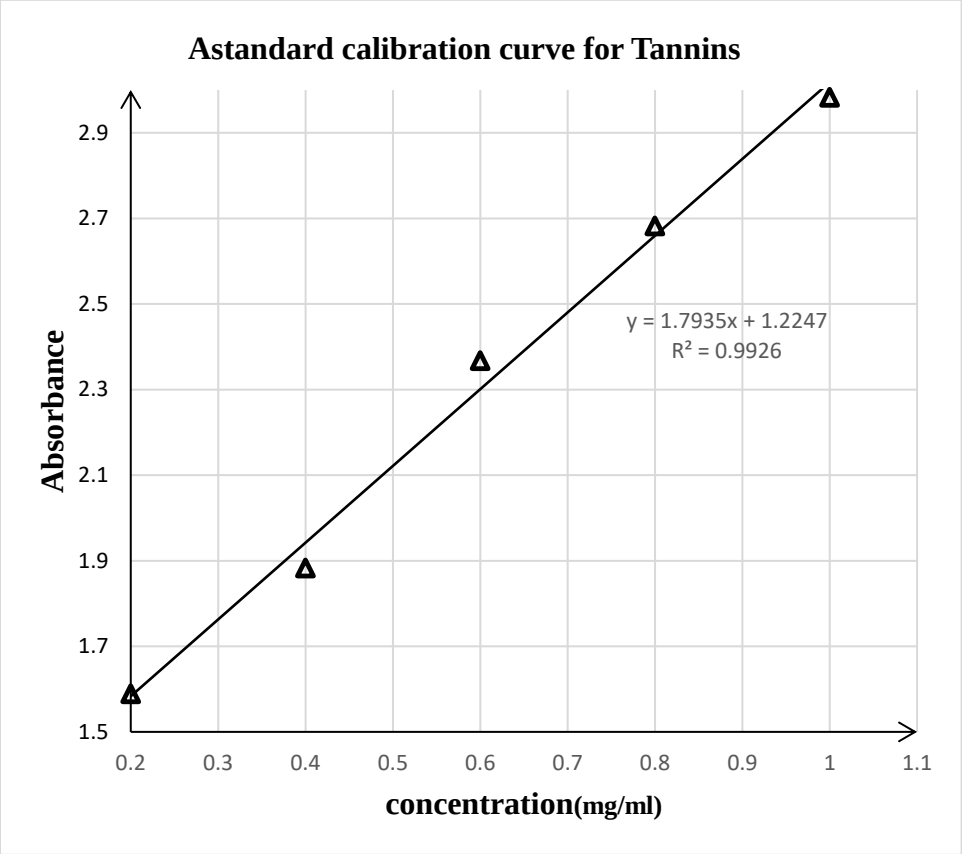


Figure 10: **A standard calibration curve for Tannins**

$$Total\ Tannins\ (\text{mg/g of the extract}) = \frac{x * DF * vol\ of\ 80\%\ methanol * volume\ added}{weight\ of\ the\ sample}$$

3.1.14 Total alkaloid content

A solution of 1 mg/mL of plant extract was prepared using dimethyl sulfoxide (DMSO). 1 mL of 2 M HCl is added to 1 mL of DMSO dissolved extracts and the resulting mixture is filtered using filter paper. The filtrate was transferred to a 250 mL separating funnel and to this solution, 5 mL of 0.1% Bromocresol green (dissolved in methanol) was added followed by 5 mL of phosphate buffer (pH 6.6). Chloroform (1 mL) was added into the separating funnel and the mixture was vigorously shaken, after which the funnel was allowed to stand to allow the mixture to separate into different layers. The lower layer is collected in a 10 mL volumetric flask. The process was

repeated with 2, 3, and 4 mL of chloroform. Atropine was used to construct a standard curve using a concentration range of 1.0–0.0625 mg/ml. The absorbance of the sample and standard solutions was recorded at a wavelength of 292nm against a reagent blank. The total alkaloid content was expressed as milligram atropine equivalent/ gram of extract (mg AE/g). All the measurements were evaluated in triplicate.

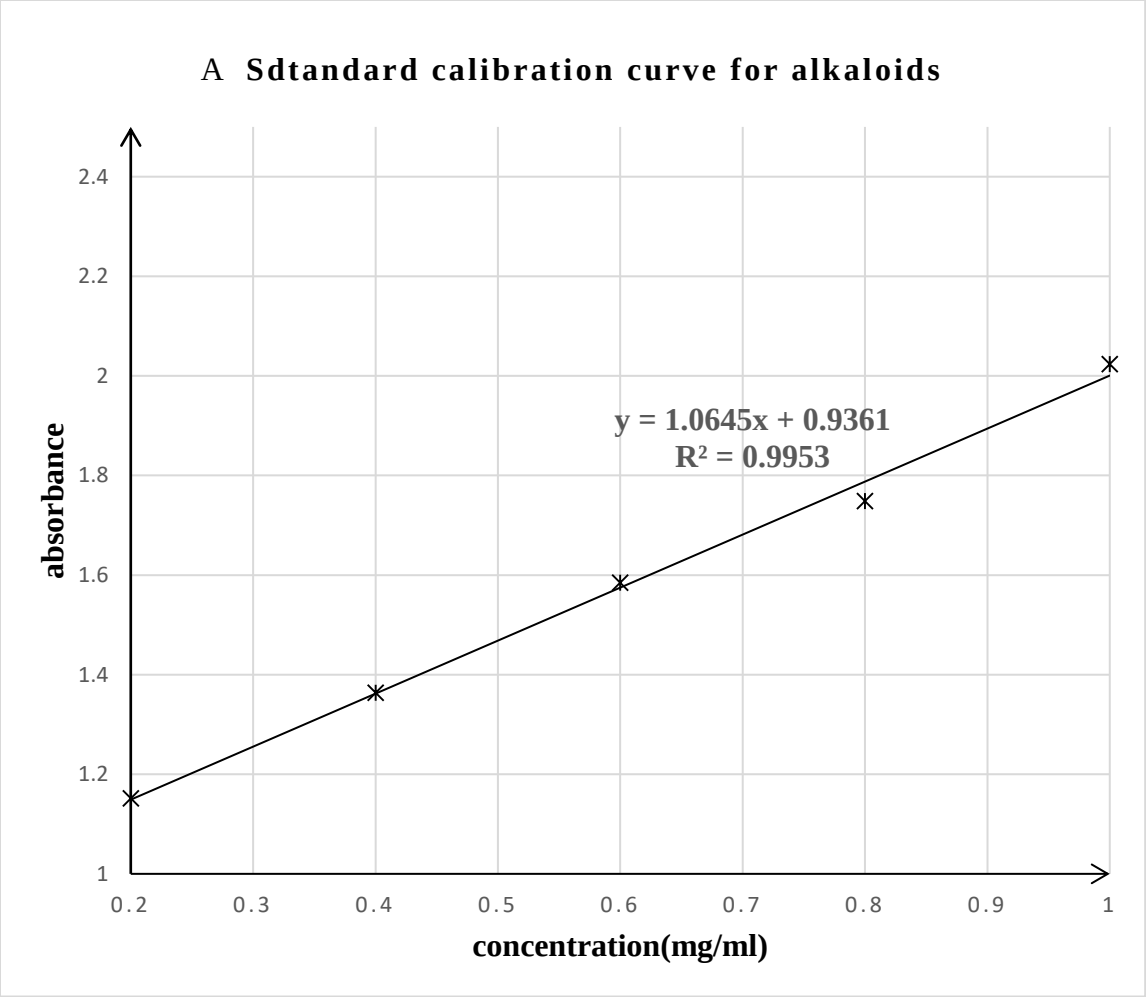


Figure 11: A **Standard calibration curve for alkaloids**

At wave length of 292nm, the average absorbance of the sample prepared

$$Total\ alkoloids = \frac{x * DF * vol\ of\ 80\%\ methanol * volume\ added}{weight\ of\ the\ sample}$$

Antifungal activity test

After identifying and quantifying the compounds of interest in the extract of *C. sumatrensis* the fungal culture was prepared by placing *Aspergillus niger* fungi on the mixture of potato dextrose agar and a small quantity of known concentrations of an organic extract of C. *sumatrensis* solidified in pedridishes. The aspergillus Niger fungi was inoculated at each center of each plate. The agar plates were incubated at a suitable condition(body temperature of 36 degrees Celsius) for three days and the fungal inhibition was observed.(Odhiambo, 2009)


(S Rashmi, 2011)

Figure 13:innoculation process

Chapter 4 RESULTS AND DISCUSSION

Phytochemical screening

The study aimed at identify the phytochemicals present in CS root extract. The results shows that alkaloids, phenols and flavonoids are present in sufficient quantities as shown in the Table 1. Which correlates with the results of research (S Rashmi, 2011)

Table 1:Results of selected phytochemical of organic extract of *C. sumatrensis*

Phytochemical	**Test**	**Quantity present**	Quantified amount (mg/g of crude extract)
Alkaloids	Wagner's test	+++	132.0
Tannins	Ferric chloride test	+	1.2
Flavonoids	Ferric chloride test	++	54.6
Saponins	Water test	+	Nd
Steroids	Chloroform and water test	++	Nd
Terpenoids	Salkowski test	-	Nd
Phenols	Ferric chloride test	+++	27.9
Glycosides	Sulphuric acid test	++	Nd

Key: strongly present +++, moderately present ++, weakly present +, absent -, Nd= not determined

Antifungal activity

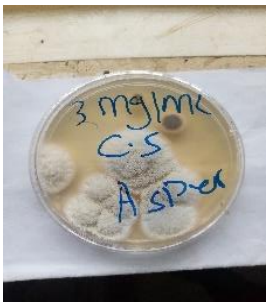


Figure 14:control experiment Figure 15:2mg/ml of *C. sumatrensis* Figure 16:3mg/ml *C. sumatrensis*

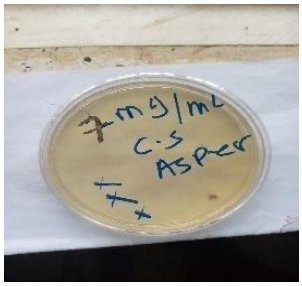


Figure 17:5mg/ml C. Sumatrensis Figure 18:7mg/ml C. sumatrensis

The presence of alkaloids, saponins, glycosides, tannins, flavonoids, and phenols in the extract suggests a potential for antifungal activity, which is supported by the inhibition of fungal growth at a concentration of 7mg/ml of the crude extract.

The control experiment showed maximum growth of fungi and sporing as evidenced in figure7 The remaining plates showed fungal inhibition depending on the concentration of the crude extract of ***C. Sumatrensis*** applied and where the fungi were able to grow didn't show any spores as seen in figure8, figure9 andfigure10.

The plate with the highest concentration (7mg/ml) showed total inhibition where there was no any fungal growth as shown in figure11 and this proved to be the best concentration to use formulation of the herbal jelly for management of ringworm.

This antifungal activity could be effective in treating ringworm, a fungal infection caused by dermatophytes, by inhibiting the growth of fungal cells, disrupting fungal cell membranes, and

interfering with fungal metabolism, nutrient uptake and stopping fungal reproduction through hindering it from sporing. The high levels of alkaloids and moderate levels of flavonoids in *C. Sumatrensis* may contribute to its antifungal properties, making it a potential natural remedy for treating ringworm infections.

Chapter 5 CONCLUSSION AND RECOMMENDATION.

CONCLUSSION

The findings of this study indicate that ***C. Sumatrensis*** is rich in various phytochemicals like flavonoids, alkaloids, phenols, tannins, saponins, and glycosides, which supports its traditional use in medicine, suggesting that these bioactive compounds may be responsible for its therapeutic properties and medicinal value. ***C. Sumatrensis*** exhibits antifungal properties, as evidenced by the inhibition of fungal growth at a concentration of 7mg/ml of the crude extract. However, the exact mechanism by which this occurs remains unknown and requires further investigation to determine the specific bioactive compounds responsible for this antifungal activity and to fully understand its mode of action.

RECOMMENDATION

This research recommends further studies to find the exact mechanism which occurs through further investigation to determine the specific bioactive compounds responsible for this antifungal activity and to fully understand its mode of action. It is also recommended that *C Sumantresis*. can be used to formulate an effective herbal jelly for managing ringworm

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