ANTIFUNGAL ACTIVITIES AND PHYTOCHEMICAL ANALYSIS OF *LAWSONIA INERMIS, SECURIDACA LONGIPEDUNCULATA* AND *ENANTIA CHLORANTHA* EXTRACTS ON SELECTED SPECIES OF DERMATOPHYTES

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MINNA

NOVEMBER, 2021

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A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA, NIGERIA IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF TECHNOLOGY IN MICROBIOLOGY

NOVEMBER, 2021

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ABBREVIATIONS

Acronyms	Meaning
MIC	Minimum inhibitory concentration
MBC	Minimum bactericidal concentration
GC-MS	Gas chromatograph interfaced to a mass spectrometer
BP	Base pair
PCR	Polymerase chain reaction
DMSO	Dimethyl sulfoxide
mm	Millimetre
CLSI	Clinical and Laboratory Standards Institute
DNA	Deoxyribonucleic acid
UZM1	*Trichophyton rubrum*
UZM2	*Trichophyton schoenleinii*
WDP	Weight of dry powder
WE	Weight of extract
PY	Percentage yield
g	Gram
LLI	Leaves of *Lawsonia inermis*,
SEC	Stem bark extract of *Enantia chlorantha*,
SSL	Stem extract of *Securidaca longipedunculata*
ND	Not Detected
mg/mL	Milligram per millilitre
WDE	Weight of dry Extract
A	Residual aqueous fraction
E	Ethyl acetate fraction
H	n-Hexane fraction
R-T	Retention Time
M.F	Molecular formula
M-W	Molecular Weight,
% Conc.	Percentage Concentration

ABSTRACT

Medicinal plants are effective source of both modern and traditional medicine. This study was aimed to determine the Antifungal activities and phytochemical analysis of *Lawsonia inermis, Securidaca longipedunculata Enantia chlorantha* extracts on some dermatophytes. The dermatophyte isolates used in this research were *Trichophyton rubrum, Trichophyton schoenleinni* and *Microsporum audouinii* and the plant material used for the study were *lawsonia inermis*, *Enantia chlorantha* and *S. ecuridaca longipedunculata.* The isolates were identified, characterized and confirmed using cultural, morphological and molecular techniques while the plants were extracted using cold maceration technique and further partitioned into n-Hexane, ethyl acetate and residual aqueous. The mean zones of inhibition of the extract revealed a significant ($p<0.05$) spectrum of activity against fungal isolates of *Lawsonia inermis* 10.05, 13.15, 13.20,13.3 and 16.65. *Enantia chlorantha* 11.55, 12.00, 13.00, 14.60, 16.90 and 18.15 and *S.ecuridaca longipedunculata* 10.85, 12.10,13.10, 13.80,14.10 and 14.80. The Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) showed at 16.65±0.35 mm at 150 mg/ml concentration and the least at 7.81 mg/ml concentration and MFC showed at 15.60 mg/ml was the least and 300 mg/ml was the highest. The GC-MS analysis of the fraction revealed 8 compounds for *L. inermis* with 9,12-octadecadienoic acid (z,z) (ethyl acetate fraction at 23.49%) and 5-hydroxymethylfurfural (residual aqueous fraction at 42.72%) with the highest peak area respectively, 3 compounds for *E. chlorantha* with n-hexadecanoidic acid (ethyl acetate fraction at 15.05%) and 1,5-anhydro-d-mannitol(residual aqueous fraction at 93.10%) with the highest peak area respectively and 6 compounds for *S. longipedunculata* with 9,12-octadecadienoic acid (z,z) (ethyl acetate fraction at 28.05%) had the highest peak area respectively. Quantitative phytochemical analysis activity of the crude and fractions were carried out using standard methods. The phytoconstituents of the fractions were determined using Gas Chromatography Mass Spectrophotometer (GC-MS). The extract contains phenols, flavonoids, tannins, alkaloids and saponins at varying concentrations *Lawsonia inermis* (822.11 mg/100g, 89.84 mg/100g, 181.95 mg/100g, 49.90 mg/100g and 346.83mg/100g respectively), *Enantia chlorantha* (416.07 mg/100g, 26.09 mg/100g, 26.45 mg/100g, 20.67 mg/100g and 117.86 mg/100g respectively) and *Sacuridaca longipedunculata* (509.68 mg/100g, 62.21 mg/100g, 87.75 mg/100g, 380.92 mg/100g and 35.28 mg/100g respectively). The study revealed that the plant material could be a good candidate for use in the management of dermatophyte, although further evaluation needs to be carried out in other to fully validate their usage.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Plants are the major source of drugs use by man for thousands of years (Ngulde *et al.*, 2019). Plants used in ethno-medicine are often used as guide to screen for an activity or to confirm the claim by the natives. Compounds with reputed activity are subjected to further scrutiny that could eventually lead to drug development. In addition, knowledge of the possible mode of action could give a scientific rationale for integration of complementary medicine into orthodox medicine (Tijjani *et al*., 2018). Medicinal plants are obtained exclusively from vegetal raw material; its efficacy and safety are validated by ethno pharmacological studies on traditional use, technical and scientific documentation (Carmon and Pereira, 2013).

Medicinal plants are being widely used, either as single drug or in combination with orthodox in health care delivery system (Borade *et al.*, 2011). These medicinal plants have been used widely around the earth to treat several disease and ailments (Gozubuyuk *et al.*, 2014). There is a popular believe among humans that green medicines are healthier and more harmless or safer than synthetic medicine (Gozubuyuk *et al*., 2014). Medicinal plants are in great demand in the developed as well as developing countries for primary healthcare because of their wide biological activities, higher safety margins and lesser costs (Gupta *et al.*, 2014).

Many microorganisms show some level of pathogenicity towards human, plants and animals which cause various diseases (Sharma *et al*., 2016). Orthodox medicines are used to treat these diseases, but due to inappropriate use of these medicines, pathogenic

microorganisms are developing resistance to the medicines and increasing the public health problems (Sharma *et al*., 2016).

Fungi are eukaryote that digests food externally and absorbs nutrients directly through its cell walls. Most fungi reproduce by spores and have a body (thallus) composed of microscopic tubular cells called hyphae (Warnock, 2017). Fungi are heterotrophs and, like animals, obtain their carbon and energy from other organisms (Brandt and Warnock, 2015). Some fungi obtain their nutrients from a living host (plant or animal) and are called biotrophs while others obtain their nutrients from dead plants or animals and are called saprotrophs (saprophytes, saprobes). Some fungi infect a living host and kill the host cells in order to obtain their nutrients; these are called necrotrophs (Carris *et al.,* 2012). Fungi are classified morphologically rather than physiologically (Warnock, 2017). There are 19387 species in 995 genera, 115 families, 39 orders and eight classes (Lucking *et al*, 2017). Fungi can be classified by method of reproduction as Zygomycetes, Basidomycetes, Ascomycetes and Deuteromycetes. Morphologically, it can be classified as Yeasts, Yeast like fungi, Molds and Dimorphic fungi (Brandt and Warnock, 2015).

Lawsonia inermis belongs to the family *lynthraceae*, the plant is commonly called henna. It is also known with various names across the world; in English (henna, samphire), Sanskrit (mendhi, mendika), Arabic (alhenna, hinna), French (hennè, alcana d'orient), Hindi (hena, mhindi) Hausa (lalle, kunshi) (Gozubuyuk, *et al*., 2014).

Lawsonia inermis (Henna) is a shrub or small tree cultivated in many regions as an ornamental and commercial dye crop (Mohammed *et al*., 2016). Leaves of this plant are 1.3-3.2 cm broadly or elliptic lanceolate (Sharma *et al.,* 2016). It is mostly found in the tropic, sub-tropic, and semi-arid zones of Africa (tropical Savannah and tropical arid zones), south Asia, and north Australia (Mohammed *et al*., 2016). Henna leaf paste has

an orange-red dye which is used to decorate hands, nails, feet and even dye hair around the world mostly during weddings, traditional or religious festivals. These leaves are used externally for skin infections or disease (such as dermatophytes, a group of fungi adapted to human and animal skin), headache, burning feet and menstrual cramps. Internally in form of decoction for sore throat (gargle), jaundice, spleen enlargement, calculus and leprosy (Gozubuyuk, *et al*., 2014).

Enantia chlorantha is commonly known as African whitewood, Moambe Jaune and Annikia chlorantha, in Yoruba, it is known as Awopa or Osu pupa (Ajani and Ibrahim, 2020). It is an ornamental tree found in the rainforests of Nigeria, Liberia, and Cote d‟ Ivoire, *Enantia Chlorantha* is a fair sized ornamental forest tree. It grows in dense shade and may be recognized by its bright yellow slash and conspicuous black fruits. It is located in the West African region and extends from southern Nigeria to Gabon, Zaire and Angola (Abike *et al.,* 2020). The tree grows to about 30m high with dense foliage and spreading crown with fluted stem which produces a sulphurous yellow dye (Atata *et al.*, 2003).

It is used locally across Africa to make unpainted furniture and veneers. In Nigeria, traditionally it is used in the treatment of several ailments such as malaria and typhoid (Chukunda *et al*., 2020), ulcers and leprous spots for quick healing, decoction is used for washing wounds, bark sap is taken as decoction against diarrhoea (Atata *et al.,* 2003).

Securidaca longipedunculata Fresen belongs to the family *Polygalaceae*, it is commonly known as violet tree and locally called Sanya or Uwar maganguna (Hausa), Ezeogwu (Igbo) and Alali (Fulani) (Ngulde *et al.,* 2019). The plant is a shrub with twisted bole or slender erect branches and grows up to 30 feet high, that grows in savannah vegetation (Tijjani *et al.,* 2018). The plant is ethno medicinally used as a remedy for numerous human ailments; the stem bark is used to treat epilepsy, skin diseases, dysentery, malaria,

typhoid, constipation, snake bites, infertility problems, flu, coughs, fever, used as sexual boost, contraceptive and abortion purposes. Other uses of the root extract are for the treatment of toothache, tuberculosis, rheumatism, pneumonia and as blood purifier (Namadina *et al.,* 2020).

1.2 Statement of the Research Problems

Fungal infections are one of the most prevalent cutaneous infections globally. Antifungal agents are difficult to formulate due to the similarities in cellular structure of fungi with humans and animals (Warnock, 2017). Antifungal agents such as griseofulvin, azole derivatives and allylamine which are used as therapeutic options are less useful in the treatment of dermatophytosis as a result of the development of fungal resistance, long treatment duration and the presence of therapeutic side effects, so additional options of antifungal therapy with therapeutic potential are needed (Erza *et al.,* 2020). The rise in the resistance not only impedes the ability treat the fungal infection in human and animals which has broader economic effects these effects include severe adverse reactions, multi drug resistance, high cost and severe infections with no therapeutic options (Woon and Fisher, 2016). Hence it is important to search for alternative antifungal agent that can counter these problems with suitable antifungal agents.

1.3 Aim and Objectives of the Study

The aim of this study was to evaluate the antifungal activities and phytochemical analysis of *Lawsonia inermis, Securidaca longipedunculata* and *Enantia chlorantha* extract on some selected species of dermatophytes.

The objectives of the study were to:

i. isolate and identify fungi from different from infected skin

ii. extract plant material from *Lawsonia inermis* leaves, *Enantia chlorantha* stem bark and *Securidaca longipedunculata* stem using methanol

iii. determine the fungal molecular characterization

iv. determine the antifungal activities of the plants (leaves and stem bark) extracts

v. determine the minimum inhibitory concentrations (MIC)

vi. determine the minimum fungicidal concentrations (MFC)

vii. fractionate the crude extract from (ii) above

viii. determine the compounds present in the purified fractions using gas chromatography- mass spectrophotometry (GC-MS) analysis.

ix. determine the qualitative and quantitative phytochemical constituents of the plants.

1.4 Justification for the Study

Because of the issue of resistance, cost, inaccessibility, and bioaccumulation of harmful substances from the persistent or over utilization of the conventional drugs, consideration is currently turning towards nature to look for lead compounds with better therapeutic potentials, modest, less harmfulness and promptly accessible for use (Innalegwu *et al.,* 2021). These plants are from natural sources; they are eco-friendly and have found application as biological control agents. This necessitate the need to re-evaluate this plants that have been studied previously but due to the difference in ecosystem and geographical location this might be responsible for the difference in its chemical composition.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Research Plants

2.1.1 *Lawsonia inermis*

Lawsonia inermis Linn. (Henna), belonging to the *Lythraceae* family, known as is a widespread medicinal plant and natural dye in the world. *Lawsonia inermis* is also known as Henna, Mhendi, Shudi, Madurang, Mendi, Manghati, Madayantika, and Goranti. The henna plant is a glabrous, much branched shrub or quite a small tree with greyish-brown bark. Leaves are opposite, subsessile, elliptic, or broadly lanceolate, entire, acute or obtuse, 2 to 3 cm long and 1 to 2 cm wide as observed in Figure 1 at the appendices. Lawsone is the chief constituent responsible for the dying properties of the plant. Dried powdered leaves of henna contain about 0.5% to 1.5% lawsone, traditionally used to produce colour fast orange, red, and brown dyes. *Lawsonia inermis* is reported to contain carbohydrates, proteins, flavonoids, tannins and phenolic compounds, alkaloids, terpenoids, quinones, coumarins, xanthones, and fatty acids. The plant has been reported to have analgesic, hypoglycemic, hepatoprotective, immunostimulant, anti-inflammatory, antibacterial, antimicrobial, antifungal, antiviral, antiparasitic, antitrypanosomal, antidermatophytic, antioxidant, antifertility, tuberculostatic, and anticancer properties (Davood *et al.*, 2018).

2.1.2 Botanical description of *Lawsonia inermis*

The plant is multi branched, deciduous shrub or small tree having 2.6 m height. Leaves of this plant are 1.3-3.2cm broadly or elliptic lanceolate. Flowers are white or rose colour, which are used as a fragrant agent in local scent. Pedicle is short less than 1.3 cm, numerous in number and slender in shape. Calyx is 3-5 mm, long broadly campanulate; lobes are 2.5-3 mm, long, suborbicular or subreniform and undulate. Stamens are 8, which are inserted in pairs on the calyx-tube. Capsules are slightly veined outside, globose and

diameter of the capsule is 6 mm. Persistent calyx support capsule with the tipped style. Pea shape and globose seed capsules which are red in colour. Seeds are brown pitted, numerous small and pyramidal in shape (Sharma *et al*., 2016)

2.1.3 Botanical classification of *Lawsonia inermis*

Kingdom: *Plantae* Subkingdom: *Viridiplantae*, Infrakingdom: *Streptophyta*, Superdivision: *Embryophyta*, Division: *Tracheophyta*, Subdivision: *Spermatophytina*, Class:*Magnoliopsida*, Superorder: *Rosanae*, Order: *Myrtales*, Family: *Lythraceae*, Genus: *Lawsonia*, Species: *inermis* (Al-Snafi, 2019).

2.1.4 Plant habitat and distribution of *Lawsonia inermis*

Lawsonia inermis is mainly cultivated for cosmetic purposes and as traditional medicine in all over the world but native place of this plant is tropical as well as subtropical regions mainly India, Sri Lanka the Middle East and other sub Saharan regions. The plantis known as laali in Nupe, in Yoruba as lali and in Igbo as uli, the plant leaves of this plant are used as dye which stains the hair, hands and feet mainly in Asian countries (Sharma *et al*., 2016). A native of North Africa and Southwest Asia, the plant is now widely cultivated throughout the world as an ornamental and dye plant (Davood *et al.*, 2018).

2.1.5 Medicinal uses of *Lawsonia inermis*

Based on the clinical trial carried out by Davood *et al.* (2018) found out that henna leaves is effective in preventing decubitus ulcers which is a local damage to skin or its underlying tissues that usually occur around bony prominences due to decubitus or a combination of decubitus and friction of sliding forces.

Henna is believed as a medicinal plant, because of its antibacterial effects especially on gram positive bacteria, antifungal activity against dermatophytes, wound healing,

antitumor effects, hypotensive, astringent and sedative effects, its paste has cooling effect hence use in treatment of fever (Yigit, 2017).

Al Saif (2016), summarizes the effects attributed to henna regarding its non-dying properties including pharmacological properties (such as its antimicrobial activity, antioxidant activity, anti-inflammatory, analgesic and antipyretic activity, immunomodulatory activity, wound healing activity, anti-carcinogenic and cytotoxic activity and hypoglycemic and hypolipidemic activity) and side effects ranging from Allergic contact dermatitis, erythema multiforme, immediate-type hypersensitivity with urticarial, post-inflammatory hypo or hyperpigmentation, hypertrichosis, keloids, hemolysis, vasculitis and renal impairment and teratogenicity.

According to a research conducted by Kavitha and Vidyasagar (2013), fungal infections are not only causing primary diseases, but also responsible for various secondary ailments due to many predisposing factors. A widespread use of broad-spectrum antibiotics and immunosuppressive drugs for these ailments has led to an increase in the incidence of systemic fungal infections due to development of resistant strains of some of the fungi. Proliferation of new classes of drugs such as, allylamines (terbinafine) and orally active triazoles (itraconazole), has been considered as the most effective in dermatophytosis therapy. The emerging antifungals of plant origin could be useful alternatives for the treatment of dermatophytoses, where a topical therapy is required (Yigit, 2017).

In an ethno botanical study conducted by Chaudhary *et al*. (2010) found that traditionally in India, Henna is applied to hands and feet which symbolize fertility. The leaves, flowers, seeds, stem bark and roots are used in traditional medicine to treat a variety of ailments as rheumatoid arthritis, headache, ulcers, diarrhoea, leprosy, fever, leucorrhoea, diabetes, cardiac disease, hepatoprotective agent and colouring agent. The dried leaves of henna plants are use in northern Nigeria to prevent Complication due to burn wounds which may

arise from the colonization of the burn site by such organisms as *Streptococci sp., Staphylococcus aureus, Pseudomonas aeruginosa, Fusarium oxosporum, Aspergillus niger* and *Candida alblicans.* These complications can usually be avoided by good initial therapy. Penicillin therapy is usually undertaken in hospital. However, in third world countries such as Nigeria where medical care is sometimes substandard, patients stay home and nurse themselves back to health using local remedies. Other common practices are the application of tea leaves, *Aloe vera* gel in its crudest form, castor oil, egg yolks, palm oil and honey (Muhammad and Muhammad, 2005).

2.2.1 *Enantia chlorantha*

Enantia chlorantha belongs to *Annonaceae* family. This plant is commonly known as African yellow wood. Among the Yorubas in Nigeria, it is known as Awopa, Osu pupa or dokita by the Igbo. In the traditional medicine, this plant has been used for a long time in many parts of the African continent to treat various ailments of the human body (Ajani and Ibrahim, 2020)

According to Abike *et al.* (2020), *Enantia chlorantha* is a well-known medicinal plant that is used all over Nigeria. It is a fair sized ornamental forest tree that can grow to heights of 30 m. It grows in dense shade and may be recognized by its bright yellow slash and conspicuous black fruits. It is located in the West African region and extends from southern Nigeria to Gabon, Zaire and Angola. It is commonly called African white wood, *Moambe Jaune* and *Annikia chlorantha*. There are over 40 different species that grow in Nigeria as is seen by the various names that it is called and the varied uses to which it is applied. *Enantia chlorantha* is used to treat a wide variety of conditions using its roots, stem bark, fruit and leaves. In Akwa Ibom, it is used to treat malaria fever, typhoid fever, jaundice, dysentery, wounds, infections, high blood pressure and convulsions. It has been used also as anti-viral, anti-candidal and for gastroenteritis.

2.2.2 Botanical description of *Enantia chlorantha*

It is an ornamental tree which may grow up to 30 m high with dense foliage and spreading crown. The outer bark which is thin and dark brown is fissured geometrically, while the inner bark is brown above and pale cream beneath as seen in Figure 2. The stem is fluted and aromatic while the elliptic leaves are about 0.14–0.15 m long and 0.05–0.14 m broad. The leaves display up to 20 pairs of prominent lateral vein and parallel secondary nerves (Nyong *et al.*, 2015).

2.2.3 Botanical classification of *Enantia chlorantha*

Kingdom: *Plantae* Order: *Magnoliales*, Family: *Annonaceae,* Genus: *Enantia,* Species: *Chlorantha* (Tcheghebe *et al.,* 2016)

2.2.4 Plant habitat and distribution of *Enantia chlorantha*

It is a dense forest tree found in the Western and Southern forest of Cameroon, Southern part of Nigeria, Gabon, Angola and Zaire. It is widely distributed along the coasts of West and Central Africa (Nyong *et al.*, 2015).

2.2.5 Medicinal uses of *Enantia chlorantha*

Enantia chlorantha is used to treat malaria fever, typhoid fever, jaundice, dysentery, wounds, infections, high blood pressure and convulsions. It has been used also as anti-viral, anti-candidal and for gastroenteritis. In traditional medicine, the plant is used in the treatment of coated tongue, typhoid fever, malaria, jaundice, ulcer, rickettsia and infective hepatitis (Abike *et al*., 2020). It is used traditionally in the treatment of malaria and other ailments of the body such as cough and wounds (Salman and Adesokan, 2008). According to Atata *et al.* (2003), traditionally the bark extract is applied to ulcers and leprous spots for quick healing, decoction is used for washing wounds and bark sap is taken as decoction against diarrhoea.

2.3.1 *Sacuridaca longipedunculata*

Securidaca longipedunculata Fresen belongs to the family *Polygalaceae*, it is commonly known as violet tree and locally called Sanya or Uwar maganguna (Hausa), Ezeogwu (Igbo) and Alali (Fulani). The plant is ethnomedicinally used as a remedy for numerous human ailments; the stem bark is used to treat epilepsy, skin diseases, dysentery, malaria, typhoid, constipation, snake bites, infertility problems among others (Abubakar *et al.*, 2020)

2.3.2 Botanical description of *Sacuridaca longipedunculata*

Securidaca longipenduculata Fres (*Polygalaceae*) is shrub with twisted bole or slender erect branches and grows up to 30 feet high, that grows in savannah vegetation as seen in figure 3 (Tijjani *et al*., 2018)

2.3.3 Botanical classification of *Sacuridaca longipedunculata*

Kingdom: *Plantae* Family: *Polygalaceae,* Genus: *sacuridaca*, Species *longipedunculata* (Chukunda *et al.*,2020)

2.3.4 Plant habitat and distribution of *Sacuridaca longipedunculata*

It grows up to 12m tall, with an often flattened or slightly fluted bole It is distributed in a wide range of climates ranging from subtropical, hot, and arid climate to summer rainfall and equatorial humid. It grows in different vegetation ranging from semi-arid scrub to dense forest, including bush habitats and gallery forests and woodland. It is sensitive to frost and resistant to bush fires (Lijalem and Feyissa, 2020).

2.3.5 Medicinal uses of *Sacuridaca longipedunculata*

According to Abubakar *et al*. (2018), it is traditionally used to treat fever, diarrhoea, dysentery, typhoid constipation, headaches, rheumatism, malaria, tuberculosis, pain, epilepsy, pneumonia, skin infections, urethral discharges, stomach problems, toothache,

sleeping sickness, cough, chest complaints, snakebite and wound dressing. The powdered stem bark exhibited antimicrobial activity against a variety of organisms including *Neisseria gonorrhea, Candida albicans, Trichomonas vaginalis* and the agent for syphilis (Hedimbi and Chinsembu, 2012).

2.4 Phytochemicals in the Plants

The therapeutic properties of medicinal plants rely on the bioactive compounds which are also known as phytochemicals. Phytochemicals means naturally occurring chemicals in plants that confer a protective function to plants against bacteria, viruses, fungi, damage by free radicals, insects and herbivores that feed on them and any other environmental threat (Shemishere *et al.,* 2020). Phytochemicals represent traditional knowledge of compounds effective in symptomatic relief and potential healing properties (Sharma and Kaur, 2020).

2.4.1 Classes of phytochemicals and their examples

Alkaloids: Strychnine, emetine, brucine, piperine, caffeine, quinine, emetine, brucine.

Sulfur-containing phytochemical: Glucosinolates from broccoli, cabbage, cauliflowers, sulforaphane, isothiocyanates (berteroin, hirsutin, phenethylisothiocyanate, alyssin and erucin).

Terpenoids: Thymol, carvacrol, eugenol, transcinnamaldehyde, β-resorcylic acid, vanillin, thymol, berberine, eugenol, cinnamaldehyde.

Carotenoid: carotenoid fractions from red paprika, Valencia orange peel, Rose hips, peel of Shation Pummelo.

Polyphenols: galangin, kaempferol, quercetin, myrecetin, galangin (Sharma and Kaur, 2020).

2.4.2 *Lawsonia inermis*

According to Yigit (2017), the phytochemical characterization of *Lowsania inermis*, included the leaves contain about 0.5-1.5% lawsone, the antimicrobial agent responsible for the dyeing propertied of the plant. The plant has contain various compounds like; gallic acid, coumarins, naphthalene derivatives, flavonoids, sterols, triterpenoids, tannins, saponins, glycosides, and xanthones

2.4.3 *Enantia chlorantha*

Phytochemical analysis of *Enantia chlorantha* bark phytochemical screening of the extract of *Enantia chlorantha* stem bark was carried by Chukunda *et al.* (2020) to contain tannins, alkaloids, saponins, glycosides and flavonoids

2.4.4 *Sacuridaca longipedunculata*

Sacuridaca longipedunculata extract and fractions were evaluated for the presence of carbohydrates, anthraquinones, flavonoids, tannins, alkaloid, saponins, cardiac glycosides, steroids and triterpenes using standard procedures (Tauheed *et al.*, 2017). According to Lijalem and Feyissa (2020), *S. longipedunculata* has different chemical constituents including methylsalicylate, flavonoids, alkaloids elymoclavine, and dehydroelymoclavine, an ergoline compound and cinnamonic acid and the xanthones: 1,7 Dimethoxy-2-hydroxy-xanthone and 1, 4-dihydroxy-7methoxy-xanthone. A number of fatty acids and triglycerols such as coriolic acid, trans-9-dienoic acid, and 9-hydroxytetradeca-cis-5, trans-7-dienoic acid have been isolated from its seed oil

2.5 Research Organisms

2.5.1 *Trichophyton schoenleinni*

Trichophyton schoenleinni is an important anthropophilic dermatophyte that causes *Tinea favosa* and it transmitted by contact between humans and it is endemic in Africa (Khaled *et al*., 2007). *Trichophyton schoenleinii* is an uncommon causative fungi of tinea corporis

since the discovery of *Achorion Schoenleinii* by Johann Lucas Schoenlein in 1839, this dermatophyte was both the first microorganism clearly attributable to a human disease and also the dominant dermatophyte in 19th century Northern Europe (Mansouri *et al*., 2012). *Trichophyton schoenleinii* is an important anthropophilic dermatophyte that causes tinea favosa and is transmitted by contact between humans. It is currently endemic in Africa. Improvements in living conditions and hygiene in developing countries after the Second World War have been associated with the almost complete disappearance of many anthropophilic species, including *Trichophyton schoenleinii. Trichophyton schoenleinii* is now rare throughout Europe, as mentioned in Korstanje's paper on tinea capitis in northwestern Europe from 1963 to 1993. In Poland, *T. schoenleinii* was isolated in 0.2% of 1,045 specimens taken from cases of *tinea capitis* during a period of 20 years. One case of *T. schoenleinii* was isolated out the 190 cases of *tinea capitis* in Spain between 1977 and 1997. In Greece, only 35 cases of *tinea capitis* were identified between 1981 and 1995, and 5.7% of them were due to *T. schoenleinii*. There has been a shift in organisms associated with *tinea capitis* in the Netherlands from *T. schoenleinii* to *T. violaceum;* this can be explained by the increase of immigrants from Mediterranean countries (Khaled *et al*., 2007).

2.5.2 *Trichophyton rubrum*

Trichophyton rubrum is the most common aetiological agent of human dermatophytoses. These infections mainly occur in keratinised layers such as skin, hair and nails because the fungus uses keratin as a nutrient source *Trichophyton rubrum* is the main causative agent of dermatophytoses, accounting for 80%-90% of these infections.

The treatment of infections caused by *T. rubrum* is time consuming and expensive and cases of recurrence are common. In addition, strains resistant to the antifungal agents used for treatment have emerged (Abreu *et al.,* 2020). The infection is usually first noticed as a

scaling or macerated area between the toes indistinguishable from *tinea pedis* due to other fungi. *Trichophyton rubrum* differs from other dermatophytes, however, in that it is much more likely to attack the nails, when their discoloured, misshapen, and broken appearance, may cause the patient much mental distress; treatment at this stage is very rarely successful. It is surprising, therefore, that the epidemiology and prevention of the disease have been so neglected (Mary, 1957).

Trichophyton rubrum has adapted to the skin of human beings. It is believed, the organism uses several different strategies. First, many infected patients cannot elicit a cell-mediated immune response to eliminate the fungus. The reasons for this are not completely clear, but trichophytin skin tests are negative at 48 hours despite persistent, chronic, and even widespread infections. Antigens are present on *T. rubrum*, just as they are on other *dermatophytes*, but differences in antigen penetration through the skin may prevent induction of immunity (Dahl and Grando, 1994). *Trichophyton* genus was accounted for 93% of dermatophytoses, which was shared by *Trichophyton rubrum* (73.3%) and *Trichophyton mentagrophytes* (19.7%), followed by *Epidermophyton floccosum* (4.2%) and *Microsporum gypseum* (2.8%). It was noted that *tinea corporis* (64.8%) is the most prevalent infection followed by *tinea cruris* (26.8%), *tinea pedis* (5.6%) and *onychomycoses* (2.8%). *Trichophyton rubrum* was the predominant species responsible for the dermatophytoses, especially tinea corporis in Chennai, Tamilnadu, India. In addition, it was also observed that *T. rubrum* was most predominant species responsible for the chronic dermatophytoses (81.8%). Further work is in progress to understand the protease profile of the isolates with relation to the chronisity of the infection. Antifungal agents such as griseofulvin, azole derivatives and allylamines may become of little use in the treatment of dermatophytoses as a result of the development of fungal resistance, prolonged duration of treatment and side effects. There exists a clear demand for additional

antifungals with therapeutic potential. In this context, attention has focused on the antifungal activity of aromatic plants and their constituents due to their potential biological properties (Fillipe *et al*., 2013)

2.5.3 *Microsporum audouinii*

Microsporum audouinii is commonly regarded as an anthropophilic dermatophyte since isolation from animals and soil is rare. Transmission is thought to be human-to-human through habitual contact. A number of risk factors for acquiring *Tinea capitis* have been reported, including ethno-cultural factors such as hairdressing mode, socio-economic factors, and climatic factors such as humidity. *Microsporum audouiniii* is predominantly isolated in Africa but is increasingly reported in Europe and the America likely as a result of population migrations. This indicates why there is a need for continuous surveillance not only to better determine the prevalence but also for the changes in causative species of dermatophytosis. Infection by *M. audouinii* is known to be associated with no or mild inflammatory response. It commonly starts as a small erythematous papule surrounding a single hair shaft, which gradually spreads centrifugally to surrounding follicles leading to patches of alopecia (Yotsu *et al*., 2021). *Tinea capitis* caused by *Microsporum audouinii* is reported herein from two Brazilian schoolchildren, which are brothers. Arthroconidia were evidenced on direct examination of scalp hair, and a fungus of the genus *Microsporum* was isolated from cultures of each patient. The isolated fungi were classified as *M. audouinii* by visualization of species-specific structures, including: pectinate hyphae, chlamydospores, and fusiform macroconidia, sterile growth with characteristic brown pigment in rice grains, and through DNA sequencing of the internal transcriber spacer region. Patients were refractory to ketoconazole, but the two cases had a satisfactory response to oral terbinafine. All *M. audouinii* infections described in this century were

reviewed, and to our knowledge, this is the first literature description of this species from South America.

Misidentification of *M. audouinii* with *Microsporum canis* can occur in this area, leading to erroneous data about the occurrence of this species (Brito-Santos *et al*, 2017). *Microsporum audouinii* is an anthropophilic dermatophyte common in Africa. It typically causes tinea capitis and tinea corporis in children. While *Microsporum canis*, a zoophilic dermatophyte, is still the most common cause of tinea capitis in Europe, an increase in anthropophilic tinea capitis has been noted, mainly in urban areas. The anthropophilic *Trichophyton tonsurans* is the most often reported etiologic agent in the UK, whereas *Trichophyton soudanense* and *M. audouinii* are most common in France. *M. audouinii* cases have also been reported in Italy, Spain and Portugal. These anthropophilic fungi are most prevalent in immigrant communities from Africa and Asia. They cause less inflammatory reactions and have an increased tendency to be associated with chronic disease as compared to infections due to *M. canis.* The latter may be the result of late detection due to the absence of subjective symptoms, even though kerion may occur (Ali *et al*, 2012).

2.6 Toxicology and Toxicity of the Plant

The median lethal dose (LD50) test was first introduced in 1927 by J. W. Trevan in order to estimate the dose of a test substance that produces 50 % death in a given species of animals. It is usually the first test conducted for every chemical before further toxicity tests are evaluated. It is used for estimating the potential hazards of substances on humans. Although, the major endpoint of LD50 is death, non-lethal acute effect may occur as signs of toxicity depending on the substance being investigated (Suleiman *et al*., 2018).

2.6.1 *Lawsonia inermis*

Reports of the European Commission, formulated by the Scientific Committees on Consumer Products and Consumer Safety provide a summary of detailed toxicity studies done on henna, as required for product registration. In acute toxicity study of henna done by Semwal *et al.* (2014), henna did not display signs of irritation potential when tested for acute dermal toxicity. A slight and transient irritation to some individuals were reported in an assay to determine the extent of mucous membrane irritation in New Zealand White rabbits, after exposing the conjunct vital sac of the eyes to henna. No reaction to henna was apparent in skin sensitization experiments in guinea pigs.

In addition, there were no indications of any effects of henna in a repeated insult path test on humans. Since henna is applied to the skin and hair, it is important to establish the degree of absorption through the skin. In-vitro assays using isolated pig skin mounted in permeation chambers were exposed to henna pulp, containing 1% lawsone. It was calculated that after exposure of the skin to henna pulp for 30 min and a follow-up period of 72 h, 0.06% of the applied lawsone remained in the skin. An absolute penetration rate of 703 ng/cm2 lawsone was established. Excised, non-viable human skin mounted in flow-through diffusion cells was exposed to henna containing C14-labelled lawsone in the form of shampoos (5 min exposure) and hair colour pastes (1 h exposure). In the case of pastes, 2.2% to 3.7% of the applied lawsone dose remained in the skin, while 3.6 to 6.8% of the lawsone from shampoos could be detected (Semwal *et al.,* 2014). According to Kaur *et al*. (2020), there were also no lesions (pathological changes) in the tissues of the animals that received doses of 200 and 500gm/kg b.w of the extract. The present finding is in agreement with the previous reports on biochemical and hematological response in albino Wister rats exposed to aqueous extract of *Lawsonia intermis*. In conclusion, ingestion of 80% ethanolic extract of *L. intermis* has no adverse effect on the tissues of the organs studied

in the rats up to dose level of 500mg/kg b.w. However, more work is suggested at the sub cellular.

2.6.2 *Enantia chlorantha*

The acute toxicity test was performed using the OECD guidelines 423. Standard two-phase approach described by Lorke was used by Ajani and Ibrahim (2020), came to a conclusion that This study concluded that *E. chlorantha* is not toxic at acute dose and that combine administration of *E. chlorantha* extract and lisinopril at sub-acute dose in diabetes may aggravate liver dysfunction associated with diabetes using the formula below:

$$LD50 \geq \text{Maximum-dose } Y = \text{number of rats per group}$$

Where Y=Sum of mean death

The medicinal properties of the plant may be due to one or more of its phytochemical constituents. However, some of these compounds may be toxic, and thus the plants containing them could confer varied levels of toxicity to an individual consuming them. A plant, *Hilleria latifolia*, which is used as analgesic and the treatment of asthma, has been reported to induce cholinergic signs like defecation, salivation and urination in experimental animals (Wonder *et al.*, 2011). Some plants may therefore be inherently dangerous, containing naturally occurring toxins, often with cytotoxic, cardiotoxic effects, or some other toxic properties (Humphrey and McKenna, 1997). *Enantia chlorantha* is not an exception. Unfortunately, most of the users of this plant do not have the knowledge of its adverse effects, toxicity, and neither of its other beneficial properties (Agbaje and Babatunde, 2005). Therefore, in order to have a standard natural plant product, preliminary studies have to be done to evaluate possible risks such as, undesirable effects, overdose or poisoning. Our study was conducted to identify the major chemical groups contained in the ethanolic stem bark extract of *E. chlorantha*, its hematologic and serum biochemistry variables, as well as histopathological changes following a single oral intake in albino rats.

This should provide preliminary safety information regarding *E. chlorantha* as well as establish the toxicological limits in rats (Olamide and Abatan, 2013).

2.6.3 *Sacuridaca longipedunculata*

According to Mongalo *et al.* (2015) the aqueous root bark extract was slightly toxic to albino rats however, acute toxicity studies of the aqueous whole root extract on mice revealed LD50 values more than the oral and intraperitoneal application routes while 80% ethanol extract of the root bark exhibited an LD50 against albino mice was higher. These findings may well suggest that the root bark extract has greater acute toxicity than the whole root extract following oral administration in mice. The medial lethal dose (LD50) of the ethyl acetate stem bark extract of *S. longipedunculata* was greater than 5, 000 mg/k g body weight, while that of aqueous stem bark extract was found to be above 2, 000 mg/kg body weight (Abubakar *et al*., 2018). Also, the acute toxicity studies of the aqueous whole root extract of the plant revealed LD50 values of 1740 mg/kg and 20 mg/kg body weight for oral and intraperitoneal application routes respectively (Adeyemi *et al*., 2010), while Keshebo *et al*. (2014) reported LD50 values of 37 mg/kg and 547 mg/kg body weight for aqueous and ethanol root extracts respectively. However, death was not recorded when different doses (300, 900 and 2700 mg/kg body weight) of the aqueous root extract were orally administered on a daily basis for 28 days (Etuk *et al*., 2006). The aqueous root bark extract was slightly toxic to albino rats with an LD50 of 0.771 g/kg (Auwal *et al*., 2012), while Agbaje and Adekoya (2012) reported an LD50 of 3.16 g/kg when administered orally to rats. Moreover, acute toxicity studies of the aqueous whole root extract on mice revealed LD50 values of 1.740 g/kg and 0.020 g/kg for the oral and intraperitoneal application routes respectively (Adeyemi *et al*., 2010). Elsewhere, the 80% ethanol extract of the root bark exhibited an LD50 of 0.547 g/kg against albino mice (Keshebo *et al*., 2014). These findings may well suggest that the root bark extract has greater acute toxicity

than the whole root extract following oral administration. In a repeated dose toxicity study, there was no mortality observed when varying doses of 0.3, 0.9 and 2.7 g/kg of the aqueous root extract were administered orally on a daily basis for a period of 28 days to Swiss albino mice (Etuk *et al*., 2006). Besides the method of preparation of the extracts, the difference in LD50 may be due to differences in collection site, geographical area and the season of collection. However, there is no data in the literature on the administration of various isolated compounds from *S. longipedunculata* to mice or rats. In the brine shrimp bioassay, the 70% methanol extract of the root exhibited a 100% mortality rate at a concentration of 1000 µg/ml (Adiele *et al*., 2013), while the 80 % methanol root extract exhibited an LC50 of 77.1 µg/ml (Moshi *et al*., 2007), suggesting that these extracts are relatively toxic. However, the brine shrimp assays have some problems as the counting of the viable larvae is performed while the live larvae are continually moving around the petri dish. The aqueous root bark extract was toxic to Ehrlich ascites tumor cells with a mortality rate of 82.5 % at 1000 µg/ml and revealed an IC50 of 67 µg/ml (Lawal *et al*., 2012). Compounds such as 1,6,8-trihydroxy-2,3,4,5-tetramethoxyxanthone and 1,6- dihydroxy-2,3,4,5,8-pentamethoxyxanthone showed potent cytotoxicity with IC50 values of 22.8 and 17.4 µM respectively against human pancreatic cancer cells (Dibwe *et al*., 2013) while the 70% methanol extract of the root bark exhibited average inhibition of cell proliferation of 22.6 % at a concentration of 1 µg/ml against HeLa cells (Runyoro *et al*., 2005). However, this is not a useful result, when compared to the IC50 which will explain the overall average concentration at which 20-50% of the cells will be inhibited by the test plant extract. *S. longipedunculata* extracts have been investigated for cytotoxicity against human pancreatic cell lines, brine shrimp larvae, Ehrlich ascites tumor cells, Hela cells and both albino rats and mice. However, there is a need to investigate the cytotoxicity of various

extracts and some compounds isolated from this species against normal human cell lines (Mongalo *et al.*, 2015).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

Chanchaga is the Local Government Area (LGA) where Minna the capital of Niger state is located. It is also bordered by Bosso LGA Council on all side. Chanchaga is located between latitudes 9°30′ to 9°40′ north of the equator and longitudes 6°30′ to 6°40′ east of the Greenwich meridian as shown in appendix E (Samuel *et al*., 2018; Lympson and Husaini, 2020).

3.2 Ethical Consideration

An ethical clearance that permitted the collection of medical samples and to conduct research was obtained from the Research Ethics and Publication Committee (REPC) of General Hospital Minna, Niger State.

3.3 Media Preparation

All the media used were prepared according to the manufacturer's instruction and are autoclaved within the validated autoclaving period i.e holding at 121℃ for 15 minutes for proper sterilization (Cheesbrough, 2006). SDA was prepared by suspending 13g of the powder into 500ml of distilled water, chloramphenicol and cyloheximide was added and shacked vigorously on heat for about a minute it was autoclaved at 121℃ for 15minutes and cooled then poured into the petri dishes.

3.4 Sample Collection and Transportation

3.4.1 Collection of test organisms

The fungal clinical isolates used in this research were from hair and skin of children living within Minna, Niger state as observed in Plate 3.1, Plate 3.2 and Plate 3.3 respectively. Using the method described by Berkow and McGowan (2019), 70% ethanol was used to

disinfect the surfaces of the infected parts of the skin; hair strands were plugged from the head into a clean envelope and for the skin; the infected skin was scraped gently using a sterile scalpels blade and the scrapings were put in clean envelope they were transported to microbiological laboratory of Federal University of Technology Minna where portions of the specimen were pressed gently on Sabouraud dextrose agar (SDA) at room temperature for 7 days. Growths were subcultured on SDA to obtain pure isolate. Isolates were used for further identification and characterization

3.4.2 Collection of plant materials

The plant materials used in this study were washed under running tap water, air-dried at room temperature and then pulverized and homogenized to fine powder and stored for use in airtight glass containers at room temperature in the dark until use as described by Shobowale *et al*. (2013).

3.5 Plant Processing and Extraction

The plants parts were extracted using cold maceration technique as described by Nenaah and Ahmed (2011). One hundred (100g) of the dried powder were soaked into 500ml of methanol in a clean sterile airtight container for three days (72 hours) at room temperature, while undergoing vigorous shaking at regular interval. The mixtures were then filtered through muslin cloth and re-filtered through Whattman`s filter paper No. 1. The filtrates were concentrated by complete evaporation of the solvent at room temperature. The concentrated extracts were subsequently transferred into clean sterile airtight glass container and stored in the refrigerator at 4°C until use. The weights were obtained and the percentage yields were calculated using the Equation 3.1 below as described by Duniya *et al.* (2018).

$$\%Yield = \frac{\text{weight of the extract}}{weight\ of\ dry\ sample}\ X\ 100 \qquad (3.1)$$

3.6 Identification of Dermatophytes

3.6.1 Colonial morphology and microscopy of isolates

Identification of the isolates was done based on the appearance of the colonies to the naked eye and the mycelium with microscopical preparations and was compared with the standard as by the Society for General Microbiology (2006). Seventy percent (70%) alcohol was placed on a microscopic glass slide, the isolates were immersed into the alcohol and a drop of lactophenol cotton blue was added, the cover slip was placed gently to avoid air bubbles and the mounted on a microscope, it was observed at x40 magnification which is the switched to x100 for clearer view.

3.7 Molecular Characterization

3.7.1 Fungi DNA extraction

Approximately 100 mg of fungi mycellia were grinded with Dellaporta extraction buffer (100 mM Trls pH 8, 51 ml EDTA pH 8, 500 mM NaCI, 10 mM mcrcaptoethanol) and DNA extracted as described briefly. Each sample was grinded in 1000 µl of the buffer in a sterilized sample bag. The mix were collected in sterile eppendorf tube and 40 µl of 20% SDS were added, this was followed by brief vortexing and incubated at 65℃ for 10 minutes. At room temperature, 160 µl of 5 M potassium acetate was then added, vortexed and centrifuged at 10000 g for 10 minutes. The supernatant was collected in another eppendorf tube and 400 µl of cold isopropanol was added and mixed gently they were kept at -20℃ for 60 minutes. Centrifugations were done at 13000g for 10 minutes to precipitate the DNA after which the supernatant were gently decanted and ensured that the pellet were not disturbed. The DNA were then washed with 500 µl of 70 % ethanol by centrifuging at 10000g for 10 minutes, the ethanol was decanted and DNA air-dried at room temperature until no trace of ethanol were observed in the tube. Pellets were then re-suspended in 50 µl of Tris EDTA buffer to preserve the suspended the DNA.

3.7.2 Fungi polymerase chain reaction analysis

ITS gene were used for characterization of fungi, ITS universal primer set which flank the ITS1, 5.8S and ITS2 region were used; PCR sequencing preparation cocktail consisted of 10 µl of 5x GoTaq colourless reaction, 3 µl of 25mM MgCl2, 1 µl of 10 mM of dNTPs mix, 1 µl of 10 pmol each ITS 1: 5' TCC GTA GGT GAA CCT GCG G 3'and - ITS 4: 5' TCC TCC GCT TAT TGA TAT GC 3'primers and 0.3units of Taq DNA polymerase (Promega, USA) made up to 42 µl with sterile distilled water 8µl DNA template. PCR was carried out in a GeneAmp 9700 PCR System Thermal cycler (Applied Biosystem Inc., USA) with a PCR condition include a cycle of initial denaturation at 940C for 5 min, followed by 35cycles of each cycle comprised of 30secs denaturation at 94℃, 30secs annealing of primer at 55℃, 1.5 min extension at 72℃ and a final extension for 7min at 72℃.

3.7.3 Integrity

The integrity of the amplified about 1.5Mb gene fragment was checked on a 1% Agarose gel ran to confirm amplification. The buffer (1XTAE buffer) was prepared and subsequently used to prepare 1.5% agarose gel. The suspension was boiled in a microwave for 5 minutes. The molten agarose was allowed to cool to 60°C and stained with 3µl of 0.5 g/ml ethidium bromide (which absorbs invisible UV light and transmits the energy as visible orange light). A comb was inserted into the slots of the casting tray and the molten agarose was poured into the tray. The gel was allowed to solidify for 20 minutes to form the wells. The 1XTAE buffer was poured into the gel tank to barely submerge the gel. Two microliter (2 l) of 10X blue gel loading dye (which gives colour and density to the samples to make it easy to load into the wells and monitor the progress of the gel) was added to 4µl of each PCR product and loaded into the wells after the 100bp DNA ladder was loaded into wells. The gel was electrophoresed at 120V for 45 minutes visualized by ultraviolet

trans-illumination and photographed. The sizes of the PCR products were estimated by comparison with the mobility of a 100bp molecular weight ladder that was ran alongside experimental samples in the gel.

3.7.4 Purification of amplified product

After gel integrity, the amplified fragments were purified with ethanol in order to remove the PCR reagents.7.6 µl of Na acetate 3M and 240 µl of 95% ethanol were added to each about 40µl PCR amplified product in a new sterile 1.5 µl tube eppendorf, mix thoroughly by vortexing and keep at -20 ℃ for at least 30 min. Centrifugation for 10 min at 13000 g and 4°C was done, followed by removal of supernatant (invert tube on trash once) after which the pellet were washed by adding 150 µl of 70% ethanol and mix then centrifuge for 15 min at 7500 g and 4°C. All supernatant was removed (invert tube on trash) and invert tube on paper tissue and was allowed to dry in the fume hood at room temperature for 10-15 min. and it was re-suspended with 20 µl of sterile distilled water and kept in -20℃ prior to sequencing. The purified fragment was checked on a 1.5% Agarose gel ran on a voltage of 110V for about 1hr as previous, the presence of the purified product and quantified were confirmed using a nano drop of model 2000 from thermo scientific.

3.7.5 Sequencing

The amplified fragments were sequenced using a Genetic Analyzer 3130 x l sequencer from Applied Biosystems using manufacturers manual while the sequencing kit used was that of Big Dye terminator v3.1 cycle sequencing kit. Bio- Edit software and MEGA 6 were used for all genetic analysis

3.8 Standardization of Inoculum

The standardization of the isolates was carried out as described by Guarro *et al*. (1998). Pure isolates were sub cultured on slants for 15 days and the surface of the slants containing the isolate inoculum were flooded with sterile distilled water, the sporulated aerial

mycelium were scrapped using glass slide. The suspension obtained was filtered using cotton guaze removing the majority of the hyphae and the inoculum were adjusted to 5 x 10^5 conidia/mL with sterile distilled water and measured with spectrophotometer at 530nm to determine the *T* values, they were further sub cultured on SDA plates and their colony forming unit per ml (cfu/ml) were determined.

3.9 Antifungal Susceptibility Testing of the Extracts

The antifungal activity of the extracts was determined using agar well diffusion method adopted from Clinical and Laboratory Standards Institute (CLSI, 2019). SDA plates was prepared for all the organisms, the plates were inoculated with the suspension of the standardized cultures of each organism separately using sterile swabs. For each organism, a sterile swab was submerged in the standardized suspension and excess fluid was blotted by pressing and rotating the swab against the wall of the test tube, the swab was then used to inoculate the entire surface of the plate with continuous rotation of the plate 60 degrees. The inoculum was allowed to dry for few minutes.

Under an aseptic condition four wells of 6mm diameter was made in each plate using 6mm sterile cork borer. The extract, 0.15g, 0.25g, 0.5.g, 1.0g, 1.5g, 2.0g, 2.5g and 3.0g were weighed separately for each plant and dissolve with 2ml of dimethyl sulfoxide (DMSO) and 10 ml of sterile distilled water, so as to attain a concentration of 15 mg/ml, 25 mg/ml, 50 mg/ml, 100 mg/ml, 150 mg/ml, 200 mg/ml, 250 mg/ml and 300 mg/ml respectively.

The plates were labeled according to each concentration and 0.1ml of each concentration was transferred to a designated labeled well with the aid of sterile syringe.

Also, some plates were prepared for control using fluconazole 50 mg/ml and ketoconazole 50 mg /ml as positive control while distilled water as negative control.

Thirty minutes (30) pre diffusion time was allowed for each plate after which they were incubated at 37℃ for 5 days. The inoculated plates for each of the extracts and control are allowed to diffuse for 30 minutes. After the 5 days incubation period, the diameter zone of inhibition (mm) was measured using a measuring ruler and the measurement was obtained by measuring the centre of well and subtracting it with the diameter of the cork borer (6 mm). Test isolate with 10mm and above zone of inhibition is considered sensitive to that extract.

3.10 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of the Crude Extracts

The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) were determined using broth dilution method as described by Johnson and Arendrup (2019). A tube dilution method was used in determining minimum inhibitory concentration (MIC), series dilution was made from each extract starting from the highest concentration which tends to have higher activity to the least concentration (300.00, 150.00, 75.00, 37.50, 18.75, 9.38 and 4.69 mg/ml) in SDB and 0.1 ml of each standardized culture of the test organisms was transferred into each labeled dilution tube. Negative and positive controls were maintained respectively in which the negative control contain the inoculum in the growth medium organisms and positive controls contained the standard drug against the isolates.

The tubes were incubated at 37 ^{0}C for 5 days and at the end of the incubation period; the tubes were read using spectrophotometer at a wavelength of 600 nm. The test tube with the lowest significant reduction in absorbance was taken as the MIC.

The minimum fungicidal concentration (MFC) was determined by sub-culturing the tubes with reduced significant absorptions further into freshly prepared SDA plates and the

plates were incubated at 37 ℃ for 5 days. Thus, the plates with minimum or no growth was recorded as the MFC.

3.11 Partitioning of the Crude Extracts

The methanolic plant extract were partitioned as described by Haruna and Cyril (2011). The extracts were weighed (15 g each) and partitioned exhaustively in increasing order of solvent polarity (n-hexane, ethyl acetate and distilled water) using separating funnel. The fractions obtained were evaporated to dryness at control temperature in water bath. Each fraction was weighed after the dryness and recorded. Furthermore, the fractions were subjected to antifungal screening to detect the active fractions; also, the MIC and MFC of the fractions were also determined using the method previously described.

3.12 Gas Chromatography-Mass Spectrophotometry (GC-MS) Analysis of the Fractions

Gas Chromatography- Mass Spectrophotometry (GC-MS) analysis was conducted at Shimadzu Training Center for Analytical Instruments (STC) Lagos. The extracts were analyzed by GCMS-QP2010SE (SHIMADZU, JAPAN) equipped with DB-5 MS (0.25 µm × 30 m × 0.25 mm). Helium was used as the carrier gas at a flow rate of 0.9 ml/min. 1.0 µl injection volume, injector temperature was 250°C; ion source temperature was 200°C. Interface temperature was 250°C. Oven temperature was 60°C held for 2 min with an increase of /'5°C/min to 120°C, ending with 300°C (15°C/min). Mass spectrometer was set to operate in electron ionization mode with an ionizing energy of 70eV as uisition mass range from 45-700 a.m.u. Total running time was about 30 min. Further identification was made by comparison of their mass spectra with those stored in the National Institute of Standards and Technology (NIST) database.

3. 13 Phytochemical Screening

3.13.1 Total phenol determination

Singleton *et al*. (1999) method was used to determine total phenol content of the extracts. 0.01g of each extract was dissolved in 10 mL of distilled water, and 0.5 mL was oxidized by 2.5mL of 10% Folin-Ciocalteu's reagent which was then neutralized by 2 mL of 7.5% sodium carbonate. The reaction mixture was incubated at 45^0C for 40 minutes. Absorbance was read at 765 nm using double beam Shimadzu UV spectrophotometer, UV-1800. Standard garlic acid was used to prepare the calibration curve.

3.13.2 Total flavonoids determination

Total flavonoid content of the extracts was determined using the method of Chang *et al.* (2002). Approximately, 0.5ml of each extract was added to a test tube containing 1.5ml of absolute methanol, 0.1ml of 10% aluminum chloride, 0.1 ml of 1M sodium acetate and 2.8 ml of distilled water and incubated at ambient temperature for 30 minutes. The absorbance was read at 415 nm with double beam shimadzu UV-spectrophotometer, UV-1800. Standard quercetin was used to prepare the calibration curve.

3.13.3 Total alkaloid determination

Total alkaloid of the extracts was determined using method of Oloyede (2005). Using this, 0.5g of each extract was weighed and dissolved in 5ml of mixture of 96% ethanol:20% H_2SO_4 (1:1) and then filtered. One (1) ml of the filtrate was then added to a test tube containing 5 ml of 60% H_2SO_4 and allowed to stand for 5 minutes. Thereafter, 5 ml of 0.5% formaldehyde was added and allowed to stand at room temperature for 3hours. The absorbance was read at wavelength of 565 nm. Vincristine extinction coefficient (E_{296}, ethanol (ETOH)= 15136 $M^{-1}cm^{-1}$) was used as reference alkaloid.

3.13.4 Total tannins determination

Tannin content of the extracts was determined using the method of Association of Official Analytical Chemist (AOAC, 1984). About 0.2g of each extract was weighed into a 50 ml beaker and 20 ml of 50% methanol was added to it and covered with para film and heated in water bath at 80^0C for 1 hour. The reaction mixture was shaken thoroughly to ensure uniformity. The extract was then filtered into a 100 ml volumetric flask, and 20 ml of distilled water, 2.5 ml of Folin-Denis' reagent, and 10 ml of sodium carbonate were added and mixed properly. The reaction mixture was then allowed to stand for 20 minutes at room temperature for the development of bluish-green coloration. The absorbance was taken at 760 nm using double beam shimadzu UV-spectrophotometer, UV-1800. Standard tannic acid was used to prepare the calibration curve.

3.13.5 Total saponins determination

Saponins content of the extracts was determined using the method of (Oloyede, 2005). 0.5g of each extract was weighed and dissolved in 20 ml of 1N HCl and boiled in water bath at 80^0C for 4hours. The reaction mixture was cooled and filtered. 50 ml of petroleum ether was added and the ether layer was collected and evaporated to dryness. Thereafter, 5ml of acetone-ethanol (1:1), 6 ml of ferrous sulphate and 2 ml of concentrated sulphuric acid were added and allowed to stand for 10minutes. The absorbance was taken at 490nm. Standard saponins was used to prepare the calibration curve.

3.14 Data Analysis

Data were analyzed using simple descriptive statistics (frequency and percentages), one-way analysis of variance (ANOVA) was used to determine significant level at 5% level of significance using SPSS software (Version 25.0.0.0 SPSS) and the differences were considered significant at $P< 0.05$.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Collection of test organisms

The isolates were collected from head, hand and feet of children within metropolis, Niger state and were identified as *T. schoenleinii*, *M. audouinii* and *T. rubrum* these were confirmed based on their growth and cultural characteristics on the culture media and their mycelium morphology using microscope as shown in Table 4.1 and observed on Plates I, II and III respectively.

Table 4.1: Site of collection of organisms and the morphological characteristics of the organisms isolated

Organism	Site of collection	Colony morphology	Microscopic morphology
T. schoenleinii	Head	Smooth, waxy, brownish	Hyphal swelling, chlamydospores, favic chandelier
M. audouinii	Leg	Velvety, brownish slow growing	Thick-walled chlamydospores, conidia are rare and irregular
T. rubrum	Head	White or creamy cottony or powdery surface, red pigment on reverse	surface, red pigment on reverse Tear-shaped microconidia

Plate I: *Microsporum audouinii* isolates on SDA plate

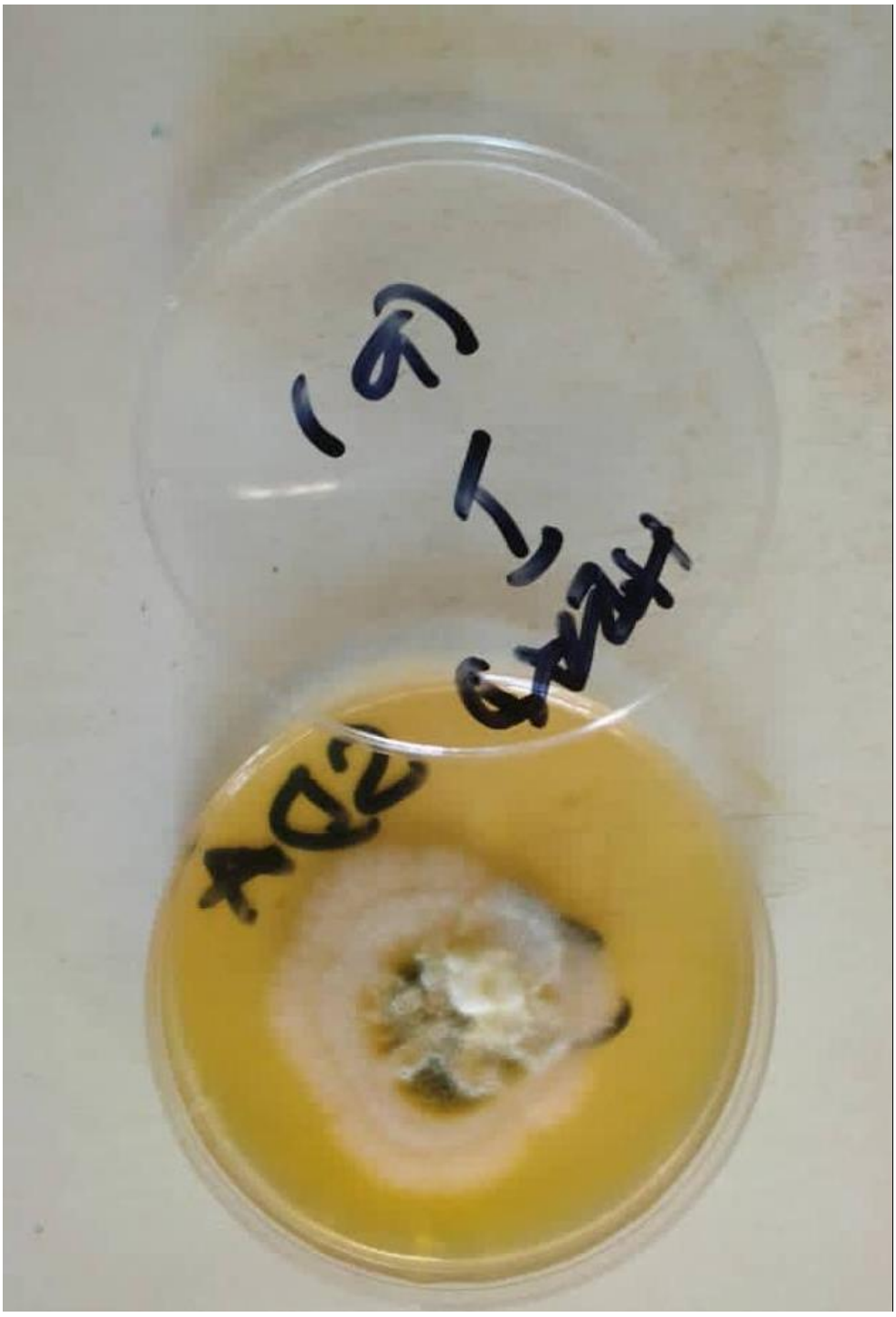

Plate II: *Trichophyton schoenleinii* isolates on SDA plate

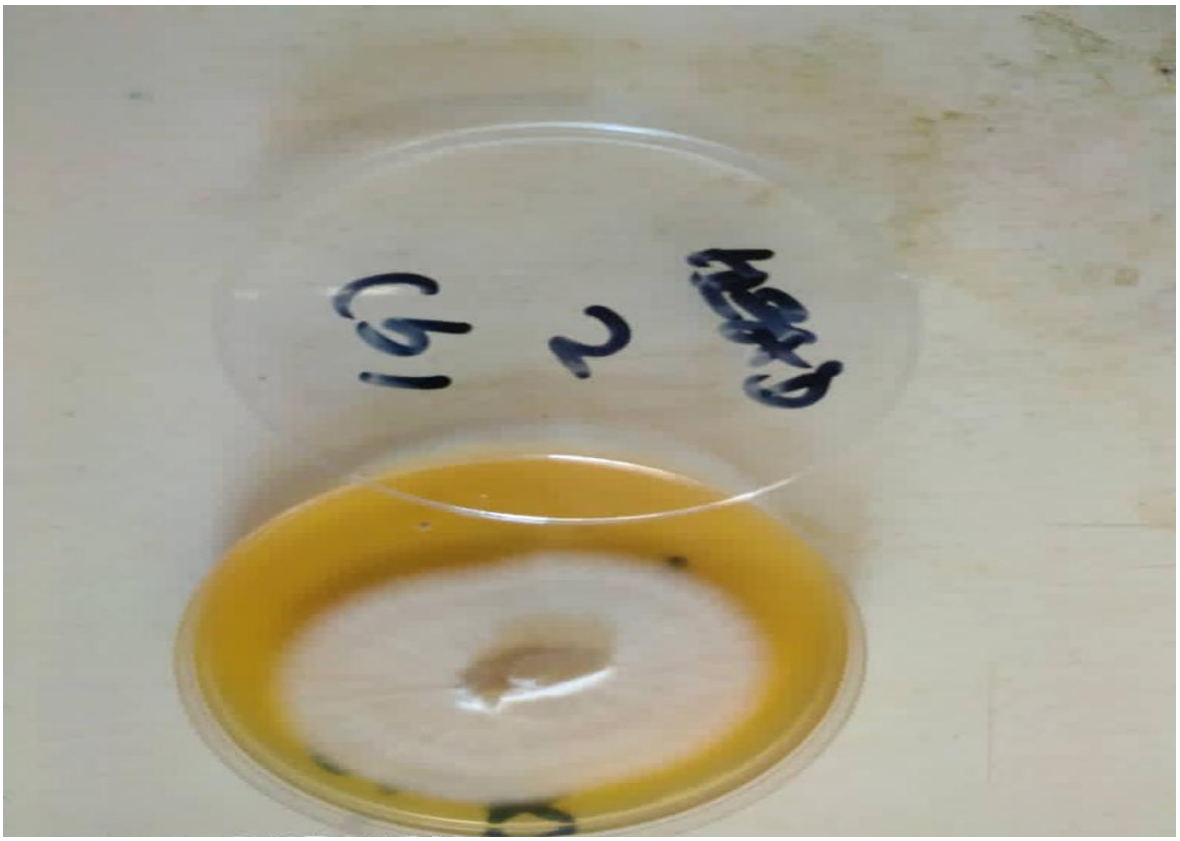

Plate III: *Trichophyton rubrum* isolates on SDA plate

4.1.2 Weight, colour and texture of the extract

The yield of the 500 g weight of the pulverized plant material extracted exhaustively with methanol were measured and recorded with the following yield *Lawsonia inermis* extract has a weight of 47 g with a percentage yield of 9.4% while *Enantia chlorantha* extract had a weight of 21 g with a percentage yield of 4.2% and *Securidaca longipedunculata* stem extract had a weight of 30 g with a percentage yield of 6% as shown in Table 4.2.

Table 4.2: Weight, percentage yield colour and texture of the extract using 500 g of the dried powder

Plant materials	**WE(g)**	**PY (%)**	**Colour**	**Texture**
Lawsonia inermis leaf	47	9.4	light green	non-sticky solid
Enantia chlorantha stem	21	4.2	dark yellow	sticky solid
Securidaca longipedunculata stem	30	6.0	dark brown	sticky solid

Key: WE= weight of extract, PY= percentage yield and g= gram

4.1.3 Molecular characterization of the fungal isolates

Polymerase chain reaction amplifications of total genomic DNA of the fungal isolates using primer pair: 5’ TCC GTA GGT GAA CCT GCG G 3’and - ITS 4: 5’ TCC TCC GCT TAT TGA TAT GC 3’ primers produced a PCR product of about 500 base pair (bp) (plate iv). The PCR products were sequenced (GenBank Accession Number: MZ209277 and MZ209278 for *Trichophyton rubrum* and *Trichophyton schoenleinii* respectively) as seen in Table 4.3. The phylogenetic tree of the isolates was also drawn to show the relations among the organisms and their origin using the NCBI database as represented in Figure 4.2.

Table 4.3: Molecular characterization of the fungal isolates

SAMPLE ID	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
1	*Trichophyton rubrum*	1236	1293	99%	0	99.56%	718	MZ209277
2	*Trichophyton schoenleinii*	34386	1157	99%	0	99.84%	681	MZ209278

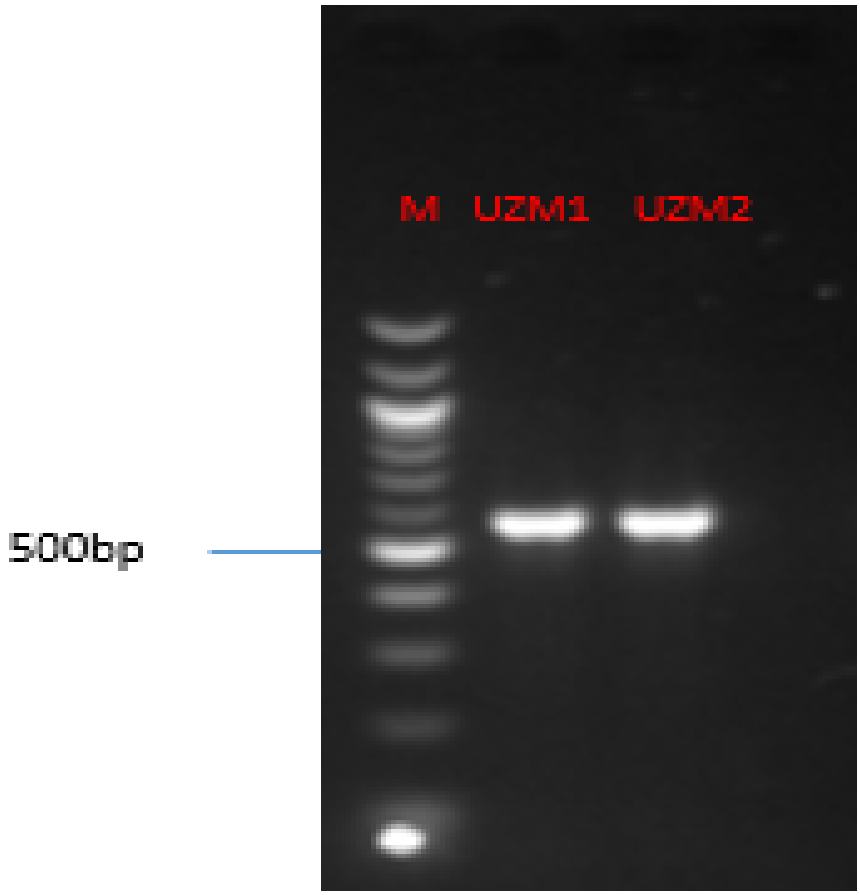

Plate IV: Polymerase Chain Reaction amplification of the DNA region of the fungal isolates using the ITS1, 5.8S and ITS2primer.

Keys: M = 500bp DNA ladder, UZM1, UZM2 = PCR reaction mixture with DNA of the samples to be identified as UZM1= *Trichophyton rubrum* UZM2=*Trichophyton schoenleinii*

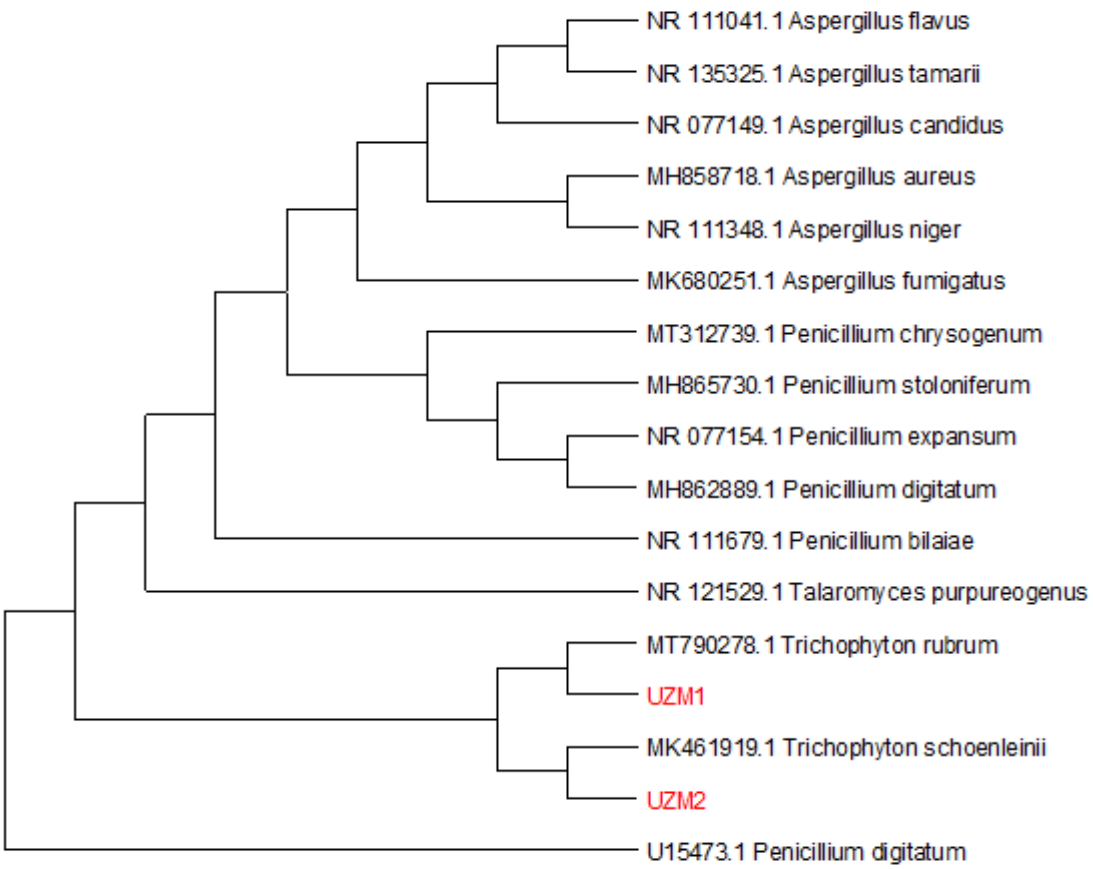

Figure 4.1: Phylogentic tree showing the relations among the fungal isolates

Key: UZM1=*Trichophyton rubrum* UZM2=*Trichophyton schoenleinii*

4.1.4 Antifungal activity of the plant extracts

4.1.4.1 Antifungal activity of the Lawsonia inermis leaf methanolic crude extract

Antifungal activity of *Lawsonia inermis* leaf methanolic crude extract at the lower concentration does not have significant activity against the three isolated organisms, significant activity was observed at 150 mg/ml (7.40± 0.20 mm), 200 mg/ml (10.05±0.05 mm), 250 mg/ml (13.15±0.85 mm) and 300 mg/ml (13.35±0.45 mm) for *T. rubrum,* 200 mg/ml (9.20±040 mm), 250 mg/ml (13.20±0.20 mm) and 300 mg/ml (16.65±0.35 mm), for *T. schoenleinii* and 250 mg/ml (5.25±0.15 mm) and 300mg/ml (5.80±0.20 mm) for *M. audouinii* as observed in Table 4.4 and these values can be compared to the positive control (fluconazole 50 mg and ketoconazole 50 mg)

4.1.4.2 Antifungal activity of the Enantia chlorantha stem methanolic crude extract

The significant activity of *Enantia chlorantha* stem extract was observed to have activity at 100 mg/ml (9.40±1.40 mm), 150 mg/ml (6.50±6.50 mm), 200 mg/ml (14.60±0.10 mm), 250 mg/ml (16.90±0.10 mm) and 300 mg/ml (18.15±0.15 mm) for *T. rubrum,* 150 mg/ml (4.10±4.10 mm), 200 mg/ml (9.80±0.20 mm), 250 mg/ml (12.00±0.30 mm) and 300 mg/ml (13.00±0.20 mm) *T. schoenleinii* and 200 mg/ml (7.00±0.20 mm), 250 mg/ml (9.45±0.50 mm) and 300 mg/ml (11.55±0.55 mm) for *M. audouinii* as observed in Table 4.5 and these values can be compared to the positive control (fluconazole 50 mg and ketoconazole 50 mg).

4.1.4.3 Antifungal activity of the Securidaca longipedunculata stem methanolic crude extract

Activity of *Securidaca longipedunculata* stem extract was observed to have activity at a relatively higher concentration for *M. audouinii* at 200 mg/ml (3.50±3.50 mm), 250 mg/ml (10.85±0.25 mm) and 300 mg/ml (14.80±0.20 mm), while for *T. rubrum* and *T. schoenleinii* were similar starting from 150 mg/ml (7.90±0.10, 7.65±0.50mm), 200 mg/ml

(9.90±0.90, 9.80±0.20mm), 250 mg/ml (12.10±0.00, 5.50±5.50mm) and 300 mg/ml (14.10±0.10, 13.80±0.10mm) respectively with no activities at lower concentration as observed in Table 4.6 and these values can be compared to the positive control (fluconazole 50mg and ketoconazole 50mg).

Table 4.4: Mean zones of inhibition of crude extract of *Lawsonia inermis* against fungal isolates

Organism	Conc (mg/ml)								Fluconazole	Ketoconazole
	15	25	50	100	150	200	250	300	50mg/ml	50mg/ml
T. schoenleinii	-	-	-	-	-	9.20±040[d]	13.20±0.20[c]	16.65±0.35[c]	29.55±0.65[a]	31.50±0.25[a]
M. audouinii	-	-	-	-	-	-	5.25±0.15[b]	5.80±0.20[b]	29.95±0. 05[a]	30.55±0.85[a]
T. rubrum	-	-	-	-	7.40± 0.20[d]	10.05±0.05[c]	13.15±0.85[b]	13.35±0.45[b]	29.25±0.55[a]	34.55±0.00[a]

Key: conc=concentration

Table 4.5: Mean zones of inhibition of crude extract of *Enantia Chlorantha* against fungal isolates

Organism	Conc (mg/ml) 15	25	50	100	150	200	250	300	Fluconazole 50mg/ml	Ketoconazole 50mg/ml
T. schoenleinii	-	-	-	-	4.10±4.10^{e}	9.80±0.20^{d}	12.00±0.30^{c}	13.00±0.20^{c}	29.55±0.65^{b}	31.50±0.25^{a}
M. audouinii	-	-	-	-	-	7.00±0.20^{e}	9.45±0.50^{d}	11.55±0.55^{c}	29.95±0.05^{b}	30.55±0.85^{a}
T. rubrum	-	-	-	9.40±1.40^{d}	6.50±6.50^{e}	14.60±0.10^{c}	16.90±0.10^{b}	18.15±0.15^{b}	29.25±0.55^{b}	34.55±0.00^{a}

Key: conc=concentration

Table 4.6: Mean zones of inhibition of crude extract of S*acuridata longipediculata* against fungal isolates

Organism	Conc (mg/ml)								Fluconazole	Ketoconazole
	15	25	50	100	150	200	250	300	50mg/ml	50mg/ml
T. schoenleinii	-	-	-	-	7.65±0.50^{d}	9.80±0.20^{c}^{d}	5.50±5.50^{e}	13.80±0.10^{c}	29.55±0.65^{b}	31.50±0.25^{a}
M. audouinii	-	-	-	-	-	3.50±3.50^{f}	10.85±0.25^{d}	14.80±0.20^{c}	29.95±0. 05^{b}	30.55±0.85^{a}
T. rubrum	-	-	-	-	7.90±0.10^{e}	9.90±0.90^{d}	12.10±0.00^{c}	14.10±0.10^{c}	29.25±0.55^{b}	34.55±0.00^{a}

Key: conc=concentration

4.1.5 Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC)

4.1.5.1 Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of Lawsonia inermis leaf extract

The MIC and MFC of the crude extracts of *Lawsonia inermis* leaf on *M. audouinii* and *T. schoenleinii* were higher with 150 mg/ml and 300 mg/ml respectively whereas *T. rubrum* had the least MIC of 37.5 mg/ml and MFC of 75 mg/ml as observed in Figure 4.2 and for the fractionated extract *M. audouinii and T. schoenleinii* were similar (37.5 mg/ml) while *T. rubrum* had 75 mg/ml for hexane fraction as seen in Figure 4.6 and similar interpretation for ethyl acetate were observed in Figure 4.7, *M. audouinii* had the highest MIC(75 mg/ml) and MFC (150 mg/ml), followed by *T. rubrum* with MIC (37.50 mg/ml) and MFC (75 mg/ml) and *T. schoenleinii* had the least MIC (9.36 mg/ml) and MFC (18.76 mg/ml) and for residual aqueous the MIC and MFC were the same *M. audouinii* and *T. rubrum* (37.50 and 75.0 mg/ml respectively) and *T. schoenleinii* had the least (18.75 and 37.50 mg/ml) as observed in Figure 4.8.

4.1.5.2 Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of Enantia chlorantha stem extract

The crude extracts of *Enantia chlorantha* stem on *M. audouinii* had the highest MIC and MFC of 37.70 mg/ml and 75 mg/ml respectively whereas *T. rubrum* had the least MIC of 18.75mg/ml and MFC of 37.50 mg/ml as observed in Figure 4.4 and for the fractionated extract for hexane in Figure 4.9 *M. audouinii* is highest MIC (75 mg/ml) and MFC (150 mg/ml) followed by *T. schoenleinii* MIC (37.5 mg/ml) and MFC (75 mg/ml) while *T. rubrum* had the least MIC (18.75 mg/ml) and MFC (37.5 mg/ml), for ethyl acetate fraction in Figure 4.10, *T. rubrum* had the highest MIC (31.25 mg/ml) and MFC (62.50 mg/ml) and *T. schoenleinii* had the least MIC (7.81 mg/ml) and MFC (15.60

mg/ml) for residual aqueous *T.rubrum* had the highest MIC (62.50 mg/ml) and MFC(125.00 mg/ml) followed by *T. schoenleinii* MIC(31.20 mg/ml) and MFC (62.50 mg/ml) and the least is *M. audouinii* MIC (7.81mg/ml) and MFC (15.60 mg/ml) as observed in Figure 4.11.

4.1.5.3 Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of Sacuridaca longipedunculata stem extract

The MIC and MFC of the crude extracts of *Sacuridaca longipedunculata* stem on *M. audouinii* were the least with MIC and MFC of 9.37 mg/ml and 18.75 mg/ml respectively in Figure 4.5 while *T. schoenleinii* had the highest with 150 mg/ml and 300 mg/ml respectively and for the fractionated extract *M. audouinii and T. rubrum* were similar with 31.25 mg/ml and 75 mg/ml respectively for ethyl acetate fraction Figure 4.12 while *T. schoenleinii* had 125 mg/ml and 250 mg/ml respectively for residual aqueous fraction in Figure 4.13.

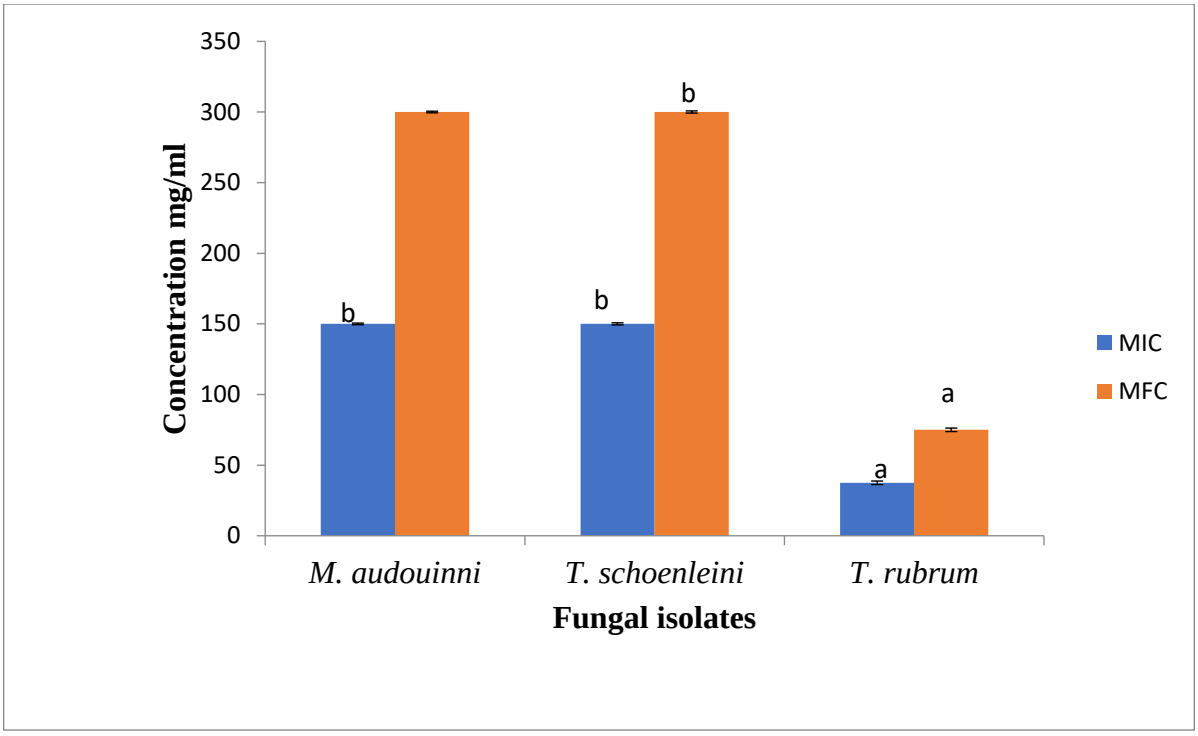

Figure 4.2: Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of methanolic crude extract of *Lawsonia inermis* leaves

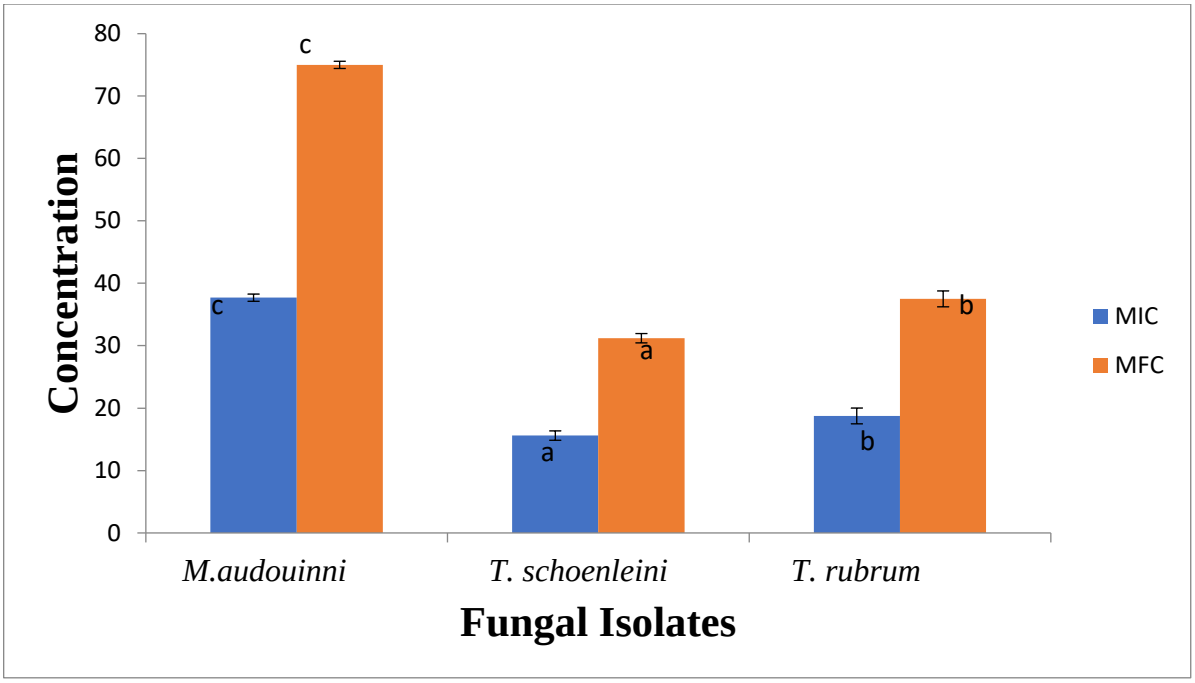

Figure 4.3: Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of crude methanolic extract of *Enantia chlorantha*

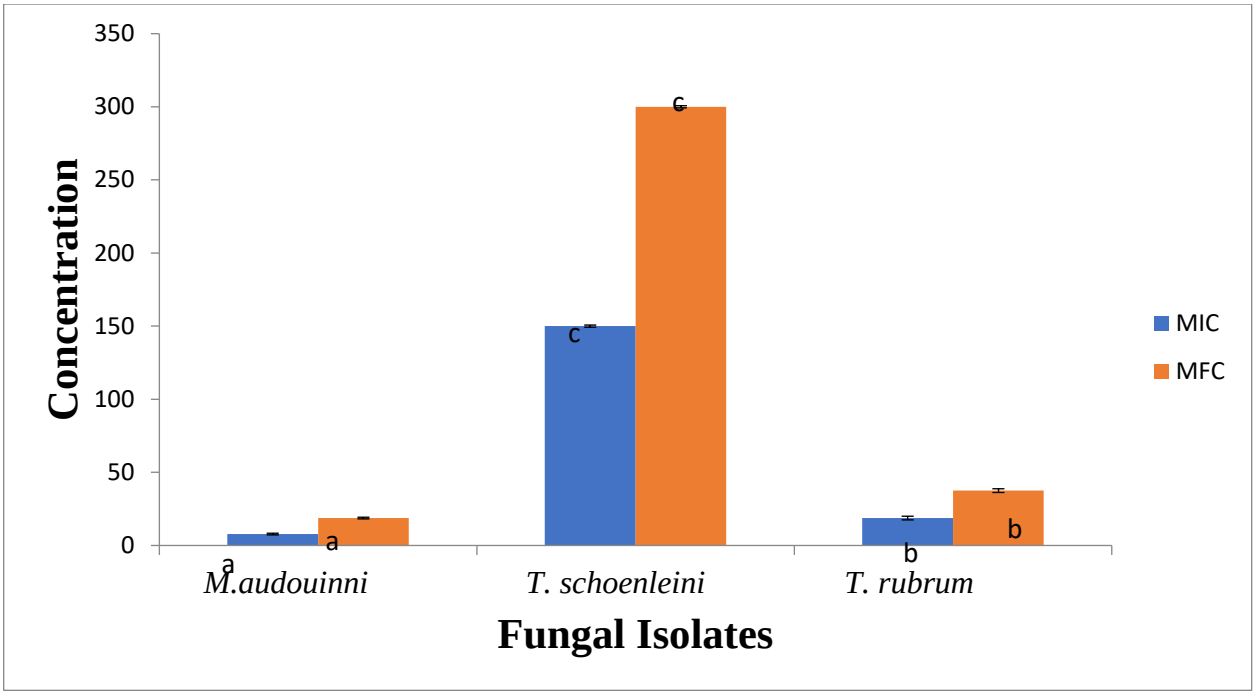

Figure 4.4: Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of methanolic crude extract of *Sacruridata longipediculata*

4.1.6 Weight and percentage yield of fractions recovered from the extract

Fractions of n-hexane, ethyl acetate and residual aqueous fraction of *Lawsonia Inermis* leaf (14%, 46.6% and 37.3% respectively), *Enantia Chlorantha* stem (8.6%, 30% and 60% respectively), and *Securidaca longipedunculata* stem (0%, 27% and 73% respectively), extract were recovered from 15g each of the crude methanolic extract.

4.1.7 Antifungal activity of *Lawsonia inermis* leaf fraction, *Enantia chlorantha* stem fractions and *Securidaca longipedunculata* stem fractions against the test organism

A significant activity were observed across almost all the concentration (150, 250 and 300 mg/ml) of both ethyl acetate and residual aqueous fraction of *Lawsonia inerm* leaves against the three clinical isolate were significant at 150, 250 and 300 mg/ml while the n-hexane fraction *T. rubrum* and *T. schoenleinni* activities were observed at 250 and 300 mg/ml no activity was observed for *M. audouinii* at all concentrations except at 300 mg/ml as observed on Table 4.10.

For *Enantia chlorantha* the zones of inhibition was significant at 250 and 300 mg/ml for *T. schoenleinni* using hexane fraction while *M. audouinii* and *T. rubrum* were only significant at 300 mg/ml, while all the concentrations (150, 250 and 300 mg/ml) were significant for the ethyl acetate and residual aqueous fraction against the three organisms as observed on Table 4.11.

For *Securidaca longipedunculata* there was no yield with hexane hence ethyl acetate and residual aqueous extract showed significance 250 mg/ml for *T. rubrum* and *T. schoenleinni* and 300 mg/ml for *M.audouinii* using ethyl acetate fraction while it showed significance for all concentration using residual aqueous fraction as observed on Table 4.12

Table 4.7: Mean zones of inhibition of *Lawsonia inermis* fractions against fungal isolates

Organism	Concentration (mg/ml)								
	H150	**H250**	**H300**	**A150**	**A250**	**A300**	**E150**	**E250**	**E300**
T. schoenleinii	-	6.20 ± 0.20^{c}	7.85 ± 0.15^{c}	7.10 ± 7.10^{c}	17.30 ± 0.20^{b}	18.15 ± 0.15^{b}	73.25 ± 59.75^{a}	17.10 ± 0.10^{b}	17.80 ± 0.10^{b}
M audouinii	-	-	5.20 ± 0.20^{b}	-	7.80 ± 0.10^{a}	7.90 ± 0.10^{a}	-	7.30 ± 0.10^{a}	7.75 ± 0.50^{a}
T. rubrum	-	7.00 ± 0.00^{e}	8.95 ± 0.50^{d}	9.15±0.15	74.00 ± 60.00^{b}	81.50 ± 66.50^{a}	8.90 ± 0.10^{d}	13.05 ± 0.15^{c}	13.85 ± 0.15^{c}

Key: H = n-hexane; A = residual aqueous; E = ethyl acetate

Table 4.8: Mean zones of inhibition of *Enantia chlorantha* fractions against fungal isolates

Organism	Concentration (mg/ml)								
	H150	**H250**	**H300**	**A150**	**A250**	**A300**	**E150**	**E250**	**E300**
T. schoenleinii	-	7.85±0.85^{d}	9.85±0.25^{c}	12.55±0.50^{b}	14.85±0.15^{ab}	16.60±0.40^{a}	12.25±0.55^{b}	14.70±0.80^{ab}	16.45±0.55^{a}
M audouinii	-	-	3.50±3.50^{f}	-	7.00±0.00^{e}	9.90±0.10^{d}	11.60±0.60^{c}	16.10±0.90^{b}	19.60±0.40^{a}
T. rubrum	-	-	4.10±4.10^{f}	8.95±0.50^{e}	10.35±0.65^{d}	14.85±0.15^{b}	8.85±0.15^{e}	12.15±0.45^{c}	17.40±0.60^{a}

Key: H = n-hexane; A = residual aqueous; E = ethyl acetate

Table 4.9: Mean zones of inhibition of *Sacuridata longipediculata* fraction against fungal isolates

Organism	Conc (mg/ml)					
	A150	**A250**	**A300**	**E150**	**E250**	**E300**
TS	9.25±0.35^{c}	15.15±0.45^{b}	18.05±0.05^{a}	-	3.40±3.40^{d}	10.00±0.00^{bc}
MA	3.60±3.60^{d}	10.10±0.10^{b}	13.10±0.40^{a}	-	-	6.65±0.55^{c}
TR	3.35±3.35^{d}	9.65±0.05^{b}	14.65±0.35^{a}	-	6.05±0.05^{c}	7.95±0.15^{c}

Key: A = residual aqueous; E = ethyl acetate; TS = *T schoenleinii*; MA = *M audouinii*; TR = *T rubrum*; conc = concentration

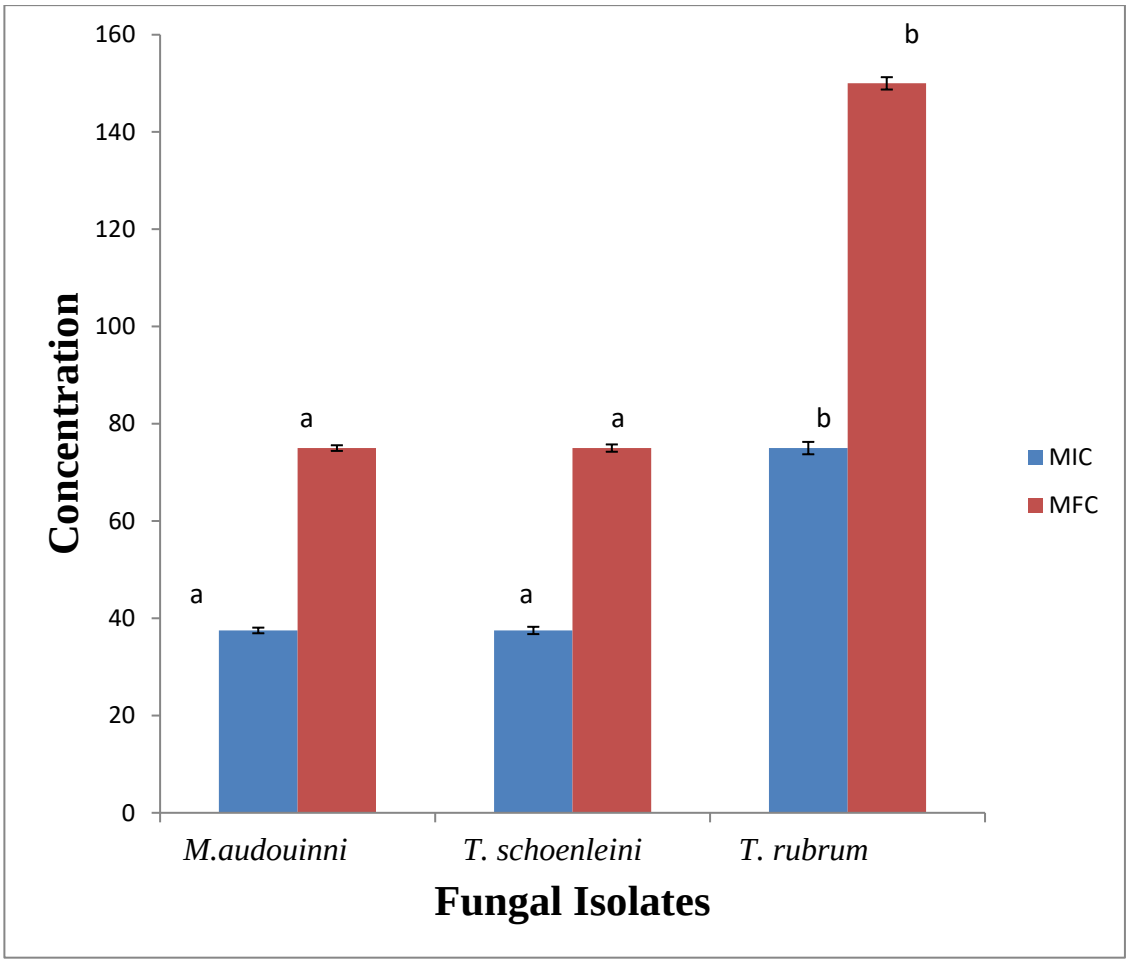

Figure 4.5: Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of hexane fraction of *Lawsonia inermis* leaves

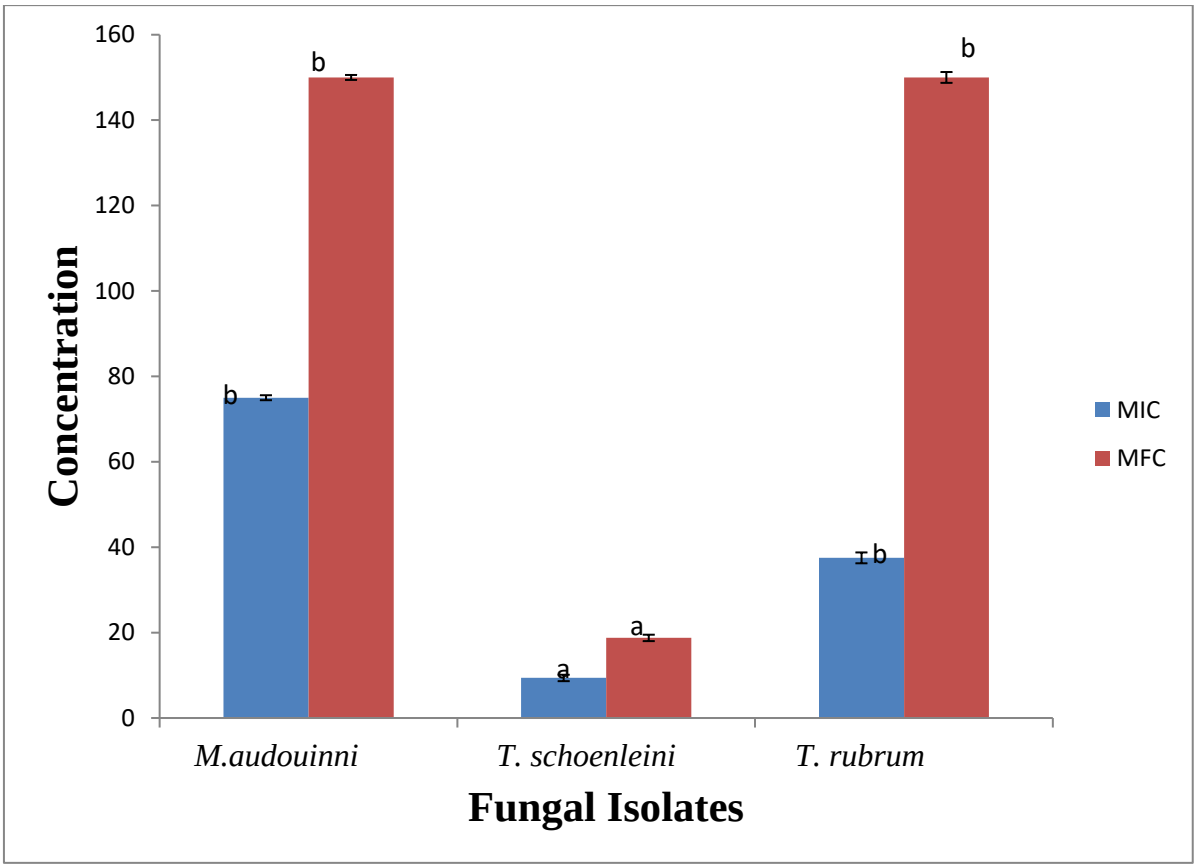

Figure 4.6: Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of ethyl acetate fraction of *Lawsonia inermis* leaves

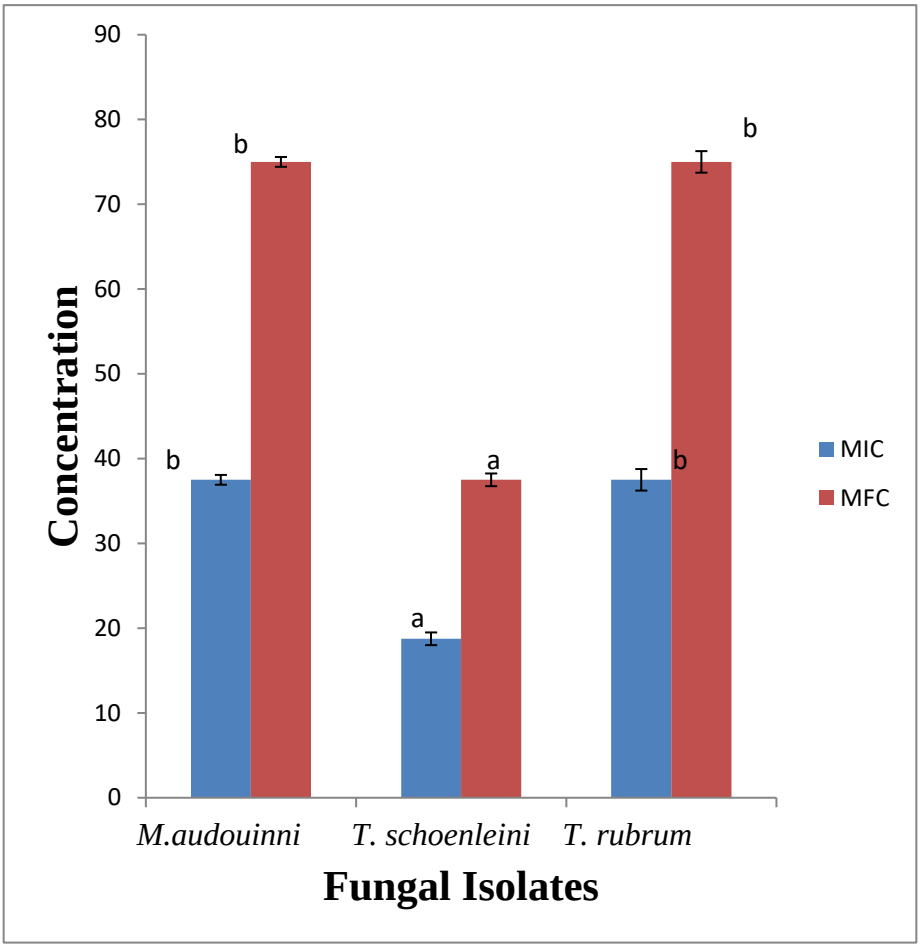

Figure 4.7: Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of residual aqueous fraction of *Lawsonia inermis* leaves

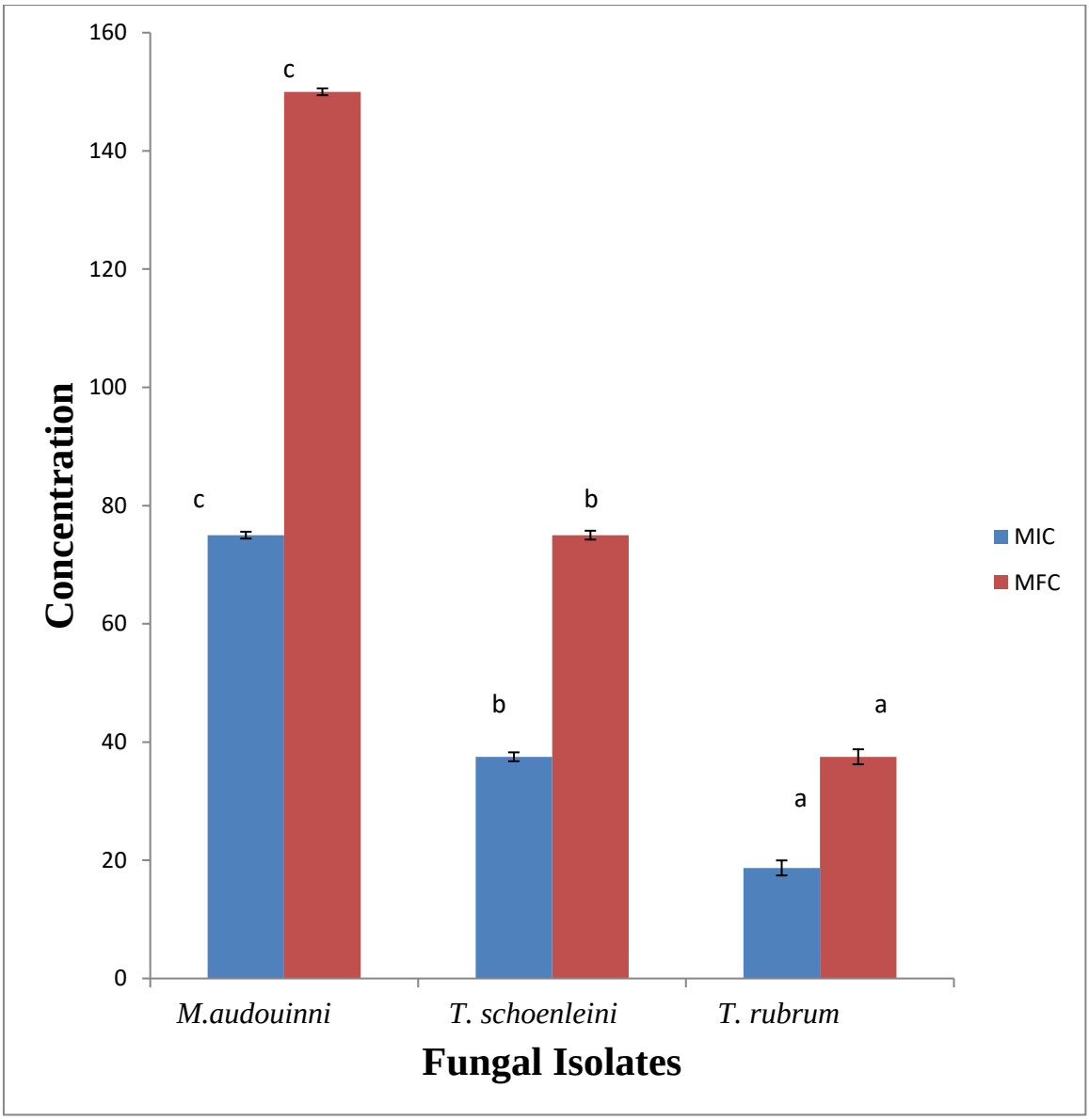

Figure 4.8: Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of hexane fraction of *Enantia chlorantha.*

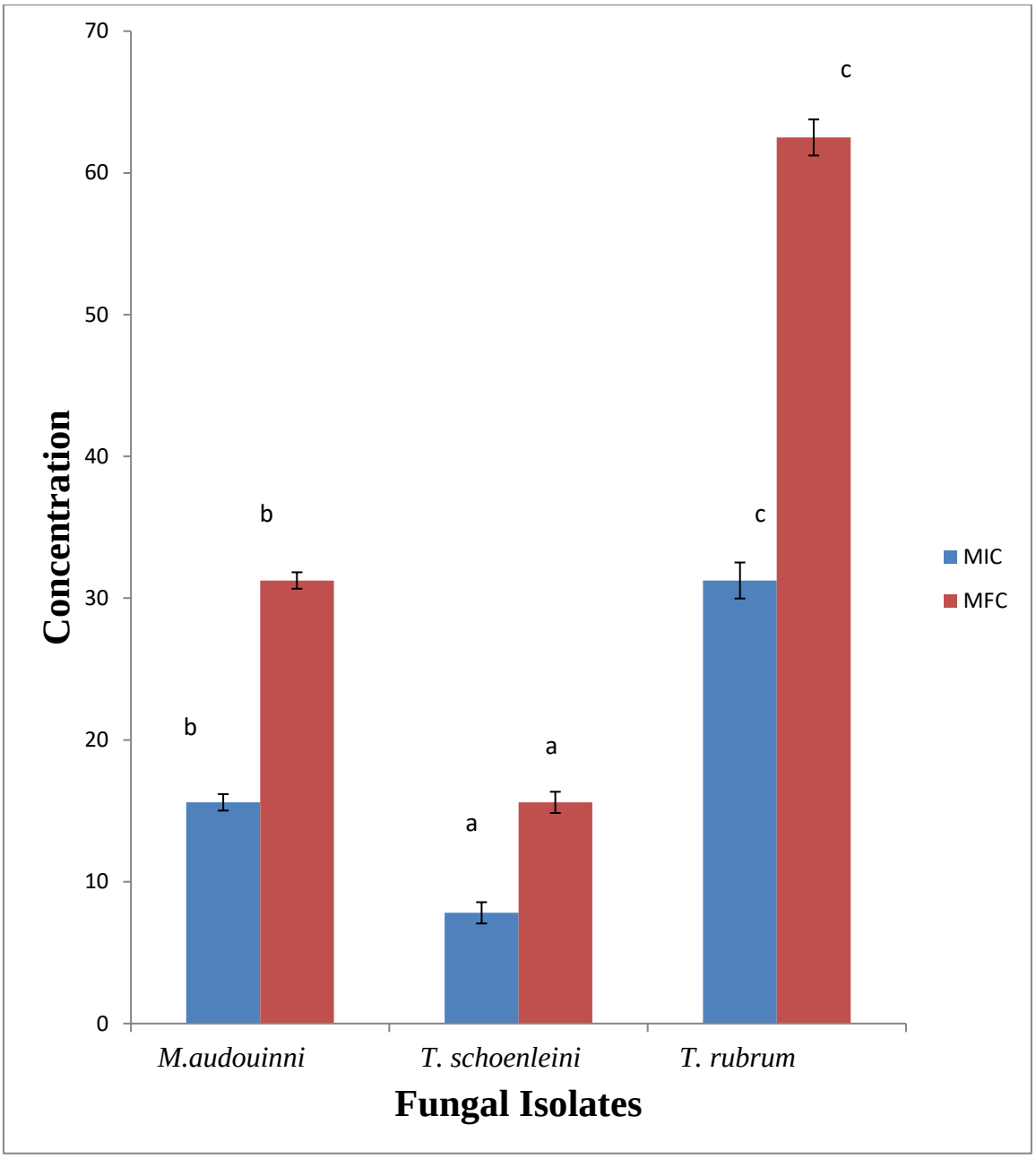

Figure 4.9: Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of ethyl acetate fraction of *Enantia chlorantha.*

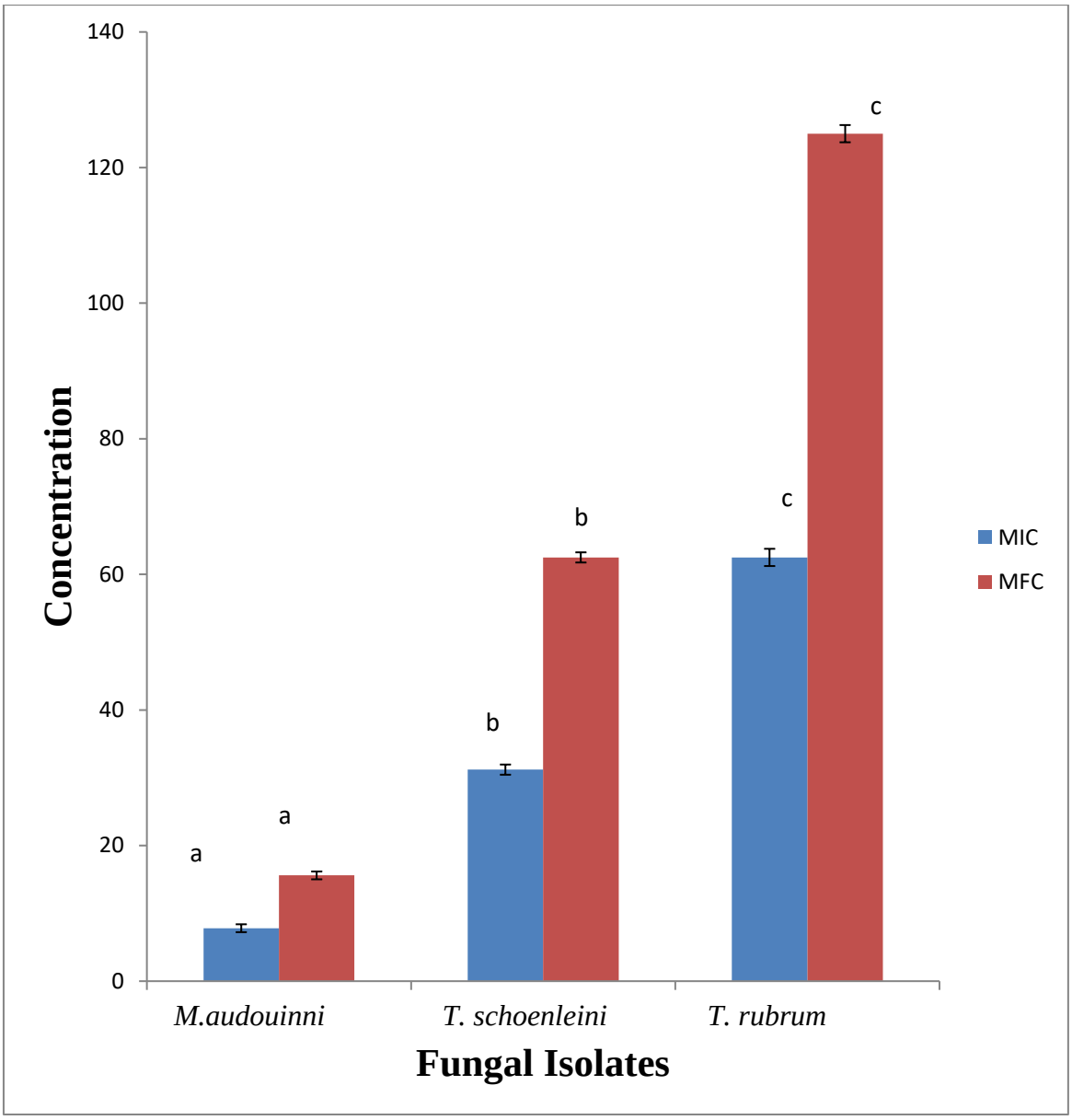

Figure 4.10: Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of residual aqueous fraction of *Enantia chlorantha*

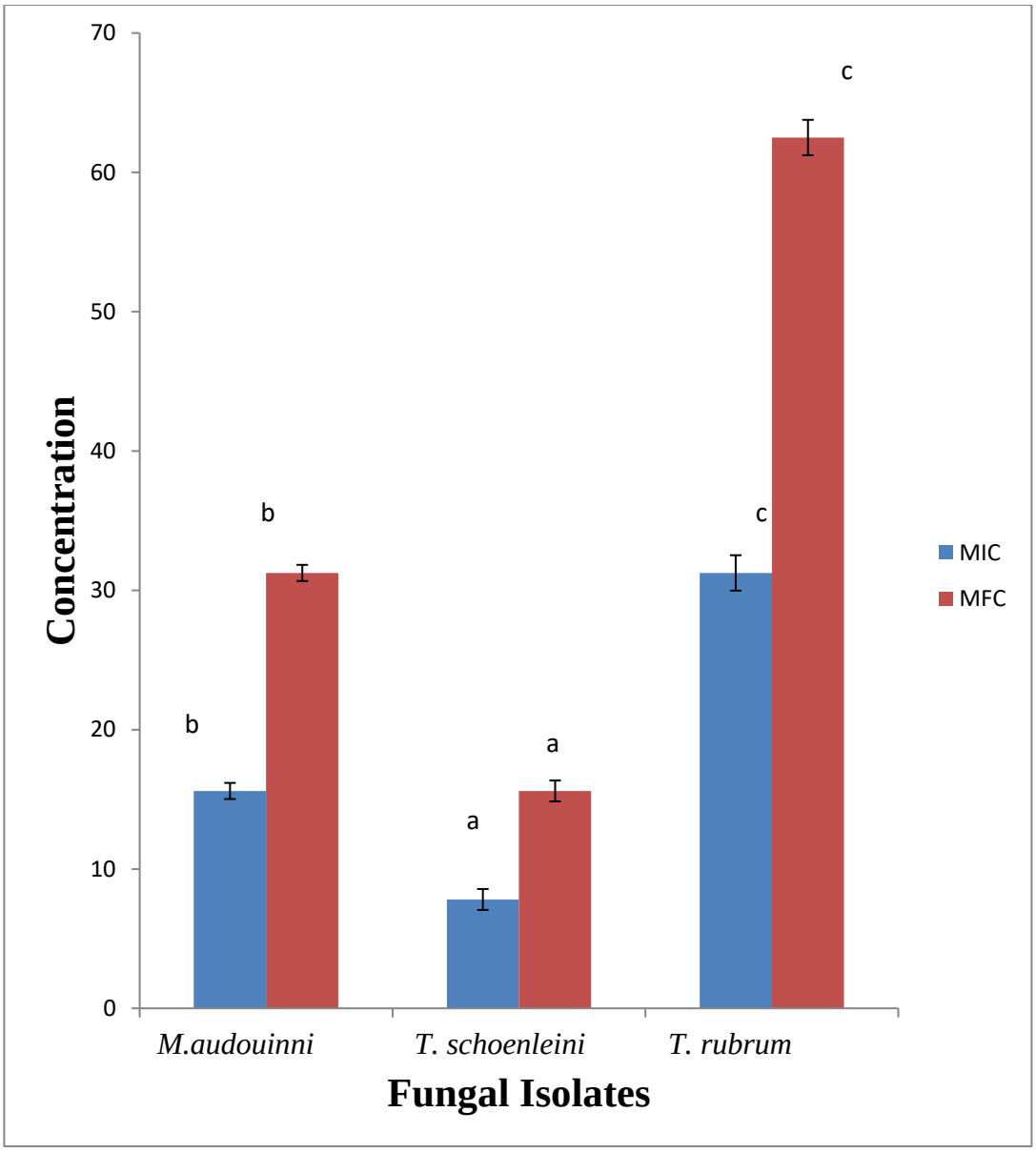

Figure 4.11: Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of ethyl acetate fraction of *Sacruridata longipediculata*

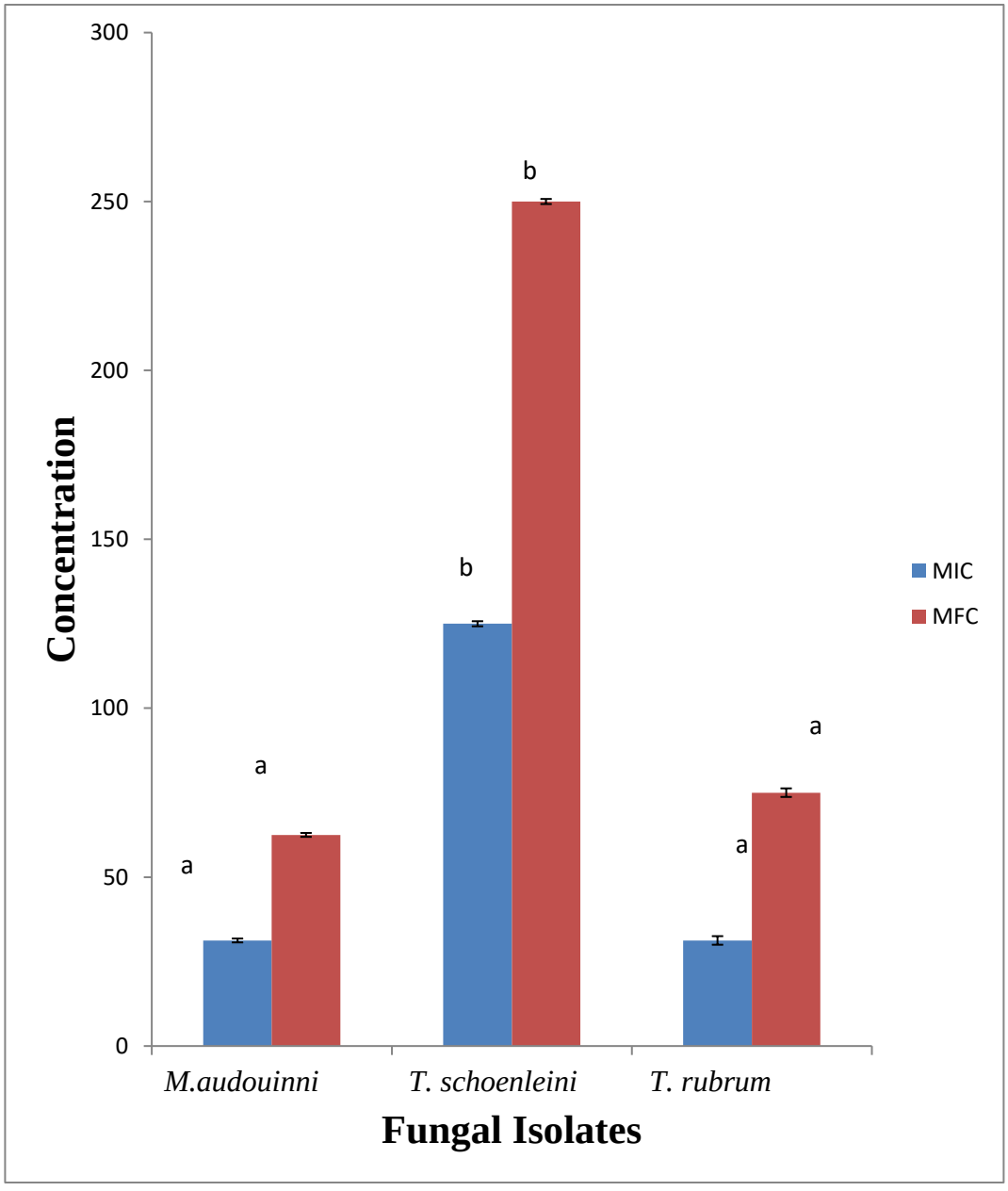

Figure 4.12: Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of residual aqueous fraction of *Sacruridata longipediculata*

4.1.8 Gas chromatography- mass spectroscopy (GC-MS) analysis

The GC-MS analysis of *Lawsonia inermis* fractions (ethyl acetate and residual aqueous) transpired 68 and 25 compounds respectively with retention time ranging from 4.541-22.943min for ethyl acetate fraction while for residual aqueous it ranged from 4.846-19.980 min in which the compounds with significant area percentage (≥3%) were summarized in Table 4.10. Analysis of *Sacuridata longipediculata* fractions (ethyl acetate and residual aqueous) transpired 45 and 16 compounds respectively with retention time ranging from 5.491-19.997min for ethyl acetate fraction while for residual aqueous it ranges from 5.772-19.978 min in which the compounds with significant area percentage (≥3%) were recorded in Table 4.11. The ethylaetate fraction of *Enantia chlorantha* has 23 components with a retention time range of 4.508-20.792 min were summarized in Table 4.12.

Table 4.10: GC-MS profiles of sub-fractions from *Lowsonia inermis* methanol extract

Peak number	Retention time(min)	Compound name	Molecular weight(g/mol)	Molecular formular	Peak Area (%)
			Ethyl acetate fractions		
15	8.450	Benzofuran, 2,3-dihydro-	12.15	C_8H_8O	8.29
46	14.972	n-hexadecanoic acid	256.4	$C_{16}H_{32}O_2$	9.67
52	16.213	9,12-octadecadienoic acid(z,z)	280	$C_{18}H_{32}O_2$	23.49
62	19.990	9,12-octadecadienoic acid(z,z)	280	$C_{18}H_{32}O_2$	8.48
			Residual aqueous fraction		
12	8.508	4H-pyran-4-one,2,3-dihydro3,5-dihydroxy	144.12	$C_6H_8O_4$	6.14
15	9.427	5-hydroxymethyl furfural	126.11	$C_6H_6O_3$	42.72

17	11.006	1,2,4-benzenetriol	126.11	$C_6H_3(OH)_3$	6.15
21	13.569	Maltose	342.1162	$C_{12}H2_2O_{11}$	26.17

Table 4.11: GC-MS profiles of sub-fractions from *Sacuridata longipediculata* methanol extract

Peak number	Retention time(min)	Compound name	Molecular weight(g/mol)	Molecular formula	Peak Area (%)
			Ethyl acetate fractions		
32	14.752	1,2-Benzene dicarboxylic acid	390	$C_{24}H_{38}O_4$	9.30
33	15.021	n-hexadecanoic acid	256.4	$C_{16}H_{32}O_2$	15.05
39	16.181	9,12-octadecadienoic acid(z,z)	280	$C_{18}H_{32}O_2$	11.99
45	19.997	9,12-octadecadienoic acid(z,z)	280	$C_{18}H_{32}O_2$	6.11
			Residual aqueous fraction		
4	7.848	Erythritol	122.120	$C_4H_{10}O_4$	3.32
13	15.933	1,5-Anhydro-d-mannitol	164.16	$C_6H_{12}O_5$	93.10

Table 4.12: GC-MS profiles of sub-fractions from *Enantia chlorantha* methanol extract

Peak number	Retention time(min)	Compound name	Molecular weight (g/mol)	Molecular formular	Peak Area (%)
			Ethyl acetate fractions		
2	5.259	Acetic acid	60.052	CH_3COOH	3.58
17	16.115	9,12-octadecadienoic acid(z,z)-	280	$C_{18}\ H_{32}O_2$	16.21
21	20.015	9,12-octadecadienoic acid(z,z)-	280	$C_{18}\ H_{32}O_2$	28.05

4.1.9 Quantitative phytochemical screening of plant extracts

Quantitative phytochemical screening of all the three extracts indicates the presence of the tested compounds such as flavonoids, phenols, tannins Alkaloid and Saponins. From Tables 4.4, *Lawsonia inermis* (LLI) leaves had the highest amount of flavonoids (89.84 mg/100 g), then *Securidaca longipedunculata* stem (SSL) (62.21 mg/100 g) while *Enantia chlorantha stem* (SEC) had the least amount of flavonoids (26.09mg/100g), similarly LLI had the highest values of phenols (822.11 mg/100 g) then SSL (509.68 mg/100 g) and SEC (416.07 mg/100 g) with the least amount, LLI had the highest values of Tanins (181.95 mg/100 g) then SSL (87.75 mg/100 g) and SEC (26.45 mg/100 g) with the least amount and observed with saponins also LLI had the highest amount (346.83 mg/100 g) then SEC (117.86 mg/100 g) and SSL (35.28 mg/100 g) with the least amount. It was observed that the alkaloidal content in SSL was highest (380.92 mg/100 g), followed by LLI (346.83mg/100g) and SEC had the least value (20.67 mg/100 g) while for the fraction in Tables 4.5 shows that SEC has the highest amount of flavonoids(156.69 mg/100 g), Tannins (163.48 mg/100 g), Saponins (232.31 mg/100 g) while LLI had the highest amount of phenols (754.54 mg/100g) and alkaloids (39.78 mg/100 g) and in Table 4.6 LLI had the highest amount of phenols (506.72 mg/100 g), saponins (290.03 mg/100 g) and alkaloids (34.24 mg/100 g) while SEC has the highest amount of flavonoids (115.00 mg/100 g) and Tannins (349.54 mg/100 g).

Table 4.13: Contents (mg/100g) of crude plant extracts

Extracts	Flavonoids	Phenol	Tannins	Alkaloids	Saponins
LLI	89.84±0.73^{d}	822.11±0.55^{a}	181.95±0.40^{c}	49.90±0.22^{e}	346.83±1.08^{b}
SEC	26.09±0.05^{c}	416.07±0.07^{a}	26.45±0.17^{c}	20.67±0.57^{c}	117.86±0.22^{b}
SSL	62.21±3.56^{d}	509.68±10.26^{a}	87.75±1.25^{c}	380.92±12.66^{b}	35.28±0.50^{e}

Keys: LLI = Leaves of *Lawsonia inermis*, SEC = Stem bark extract of *Enantia chlorantha*, SSL = Stem extract of *Securidaca longipedunculata*

Table 4.14: Quantitative phytochemical contents (mg/100g) residual aqueous of plant extracts

Extracts	Flavonoids	Phenol	Tannins	Alkaloids	Saponins
LLI	46.75 ± 1.07^{d}	754.54 ± 0.42^{a}	74.38 ± 0.29^{c}	39.78 ± 0.21^{e}	213.76 ± 1.02^{b}
BEC	156.69 ± 1.39^{c}	174.23 ± 0.23^{b}	163.48 ± 0.76^{b}	27.88 ± 0.03^{d}	232.31 ± 0.54^{a}

Keys: LLI = Leaves of *Lawsonia inermis*, SEC = Stem bark extract of *Enantia chlorantha*

Table 4.15: Quantitative phytochemical contents (mg/100g) of ethyl acetate fraction of plant extracts

Extracts	Flavonoids	Phenol	Tannins	Alkaloids	Saponins
LLI	67.95±0.74^{c}	506.72±0.04^{a}	280.42±0.17^{b}	34.24±0.56^{d}	290.03±0.50^{b}
BEC	115.00±0.11^{c}	124.04±0.18^{c}	349.54±0.14^{a}	27.98±0.06^{d}	199.03±0.49^{b}

Keys: LLI = Leaves of *Lawsonia inermis,* SEC = Stem bark extract of *Enantia chlorantha*

4.2 Discussions

The resistance of fungi to usual antimicrobials has led to a high incidence of treatment failures and a considerable increase in treatment cost (Etame *et al*., 2018). Medicinal plants could be used as an alternative as their pharmacological properties especially its antimicrobial activities. Several studies have shown that efficacy of the plant extracts against the test isolates could be attributed to the presence of some bioactive compounds in the plants (Yusha'u *et al*., 2011).

4.2.1 Collection of test organisms

The organisms were isolated from the nails, feet and head which was similar to the study by Khan *et al.* (202l) showing that dermatophytes are superficial fungal infection which can be easily identified by direct microscopy or culture method

4.2.2 Weight, colour and texture of the extract

The weight and colour of the crude extracts is influenced by the type of plants, solvents used and the phytochemicals present.

4.2.3 Molecular characterization of the fungal isolates

The molecular characterization of the organism conforms with that of national institute of standard technical data base with the same base pair and same on the phylogenetic tree

4.2.4 Antifungal activity of the plant extracts

According to Clinical and Laboratory Standard Institute (2019) when the zone of inhibition is 10 mm and above it means that the test the chemical significantly inhibits the test isolates.

4.2.4.1 Antifungal activity of the Lawsonia inermis leaf methanolic crude extract

In this studies it shows that only *T. schoenleinii* and *T. rubrum* were sensitive to the crude leaf extract of *L. inermis* at the concentration of 250 mg/ml and 300 mg/ml while for the fractions *L. inermis* does not have any significant activities with n-Hexane on the three isolate, with residual aqueous only *T. rubrum* has a significant value at 250 mg/ml and 300 mg/ml and for ethyl acetate fraction both *T. schoenleinii* and *T. rubrum* are having significant values at 250 mg/ml and 300 mg/ml with no activity for *M. audouinii*.

4.2.4.2 Antifungal activity of the Enantia chlorantha stem methanolic crude extract

The crude extract of *Enantia chlorantha* had significant activity on *T. schoenleinii* at 250 mg/ml and 300 mg/ml, for *M. audouinii* it was significant at 300 mg/ml only and for *T. rubrum* it was significant at 200, 250 and 300 mg/ml. The fractions of *Enantia chlorantha* shows that there were no significant activity using n-Hexane for the three isolates using the residual aqueous activities were observed at 150 mg/ml, 250 mg/ml and 300 mg/ml for *T. schoenleinii* while *T. rubrum* was significant at 250 and 300 mg/ml and for ethyl acetate activities were observed at 150 mg/ml, 250 mg/ml and 300 mg/ml for the three isolates.

4.2.4.3 Antifungal activity of the Securidaca longipedunculata stem methanolic crude extract

Sacuridata longipediculata crude extract was only significant 300 mg/ml for *T. schoenleinii* and for *M. audouinii* and *T. rubrum* there were activity at 250 mg/ml and 300 mg/ml concentration. The fractionated extract shows significant activities at 250 mg/ml and 300 mg/ml for *T. schoenleinii* and *M. audouinii* using residual aqueous fraction while *T. rubrum* was significant at 300 mg/ml the ethyl acetate fraction was only significant on *T. schoenleinii*

at the concentration of 300 mg/ml. The lack of activities on other concentrations and fractions might be due to suboptimal concentration or the over growth of the organism.

4.2.5 Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC).

4.2.5.1 Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of Lawsonia inermis leaf extract

The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) for *Lawsonia inermis* showed that n-hexane and residual aqueous fractions had the least values (37.5 mg/ml MIC and 75 mg/ml MFC) followed by ethyl acetate fraction (75mg/ml MIC and 150mg/ml MFC) and the highest value (150 mg/ml MIC and 300mg/ml MFC) were observed with the crude extract when tested on *M. audouinii*, similar pattern were observed when *T. schoenleinii* was tested with ethyl acetate fraction had the least value followed by residual aqueous fraction then n-hexane fraction and the crude has the highest values. While the plant extract was tested on *T. rubrum* it was observed that the crude extract, residual aqueous and ethyl acetate fraction have the same values and the least values were seen with n-hexane fraction. This might suggest that polarity might have effect on the extracts and eventually its activity.

4.2.5.2 Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of Enantia chlorantha stem extract

Enantia chlorantha extract MIC and MFC were observed on *M. audouinii*, the residual aqueous fraction has the least values (7.81 mg/ml MIC and 15.60 mg/ml MFC), then ethyl acetate fraction followed by the crude extract and n-hexane has the highest values (75 mg/ml MIC and 150 mg/ml MFC), when tested on *T. schoenleinii* ethyl acetate had the least value

followed by the crude extract, residual aqueous and n-hexane had the highest value and on *T. rubrum* the least value were observed on both the crude extract and n-hexane fraction followed by ethyl acetate fraction and the highest were observed on the residual aqueous fraction.

4.2.5.3 Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of Sacuridaca longipedunculata stem extract

Sacuridata longipediculata extract showed an MIC and MFC on *M. audouinii* the crude extract has the least (9.37 mg/ml MIC and 18.75 mg/ml MFC) value followed by ethyl acetate fraction and the residual aqueous has the highest value (with 150 mg/ml MIC and 300 mg/ml MFC), tests on *T. schoenleinii* ethyl acetate fraction has the least value followed by the residual aqueous fraction and the crude extract has the highest values and when tested on *T. rubrum* the crude extract has the lowest value while the residual aqueous and ethyl acetate fraction have the same value. *Sacuridata longipediculata* extract the n-hexane fraction had poor yield and the MIC and MFC could not be determined.

4.2.6 Weight and percentage yield of fractions recovered from extracts

Previous studies showed that the weight and percentage yield of extracts greatly depends on the type of plant, plant part and the type of solvent used. The chemical nature of each constituent of the plants parts varies hence their solubility is in a given the least polar solvent had the least percentage. The percentage yield of two plants under study proves that the plants possess high potential source for the Phyto compounds. The yield bases on the polarity of the solvents an indication of the plants pharmacological importance.

The extracts also show difference in nature and colours with extracting solvents. This indicates the difference in the composition of the extracts and each solvent extract varied in

component and quantities. The fraction of the crude extracts of hexane, ethyl acetate, methanol and residual aqueous (Nuhu and Magashi, 2017).

4.2.7 Antifungal Activity of *Lawsonia inermis* leaf fraction, *Enantia chlorantha* stem fraction and *Sacuridaca longipedunculata* stem fraction against the test organism

The antifungal activity of *Lawsonia inermis* leaf fraction, *Enantia chlorantha* stem fraction and *Sacuridaca longipedunculata* stem fraction against the test organism were having similar pattern as the crude following an increase in polarity with residual aqueous fraction having a better antifungal activity followed by ethyl acetate fraction and then least n-haxane fraction which agrees with the study of Okogbenin *et al.* (2014).

4.2.8 Gas chromatography- mass spectroscopy (GC-MS) analysis

The GC-MS analysis of fraction of the three plants revealed there are different compounds which might be responsible for its biological activities (Innalegwu *et al*., 2021).

Lawsonia inermis having 68 compounds and the major compound found in the ethyl acetate fraction was 9, 12-octadecadienoic acid(z,z)-(23.49%), in a study carried out by Adeoye-isijola *et al*. (2018) showed that 9, 12-octadecadienoic acid (Z,Z) is a polyunsaturated essential fatty acid in mammalian nutrition and is used in biosynthesis of prostaglandins and cell membranes having anti-inflammatory, hepatoprotective, anti-arthritic, antihistamine activities. In another studies conducted by Ali *et* al. (2017) stated that 9, 12-octadecadienoic acid (Z,Z) possess antifungal properties while Sudha *et al.* (2013) reported 9, 12-octadecadienoic acid (Z,Z) to be hypocholesterolemic, nematicide, antiarthritic, hepatoprotective, a ntiandrogenic, 5-alpha reductase inhibitor, antihistamic, antieczema, antiacne. While for the residual aqueous has a total of 25 compounds and 5-

hydroxymethylfurfural (42.72%) was observed to have the highest concentration from studies it found to have antioxidative, anti-inflammatory, antihypoxic, inhibiting sickling of red blood cells, antimicrobial effect, anticarcinogenic effect, genotoxicity, improvement of learning and memory, relieve fatigue, protect nerve cells (Goa *et al*., 2015).

Sacuridata longipediculata with a total of 45 compounds with the major compound found in the ethyl acetate fraction was n-hexadecanoic acid (15.05%) in the study by Praveen *et al*., (2010) indicated that n-hexadecanoic had been previously proved to have anti-inflammatory, antioxidant, hypocholesterolemic, flavour, nematicide, pesticide, antiandrogenic activities. Abubakar and Majinda (2016) states that n-hexadecanoic acid had antibacterial and antifungal properties. For the residual aqueous with a total of 16 compounds has 1, 5-anhydro-d-mannitol (93.10%) with the highest concentration has 1, 5-anhydro-d-mannitol this might be because it inhibits gluconeogenesis from lactic acid as reported by Hervey *et al.* (1997). It was also reported to have anticancer activities (Alagammal *et al*., 2012). *Enantia chlorantha* ethyl acetate fraction with a total of 23 compounds had 9,12-octadecadienoic acid(z,z)-(28.05%) with the highest concentration.

4.2.9 Quantitative phytochemical screening

The quantitative phytochemical composition showed that the methanolic extract of *Lawsonia inermis, Enantia chlorantha* and *Securidaca longipedunculata* had at least five classes of phytochemicals. Alkaloids, phenols, flavonoids, tanins and saponins were found in all fractions, it was observed that in all the crude extract of the plants phenol (822.11±0.55 mg/100mg, 416.07±0.07 mg/100mg and 509.68±10.26 mg/100mg respectively) were found to be in the highest quantity and the least quantities for *Lawsonia inermis* (49.90±0.22mg/100g) and *Enantia chlorantha* (20.67±0.57mg/100g) were observed with

alkaloid for *Securidaca longipedunculata* Saponin (35.28±0.50mg/100g) was observed to be the least in quantities a significant quantities for all, according to Elgharbawy *et al*. (2020) phytochemicals such as phenol, courmarin, alkaloids, tannin and saponin found in several plant extracts (especially using polar solvents) are natural sources that inhibits fungi. This might be the reason why some level of activities is seen in this study. Phenolic compounds denature and coagulate proteins causing the cell cycle to stop during the replication phase, and damage the mitochondria so that fungal cell growth is inhibited (Dewi *et al*., 2020). Alkaloid is an alkaline compound with a pH> 7 (Lutfiyanti *et al.*, 2012). Most fungi grow optimally at pH 6 so when the fungi placed at pH> 7 growth will be depressed (Kadhim and Al-Hamadani, 2015). Flavonoids as antifungal are as an inhibitor of fatty acid synthase (FAS), an anti-fungal target, by down regulating genes that express FAS (Erza *et al*., 2020). Similarly, to the studies carried out by Legaspi and Maramba-Lazarte, (2020) found that the nature of the phytochemical constituents found in extracts might be the reason for the possible antifungal activity. Compounds such as the flavones 2,5,7,4'-tetrahydroxy isoflavone and 3,5,7,4'-tetrahydroxy flavone effectively inhibited *T. schoenleinii*,.in addition, anthraquinones such as aloe-emodin and emodin are found to inhibit the growth of *T. rubrum* (Legaspi and Maramba-Lazarte, 2020).

CHAPTER FIVE

5.0 CONCLUSION, RECOMMENDATIONS AND CONTRIBUTION TO KNOWLEDGE

5.1 Conclusion

The dermatophyte isolated in the research were *T. schoenleinii, M. audouinii* and *T. rubrum* which were confirmed morphologically and molecularly.

The plant material extracts tested against the pathogens are leaves of *Lowsonia inermis*, stem bark of *Enantia chlorantha* and *Sacrudata longipedunculata* stem.

The plant extracts, showed antifungal activity against the tested isolates and a low level of MIC and MFC was observed. The crude extract of the plants extract showed a higher level of antifungal activity with more potency in MIC and MFC when compared to the fractions.

The compounds extracted using GC-MS analysis showed the plants effectiveness in the treatment of dermatophyte because of the therapeutic and biological potential and prove to be medicinally valuable.

The qualitative and quantitative phytochemical screening of the plants showed that alkaloidal component was the highest in all the plants which might be responsible for it antifungal activity.

This showed that the plants have potential in the treatment of dermatophyte and further research of the active biological constituent should be identified to develop a drug which will be safe for use.

5.2 Recommendations

Based on the result obtained from this study, the following are recommended:

i. These plants have antifungal activities against dermatophytes, therefore it use locally should be encouraged

ii. Further studies should be done to ascertain the specific compound having the antifungal activity.

iii. Other parts of the plant materials which are not used in this study can be used to determine its antimicrobial activity. This is because the phytoconstituents which are the sole determinant of the therapeutic efficacy of plants varies from one location to another within the plant.

iv. Different method of extraction and different solvent with various polarity from those used in the study should be employed for extraction to fully harness the phytochemical qualities of the plant.

v. Extract of these plants should also be screen against other bacterial and fungal pathogens so as to validate the efficacy of these plants against different microbial pathogens.

vi. further purification and isolations of active compounds from this plant extract is also recommended to determine their mechanisms of actions against these fungi pathogens.

5.3 Contribution to Knowledge

This study established that *Lawsonia inermis, Securidaca longipedunculata* and *Enantia chlorantha* extracts possess significant antifungal activity on dermatophytes such as *T. schoenleinii, M. audouinii* and *T. rubrum*.

The study also contributed that some important compounds and phytochemical constituents of *Lawsonia inermis, Securidaca longipedunculata* and *Enantia chlorantha* ----such as esters, carbohydrate and oleic acid with 9,12-octadecadienoic acid (z,z), 5-hydroxymethylfurfural, benzofuran, 2,3-dihydro-, n-hexadecanoic acid, 9,12-octadecadienoic acid(z,z), 9,12-octadecadienoic acid(z,z), 4H-pyran-4-one,2,3-dihydro3,5-dihydroxy, 5-hydroxymethyl furfural, 1,2,4-benzenetriol, maltose, 1,2-Benzene dicarboxylic acid, n-hexadecanoic acid, 9,12-octadecadienoic acid(z,z), Erythritol, 1,5-Anhydro-d-mannitol, acetic acid and 9,12-octadecadienoic acid(z,z) are responsible for the plants biological activities

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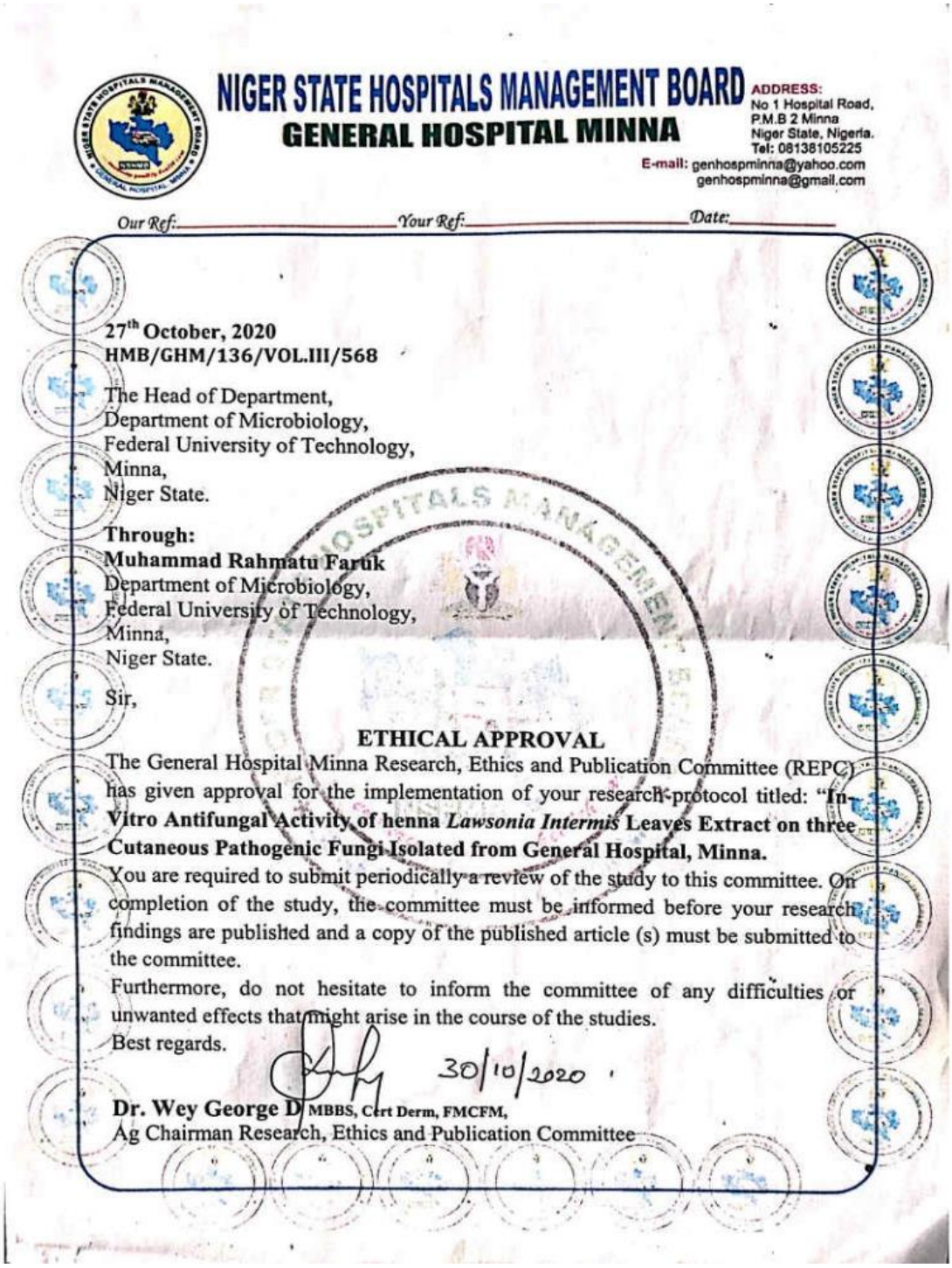
APPENDICES

APPENDIX A

ETHICAL CLEARANCE



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*Our Ref:*_____ *Your Ref:*_____ *Date:*_____

27th October, 2020
HMB/GHM/136/VOL.III/568

The Head of Department,
Department of Microbiology,
Federal University of Technology,
Minna,
Niger State.

Through:
Muhammad Rahmatu Faruk
Department of Microbiology,
Federal University of Technology,
Minna,
Niger State.

Sir,

ETHICAL APPROVAL

The General Hospital Minna Research, Ethics and Publication Committee (REPC) has given approval for the implementation of your research protocol titled: "**In-Vitro Antifungal Activity of henna *Lawsonia Intermis* Leaves Extract on three Cutaneous Pathogenic Fungi Isolated from General Hospital, Minna.**

You are required to submit periodically a review of the study to this committee. On completion of the study, the committee must be informed before your research findings are published and a copy of the published article (s) must be submitted to the committee.

Furthermore, do not hesitate to inform the committee of any difficulties or unwanted effects that might arise in the course of the studies.

Best regards.

30/10/2020

Dr. Wey George D MBBS, Cert Derm, FMCFM,
Ag Chairman Research, Ethics and Publication Committee

APPENDIX B

DNA sequencing of the organisms

MZ209277 *Trichophyton rubrum* strain UZM1

CATGTGAACCTGCGGAAGGATCATTAACGCGCAGGCCGGAGGCTGGCCCCCCACGA
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MZ209278 *Trichophyton schoenleinii* strain UZM2

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GCCCGAAAAGCAGTGGCCAGGCCGCGATTCCGGCTTCCTAGGCGAATGGGCGCAAC

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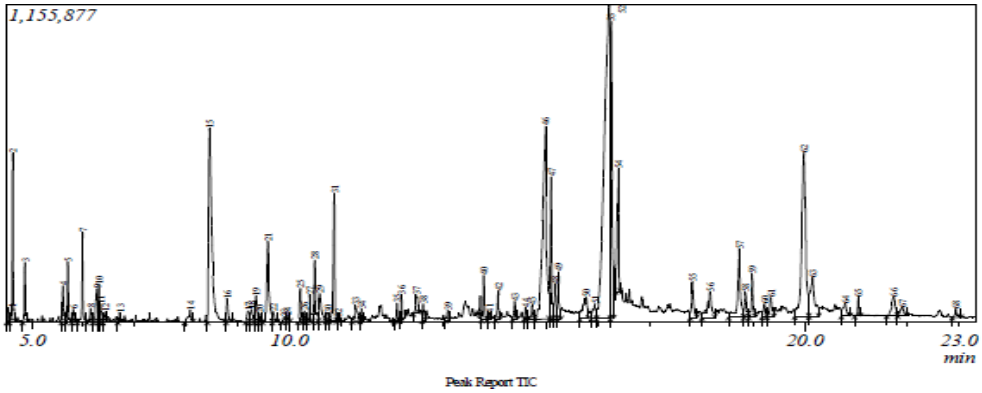
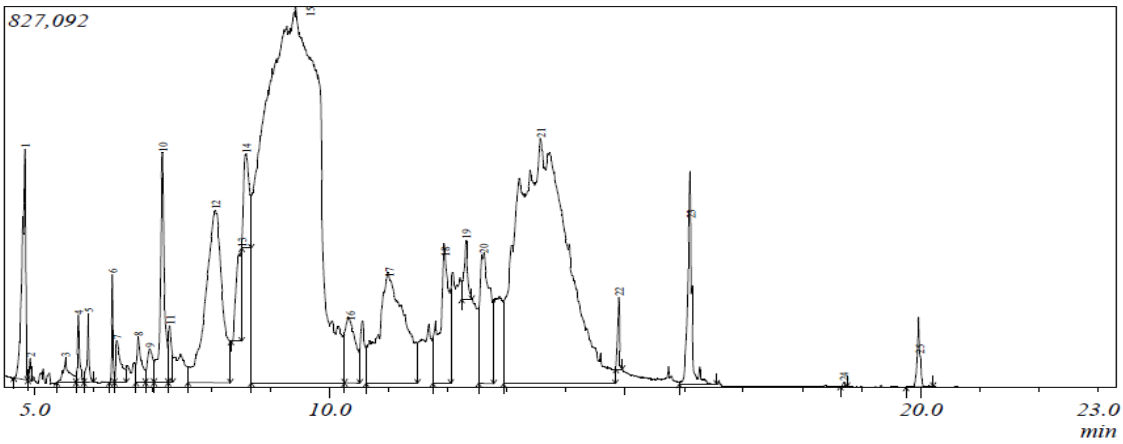
APPENDIX C

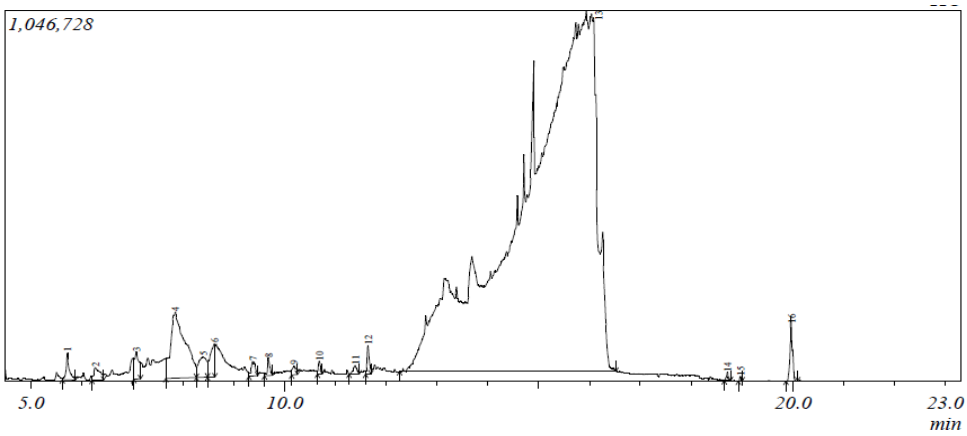
CHROMATOGRAM



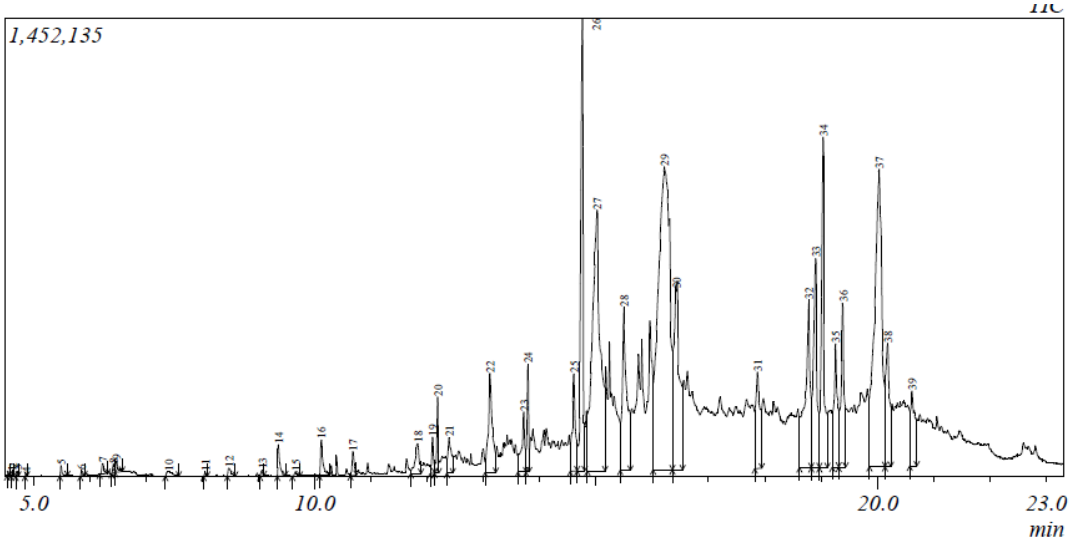
Chromatogram of ethyl acetate fraction of *Lawsonia Inermis*



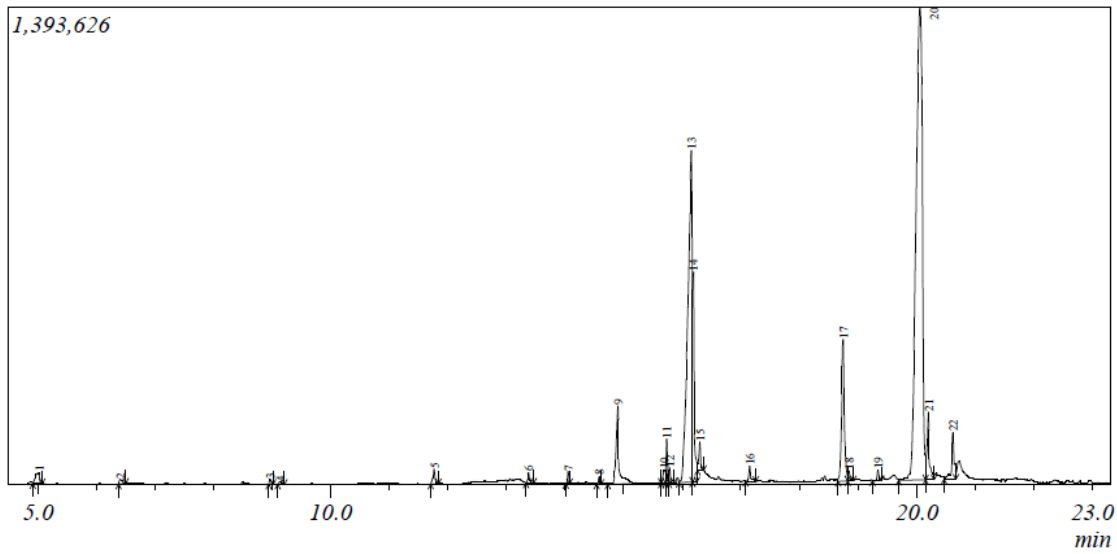
Chromatogram of Residual aqueous fraction of *Lawsonia Inermis*



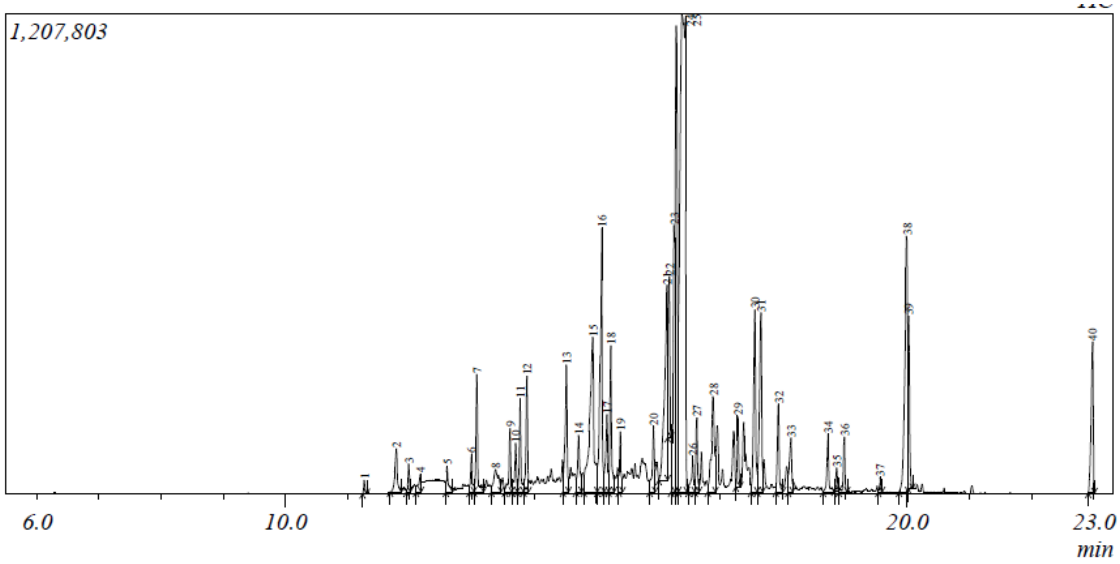
Chromatogram of ethyl acetate fraction of *Sacuridata longipediculata*



Chromatogram of residual aqueous fraction of *Sacuridata longipediculata*



Chromatogram of ethyl acetate fraction of *Enantia Chlorantha*

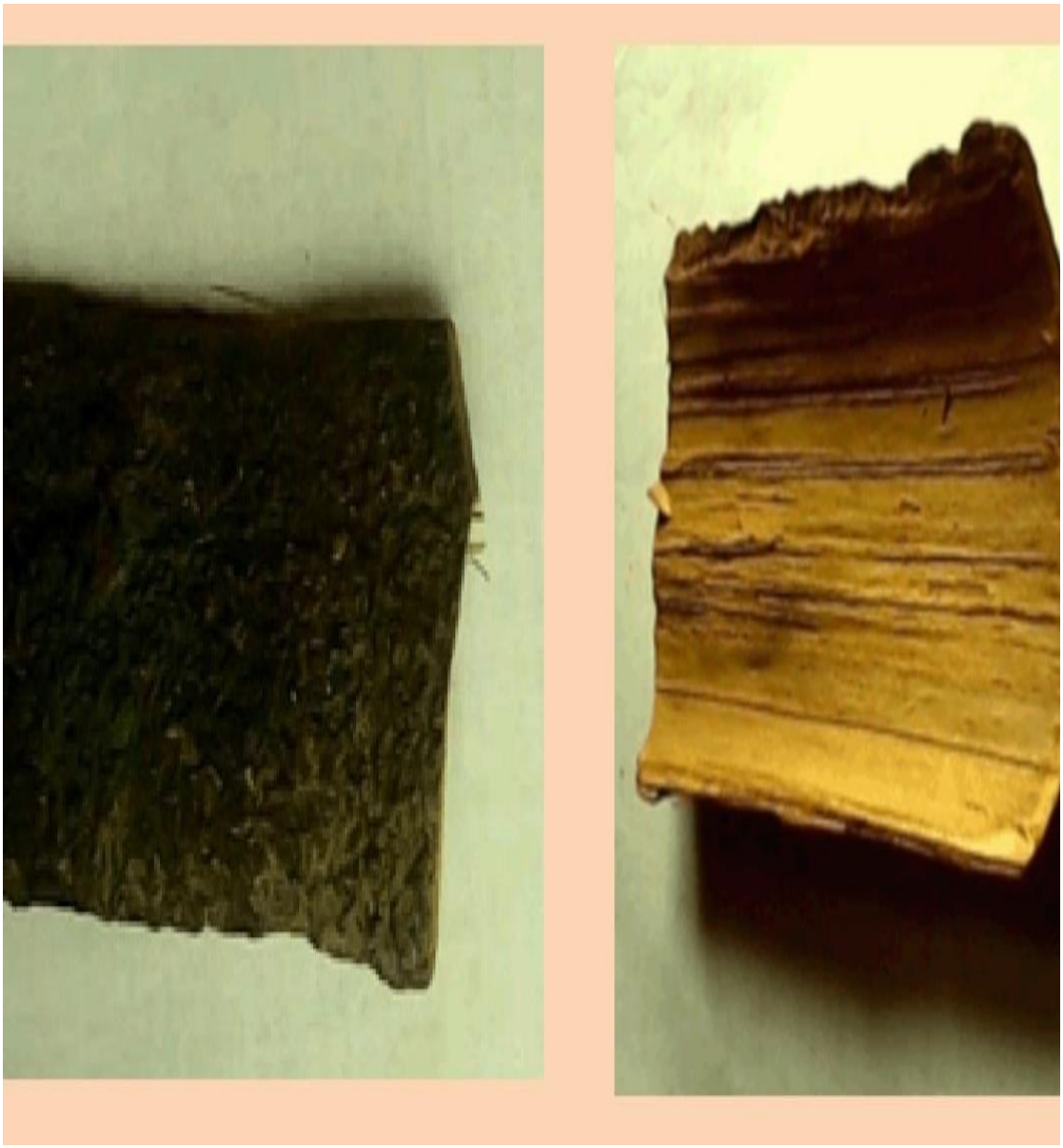


Chromatogram of residual aqueous fraction of *Enantia Chlorantha*

APPENDIX D

PLANTS

Shrub of *Lawsonia Inermis* showing the leaves (Source: Kamal and Jawaid, 2015).



The stem bark of *E. chlorantha*

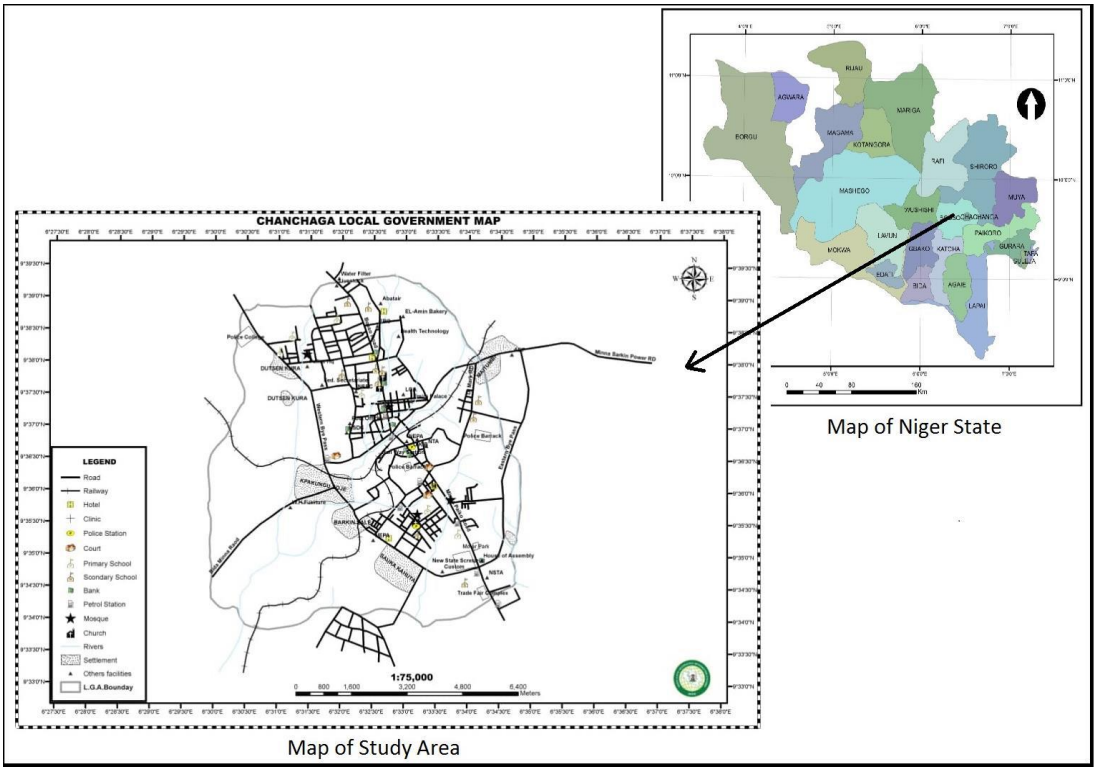
(Source: Tcheghebe *et al.*, 2016)

Sacuridaca longipedunculata leaves, stem and flower

(Source: Mongalo *et al.*, 2015)

APPENDIX E

MAP OF THE STUDY AREA

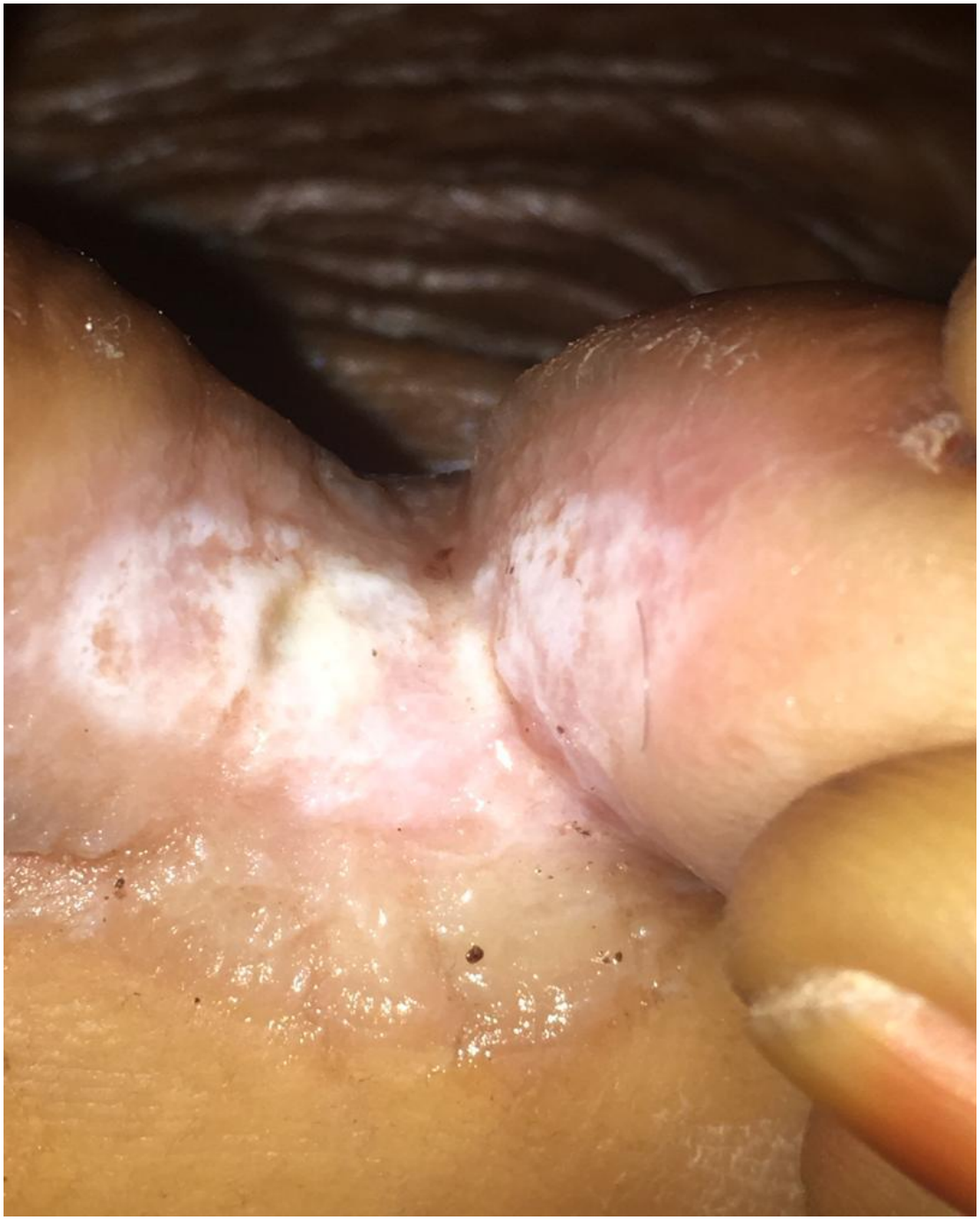


Map showing Chanchaga Local Government Area in Niger state.

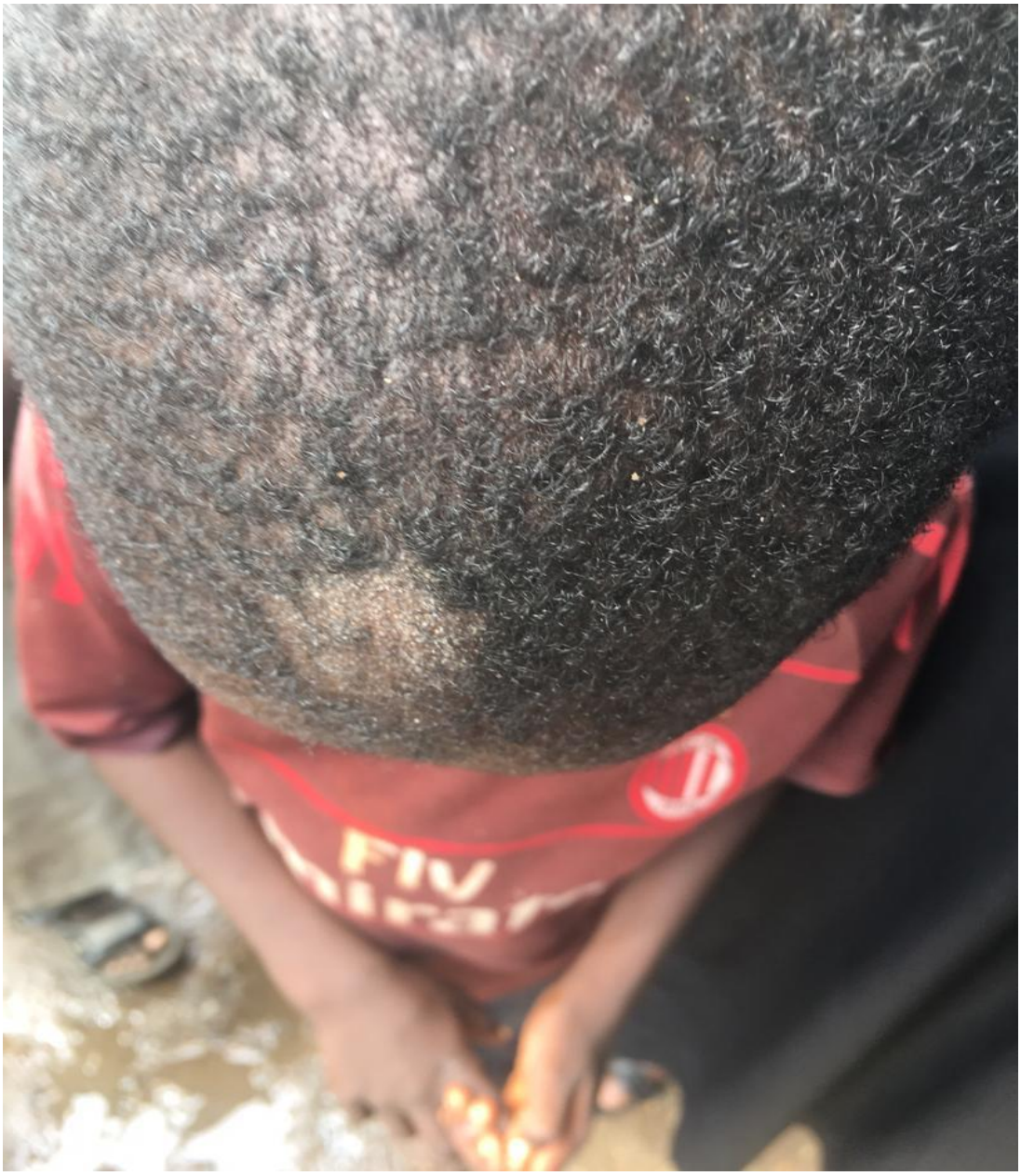
Source: Lympson and Husaini (2020)

APPENDIX F

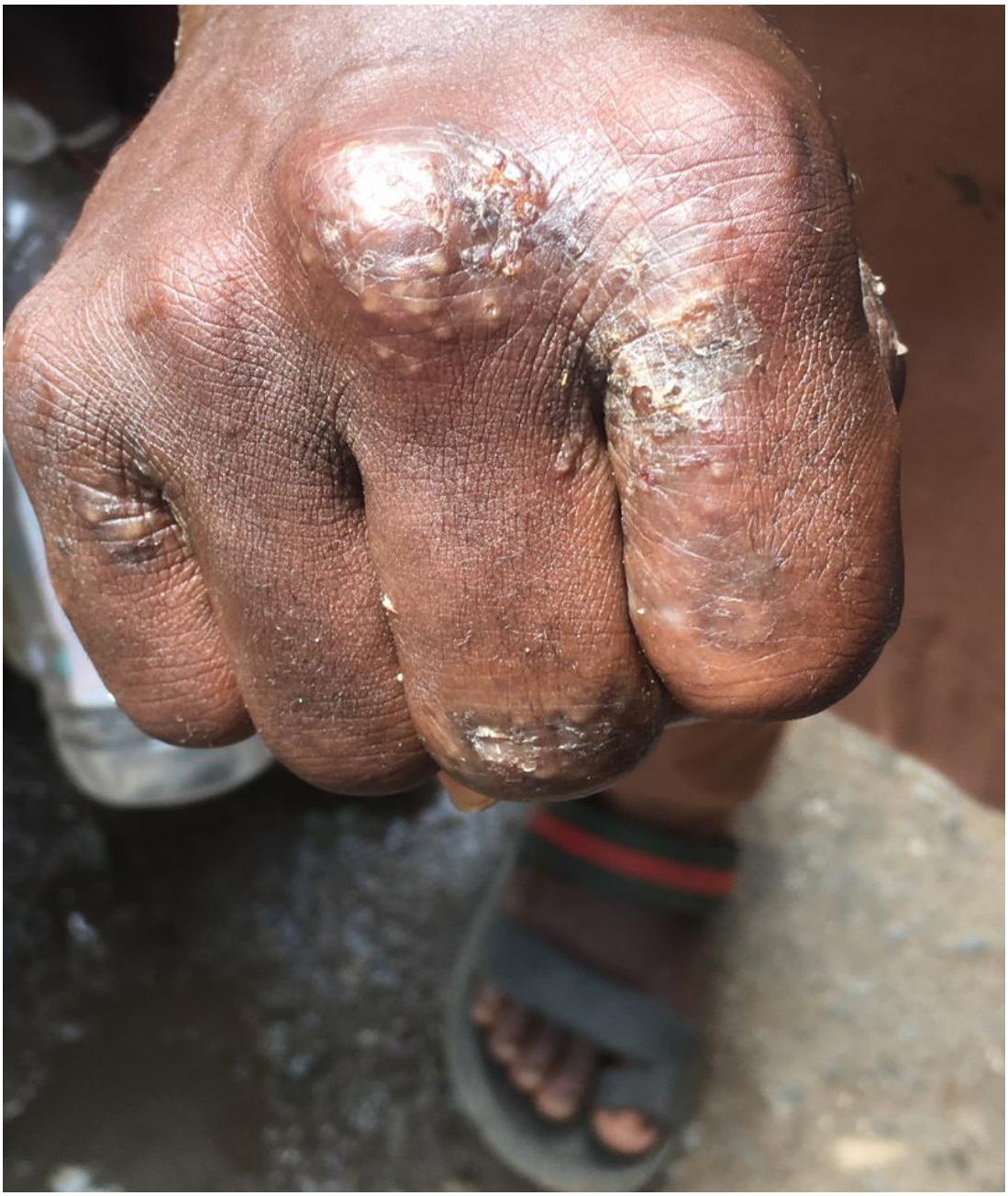
SITES OF INFECTIONS



Site of infection (foot). *Tinea pedis*



Site of infection (head). ***Tinea capitis***



Site of infection (hand). ***Tinea manuum***