

## Evaluation Of Phytochemical And Antifungal Properties Of *Afrostryrax Lepidophyllus*, *Monodora Myristica* And *XylopiA Aethiopica* On *Trichophyton Rubrum*

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<p><b>Corresponding Author</b> Folifack Nguefack Hermann Steve</p> <p>Department of clinical sciences, Specialty: Medical laboratory scientist, University of Bamenda Cameroon</p> <p><b>Article History</b></p> <p>Received: 10 / 12 / 2024 Accepted: 25 / 12 / 2024 Published: 29 / 12 / 2024</p>	<p><b>Abstract:</b></p> <p><b>Introduction:</b> Antifungal resistance is one of the main problems that continue to challenge the healthcare section in a large part of the world particularly in Cameroon. The rapid increase rate of fungal community related infection of the skin and increase resistance to antifungal synthetic drugs has stimulated the need to research on new substances that can be used to solve this problem of resistance. This has been sparingly reported to the best of our knowledge.</p> <p><b>Objective:</b> To evaluate the phytochemical and antifungal properties of <i>Afrostryrax lepidophyllus</i>, <i>Monodora nyrisca</i> and <i>XylopiA aethiopica</i> Sampling at Bamenda food-market on <i>Trichophyton rubrum</i> .</p> <p><b>Materiel and Methods:</b> The experimental study was carried out from February 24th to march 25th 2022, in order to sought out new antifungal agents from spices, by assessing the antifungal property of <i>Afrostryrax lepidophyllus</i> (country onion), <i>XylopiA aethiopica</i>( bush pepper) and <i>Monodora myristica</i> (groundnut-spices) spices on <i>Trichophyton rubrum</i>. To identify the different compounds present in these spices, qualitative phytochemical screening was also carried out. This was accomplished by purchasing dried spices of <i>Afrostryrax lepidophyllus</i>, <i>XylopiA aethiopica</i>, and <i>Monodora myristica</i> from various locations in the Bamenda food market and aseptically grinding them into powder. The resulting powder underwent independent ethanolic and aqueous extraction processes.</p> <p><b>Results:</b> The results showed that <i>Trichophyton rubrum</i> was sensitive to all aqueous extraction of the three spices using agar dilution technique after 48hrs of incubation at room temperature, while <i>Trichophyton rubrum</i> was sensitive only to alcoholic extract of <i>Afrostryrax lepidophyllus</i> under the same growth conditions of incubation. The phytochemical screening results of <i>Afrostryrax lepidophyllus</i> and <i>XylopiA Aethiopica</i> spices indicated the presence of alkaloids, flavonoids, Saponin, resins, tannins (except for <i>Afrostryrax lepidophyllus</i>) and the absence of glycosides in all the spices. <i>Monodora myristica</i> indicated the presence of saponin and flavonoids only.</p> <p><b>CONCLUSION:</b> The objective of the study on the evaluation on the phytochemical and antifungal properties of <i>Afrastyrax lepidophyllus</i>, <i>XylopiA aethiopica</i> and <i>Monodora myristica</i> on <i>Trichophyton rubrum</i>, revealed sensitivity to the aqueous extracts of all the spices. The ethanolic extract of <i>A lepidophyllus</i> also revealed sensitivity to <i>T.rubrum</i>. This proves that all the spices have antifungal properties and as such can be used as alternative treatment for fungal skin infection cause by <i>T. rubrum</i>.</p> <p><b>Keywords:</b> Phytochemical, ethanolic extractions, aqueouse extraction, agar dilution technique.</p>
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## 1.0 Introduction

### 1.1. Background

Spices have been used to improve the flavor of food for hundreds of years. Spices have items of international commerce for many hundreds of years. Their popularity in the interest of the taste to many foods such as Achu-soup, groundnut food and many others food items. Apart that, spices give others advantages like the can be using for fabrication of perfum(distillation) and nutritional value and other has efficiency among certain fungi. This have been proved by Diego et al.,(2014) Apart from using some spices to make the food tasteful, Many local people and traditional healers use spices in combinations with different plant to cure several diseases and also for food preservatives Pavela et al.(2016). The use of spices goes beyond medical importance, The use of spices goes beyond medical importance, this is why it is important to carry out the antifungal properties of *Afrostryrax lepidophyllus*, *Monodora myristica* and *Xylopiya ethiopia* on dematophytes in mezam division.

### 1.2. Statement of problem

All serious fungal infections need appropriate antifungal therapy for successful drugs are available, then the emergence of resistance to single drug classes and now multi drug resistance creates difficulties in patient management. This is why Scott et al (July, 2017) have proved the Azoles resistances among *Candida*, *Aspergillus* spices is one of the greatest challenges to clinical success. To try to solve the problem many research are already carry out to prove the efficacy of spices to treat disease. In the same order of idea this study will evaluate the antifungal effect of some selected spices mostly use in Mezam division in Bamenda-Cameroon to see how efficiency it have inside welfare in the body.

### 1.3. Reseach question(s)

- Does *Afrostryrax lepidophyllus*, *Monodora nyrisca* and *Xylopiya aethiopia* has antifungal effect on *T rubrum*?
- What is the phytochemicals contents of *Afrostryrax lepidophyllus*, *Monodora nyrisca* and *Xylopiya aethiopia* ?

### 1.4. Objectives

#### 1.4.1. General objectives

To evaluate the phytochemical and antifungal properties of *Afrostryrax lepidophyllus*, *Monodora nyrisca* and *Xylopiya aethiopia* on *T rubrum*.

#### 1.4.2. Specific objectives

- To evaluated the antifungal properties of *Afrostryrax lepidophyllus*, *Monodora nyrisca* and *Xylopiya aethiopia*.
- To evaluate the phytochemicals substance of *Afrostryrax lepidophyllus*, *Monodora nyrisca* and *Xylopiya aethiopia*

### 1.5. Hypothesis /impact of research

Spices adds taste and flavour in many food items and they also have nutritional value and are effective against some fungi infections.

### 1.6. Significance Of The Study

The outcome of our research work will be very usefull for medicinal purpose since it add not only taste and flavour to food, but also improved nutritional value and protect against some fungal infections.

## 2.0 Literature Review

### 2.1 Ethnobotanical uses of medicinal plants in cameroon

Since the vast majority of people (about 80%) rely on traditional medicine for their primary medical requirements, it is essential to Black African culture (WHO, 2002). Herbal medicine has a long history of being used in Cameroon to treat a variety of illnesses. Unfortunately, because of its disarray, traditional medicine's incorporation into the healthcare system is now ineffective (Nkongmeneck et al., 2007). But according to government health policies, traditional medicine should be organized to give the primary trends for its advancement and integration (Anonymous, 2006). Although there is still more to be done to document the current ethnobotanical knowledge, Adjanohoun et al. (1996) provide a helpful overview of Cameroon's traditional use of medicinal herbs. Jiofack et al. (2010) also revealed the traditional use of 289 plant species from 89 families against 220 illnesses. Sixty-eight percent of the known herbs are used to cure more than twenty serious disorders. They are used for decoction, infusion, maceration, powder, powder blends, plaster, calcinations, squeezing in water, boiling, cooking with groundnut paste, lamb or young cock meat, direct consumption, juice, fumigation, and sitz baths (Jiofack et al., 2010). Among the most commonly treated illnesses or conditions are typhoid, male sexual disorders, malaria, gonorrhoea, gastritis, rheumatism, fever, dysentery, diarrhea, dermatitis, boils, cough, wounds, syphilis, ovarian cysts, ovarian cysts, and amoebiasis. According to Jiofack et al. (2010), around two hundred herbs are utilized to cure certain ailments.

### 2.2 Dermatitis fungi

Typically, fungi live in wet places where skin surfaces contact, such as the vaginal area, under the breasts, and between the toes. Dermatophytes like *Trichophyton*, *Microsporum*, and *Epidermophyton* are the main culprits behind common fungal skin infections., or yeasts such as *Candida* or *Malassezia furfur*. Many of these fungi only live in the stratum corneum, which is the epidermis' outermost layer; they don't go deeper. Obese people are more prone to these conditions because they have more skinfolds, especially if the skin inside a skinfold becomes irritated and damaged (intertrigo). Denise et al. (2020) state that people with diabetes are also more likely to get fungal infections. Curiously, rashes on non-infected body areas can result from fungal infections on other body parts. For instance, an itchy, bumpy rash on the fingers could be the result of a fungal infection on the foot. These eruptions are allergic reactions to the fungus and are also known as dermatophytids or identity or id reactions. They don't happen when you contact the affected area. In 2020, Denise et al.

### 2.3 2.3 *Trichophyton rubrum*

The dermatophyte *Trichophyton* lives in earth, people, and animals. Anthropophilic, zoophilic, and geophilic species that are related to their natural habitats are found in this genus. Some species are cosmopolitan. Some have a restricted geographic range. For instance, *Trichophyton concentricum* is indigenous in Central America, Southeast Asia, and the Pacific Islands. One of the main culprits behind human infections of the skin, hair, and nails is *Trichophyton*. The genus *Arthroderma* is home to the teleomorphic forms found in the majority of *Trichophyton* species.

#### 2.3.1 Taxonomic Classification

- Kingdom: Fungi
- Phylum: Ascomycota
- Order: Onygenales
- Family: Arthrodermataceae
- Genus: *Trichophyton* (*Arthroderma* teleomorph)
- Species: *Rubrum Trichophyton* Hoog et al. (2000).

#### 2.3.2 Macroscopic and microscopic features Macroscopic Features

##### 2.3.2.1 Botanical description and classification

*Afrostryrax lepidophyllus* trees are 20 meters tall and straight, and they are found in forests in Ghana, Cameroon, especially in the south-west region of Limbe, and Congo (Brazzaville). The fruit and foliage of the tree have a pungent smell. Colonies of *Trichophyton* grow slowly to relatively quickly. It has a waxy, glabrous, to cottony texture. White to brilliant yellowish beige or crimson violet are the colors seen from the front. According to Hoog et al. (2000), the reverse might be pale, yellowish, brown, or reddish-brown.

#### Microscopic Features

Observations include conidiophores, hyaline hyphae, septate, microconidia, macroconidia, and arthroconidia. It is also possible to manufacture chlamydospores. The hyphae and conidiophores are not well distinguished. Microconidia are pyriform or spherical, one-celled organisms. They are widely distributed and can be found alone or in groups. The majority of *Trichophyton*'s conidia are frequently microconidia. Macroconidia are multicellular (two or more cells), cylindrical, clavate, or cigar-shaped, and have smooth, thin, or thick walls. They are typically not created or manufactured in large quantities. Certain species could be sterile, and sporulation requires the usage of particular media. In 1995, Hoog et al. and Larone et al.

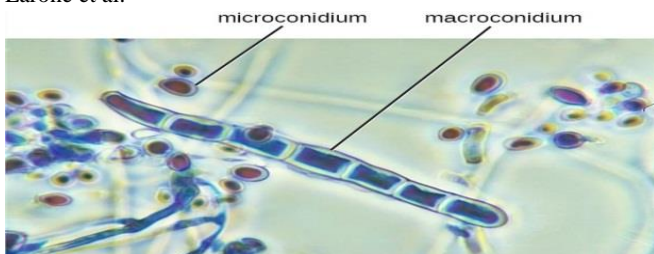


Figure 6: microscopy view *Trichophyton rubrum* (Source :[http://images .app.gl/2sgXS2zye6](http://images.app.gl/2sgXS2zye6))

#### 2.4 Disease most common associate

Dermatophytosis is caused by *Trichophyton*, *Microsporum*, and *Epidermophyton*, which infect the skin, hair, nails, and athlete's foot. Aman et al. (2001) and Aly et al. (2000) *Trichophyton* is a © Copyright IRASS Publisher. All Rights Reserved

keratinophilic filamentous fungus, just like the other two genera. The primary virulence features of these fungi are their capacity to infiltrate keratinized tissues and their abundance of various enzymes, including keratinases, elastases, acid proteinases, and other proteinases. The most frequent cause of dermatophytoses (*tenia incognito*, dermatophytes abscess) in the world is *Trichophyton rubrum* (Arenas et al., 1995).

## 2.5 Classification ,botanical description and medical importance of the spices.

### 2.5.1 *Afrostryrax lepidophyllus* nsive smell of onion or garlic (Moukette et al.,2015).

The botanical classification of *Afrostryrax lepidophyllus* belongs to the following category

- Kingdom: Plantae
- Clade: Tracheophytes
- Order: oxalidales
- Family: Huaceae
- Genus: *Afrostryrax*
- Specie: *Afrostryrax lepidophyllus* (Moukette et al., 2015).

### 2.5.2 Medical importance

- Commonly referred to as "country onion," *Afrostryrax lepidophyllus* is a non-timber forest product belonging to the Huaceae family that grows in the green forests of Ghana, Cameroon, and the Republic of Congo.
- The leaves and fruits have very offensive smell of anion or garlic. This fruit is used as spices in traditional african cuisine. In folk medicine the root and barks decoctions are drink as anthelmintic against vomitting or as enema against urinary infection (Kambu.,1990), (Olivier.,1986).
- The inner barks are applied locally after incision and in case of snake bites (Muganza D et al.,2012).
- The essential oils from the seeds(spices) of the plant demonstred cytotoxic, antimicrobial activities ( Fogang et al., 2014).
- The antioxydant effecincy and present of phytochemical value like flavonoid and have peroxidase activity (Moukette et al., 2015).

### 2.5.3 *Monodora myristica*

#### 2.5.3.1 Botanical description and classification

*Monodora myristica* (*M. myristica*) grows naturally in evergreen forest from Liberia to Nigeria ,Cameroon,Angola ,Uganda and west Kenya.(Bakarnga et al., 2014).

#### 2.5.3.2 Medicinal importance

- *Monodora myristica* (*M. myristica*) fruits and seeds are dried and sold in whole or ground to be used in stews,soups,cakes and dessert. They are used as stimulants, stomachic ,against headaches, sores and also as insect repellent .
- in the folk medicine, the bark is used in treatment of stomach-aches, febrile pains , eye diseases and haemorrhoids, (Bakarnga et al., 2014).

- The exploration, characterization and application of natural antioxidants are the focus of several research teams in the sub saharan africa (Biapa et al., 2011).
- The antioxidant properties and presence of polyphenol in this spices was also demonstrated by (Moukette et al., 2015).

#### 2.5.4 *Xylopi*a *aethi*o*pica* (Negro pepper).

##### 2.5.4.1 Botanical description and classification

An explanation of botany, *Xylopi*a *aethi*o*pica* is a fragrant tree that can grow to a height of 15–30 m and a diameter of 60–70 cm. It is native to the lowland rainforest and moist fringe forest in the savanna zones of Africa, despite being mostly found in West, Central, and Southern Africa. According to Orwa et al. (2009), these trees are widespread in humid forest zones, especially around rivers in the drier parts of the region.

Its creamy-green, bisexual blooms can be solitary, in 3-5 flowered fascicles, or in odd, sinuous, branched spikes or cymes that measure up to 5.5 by 0.4 cm. The dark brown, cylindrical, 2.5–5 cm long, and 4–6 mm thick fruits of *Xylopi*a *aethi*o*pica* resemble tiny, twisted bean pods. Each pod houses about 5 to 8 kidney-shaped seeds grains of approximately 5 mm length. (Orwa, et al., 2009).

*Aethi*o*pica* denotes its Ethiopian provenance, whilst *xylopi*a is a Greek word (*xylon pikron*) for "bitter wood." African pepper, Guinea pepper, spice tree, negro pepper, West African pepper, and Senegal pepper are some of its common names (Jirovetz et al., 1997). Smoking Negro pepper throughout the drying process results in a pleasingly peppery flavor. (Erhirhie and others, 2014).

The botanical classification of *Xylopi*a *Aethi*o*pica* belongs to the following category;

- Kingdom: Plantae
- Order: Magnoliids
- Family: Annonaceae
- Genus: *Xylopi*a
- Specie: *Xylopi*a *aethi*o*pica* (Erhirhie et al., 2014).

##### 2.5.4.2 Medical importance

Ethnomedical principles In the Congo, rheumatism can be well managed by combining palm wine and the bark of *Xylopi*a *aethi*o*pica*. Asthma, bronchitis, stomachaches, and stomach disorders can all be treated with a fruit or bark decoction. In Nigeria, the powdered root is applied locally to cure cancer and as a bandage for sores and gums to treat pyorrhea. A mixture of salt and *Xylopi*a *aethi*o*pica* is used to treat constipation. In Gabon, its infusion is used as an emetic and to treat rheumatism (Burkill, 1985). To treat epileptic fits, kola nut and leaf sap are combined (Burkill, 1985). It is administered to facilitate childbirth and promote fertility. *Xylopi*a *aethi*o*pica* is crushed and applied to the forehead as a treatment. Additionally, a seed extract is utilized as a roundworm vermifuge (Dalziel, 1973). The advancement of *Xylopi*a *aethi*o*pica*'s bioactivities as beneficial medications and the confirmation of some obscure folklore claims that have not yet received scientific validation (Erhirhie et al., 2014).

## 2.6 Phytochemical constituent of plant

The phytochemical components of medicinal plants essentially determine their wide range of pharmacological actions. Generally © Copyright IRASS Publisher. All Rights Reserved

speaking, the phytochemical components of plants can be divided into primary and secondary metabolites according to their function in fundamental metabolic processes. Primary plant metabolites are more or less the same in all living cells because they are involved in fundamental life activities. Conversely, the shikimic acid route and other subsidiary processes produce secondary plant metabolites. According to research, the secondary plant metabolites are the focus of herbal remedies' therapeutic effects (Rehab et al., 2018).

### 2.6.1 Brief study of secondary metabolites

#### Tannins

Proteins can precipitate from polyphenols known as tannins. These materials have been used for many years to make leather from raw animal hides. During this process, tannin molecules crosslink the protein, strengthening its defense against bacterial and fungal attack. Due to their structure and biosynthetic origin, many compounds that are currently categorized as tannins have little to no potential for use in the production of leather. The two primary types of tannins are hydrolyzable tannins and condensed tannins. Hydrolyzable tannins are produced by ester-bonding a number of phenolic acid molecules, such as gallic and hexahydroxydiphenic acids, to a core glucose molecule. The two primary types of hydrolyzable tannins are gallotannins and ellagitannins, which are composed of gallic acid and ellagic acid units, respectively. Tannin-containing drugs have been utilized as anti-diarrheal drugs and as antidotes for heavy metal and alkaloids poisoning. Epigallocatechin-3-gallate, the primary component of tea, has been shown to have antiangiogenic effects in mice. A randomized, double-blind, placebo-controlled trial on 153 older women showed that cranberry juice, or *Vaccinium oxycoccos*, has long been used as a urinary antiseptic [18] (Rahab et al., 2018).

#### Flavonoids

The greatest class of phenols found in nature are flavonoids. There are currently over 2000 of these compounds known to exist, with about 500 of them existing in their free state. In positions 2, 3, or 4, a chroman ring with an aromatic ring is part of the structural skeleton of flavonoids. The degree of oxidation of the center ring (ring C) can be used to categorize flavonoids into different classes. Among these, anthocyanins, flavones, and flavonols are the most prevalent. Yellow is a common color for flavones and their related relatives (Latin *flavus*, yellow). Although they are found throughout nature, higher plants and immature tissues—where they are found in the cell sap—are where they are most prevalent. In the Polygonaceae, Rutaceae, Leguminosae, Umbelliferae, and Compositae families, they are widely distributed. Recent studies have shown the therapeutic benefits of medications containing flavonoids, such as *Ginkgo biloba* (gingko), *Chamaemelum nobile* (Roman chamomile), and *Glycyrrhiza glabra* (licorice root). Many herbs that contain flavonoids are now listed in the British Pharmacopeia. Some of these include horsetail (*Equisetum ramosissimum*), motherwort (*Leonurus cardiaca*), passion flower (*Passiflora edulis*), horsetail (*Betula pendula*), calendula officinalis flower, elder flower (*Sambucus nigra*), and lime flower (*Tilia cordata*). According to Rahab et al. (2018), the group is well-known for its anti-inflammatory and antiallergic effects, antithrombotic and vasoprotective qualities, ability to block the propagation of tumors, and ability to protect the stomach mucosa.

## Alkaloids

Alkaloids are organic compounds with at least one nitrogen atom in a heterocyclic ring. Since they do not reflect a homogenous set of molecules from a chemical, biochemical, or physiological standpoint, their definition is difficult. Apart from the fact that they are all compounds that include nitrogen, no one definition can be applied to all alkaloids. Based on their basic chemical structure, alkaloids can be divided into a variety of groups. The basic types of alkaloids include pyrrolidines, pyrrolizidines, pyrroloindoles, pyridines, ephedras, carbolines, imidazoles, indoles, bisindoles, indolizidines, manzamines, oxindoles, quinolines, quinozolines, phenylisoquinolines, phenylethylamines, piperidines, purines, pyrrolidines, pyrrolidines, pyrroloindoles, pyridines, and simple tetrahydroisoquinolines. Despite the fact that people have been utilizing plants that contain alkaloids as teas, medications, and mixtures for at least 3,000 years, the elements responsible for the action were not recognized and isolated until the nineteenth century. Alkaloids are uncommon in lower plants. It has been discovered that fungi contain lysergic acid derivatives and sulfur-containing alkaloids, including gliotoxins. Alkaloids from pteridophytes and gymnosperms that have been used medicinally include lycopodium, ephedra, and taxus alkaloids. Alkaloids have a wide range of pharmacological activities, including analgesia, local anesthetic, cardiac stimulation, respiratory stimulation and relaxation, vasoconstriction, muscular relaxation and toxicity, antineoplastic, hypertensive, and hypotensive characteristics (Rahab et al., 2018).

## Saponins

A polycyclic aglycone molecule is linked to a carbohydrate unit (a monosaccharide or oligosaccharide chain) by a triterpenoid (triterpenoidal saponins) or a steroid (steroidal saponins) to form saponins. These sugar units may consist of hexoses, pentoses, or uronic acids. These compounds have a hydrophobic-hydrophilic asymmetry, which makes them soap-like and capable of lowering surface tension. They create foam in aqueous solutions and hemolyze blood erythrocytes in vitro. The aglycone component of the saponin molecule is known as the genin or sapogenin. Saponins have been found in over 500 plants from at least 90 different families, indicating how common these substances are in plants; These substances have been isolated from all parts of plants, including leaves, stems, roots, bulbs, flowers, and fruits, even though they are concentrated in the roots of many species, such as *Digitalis purpurea* (foxglove), *Dioscorea villosa* (wild yam), *Eleutherococcus senticosus* (Siberian ginseng), *Gentiana lutea* (gentian), *Glycyrrhizaspp.* (licorice), and *Panax ginseng* (Korean ginseng) (Rahab et al., 2018).

## 2.7 Materials and Methods

### 2.7.1 Materials

For example, Sabouraud Dextrose Agar (SDA), powdered spices, distilled water, a weighing balance, a funnel, a sieve, an incubator, an autoclave, a water bath, a measuring cylinder, Petri plates, a Bunsen burner, culture bottles, gloves, fungus isolates, syringes, and bechers were among the many materials used in this study.

## 2.7.2 Methods

### 2.7.2.1 Study design and duration

This experimental study design was done from the 18th of February to the 27th of March 2022

### 2.8 Procedure for extraction, antifungal sensibility and phytochemical screening of the spices

#### 2.8.1 Extraction procedure

#### 2.8.2 Preparation of dry spices powder

The dry spices bought from food market was transported to science for life foundation laboratory, the capsule walls were completely removed and the plants crushed to powdered form.

#### 2.8.3 Alcoholic and aqueous extraction.

Extraction is a method of removing active constituents from a solid or liquid by means of liquid solvent. for this research aqueous and alcoholic extraction are used:

#### Procedure

- A 200 mL conical flask containing 25 grams of powdered spice material was filled with 100 mL of solvent, such as ethanol and distillate water, separately. The mouth of the conical flask was covered with cotton wood and kept in a reciprocating shaker for 24 h for continuous agitation at 150 rev/min for thorough mixing and also complete elucidation of active materials to dissolve in the respective solvent.
- Next, a little cotton was used to filter the extract, and then Whatman No. 1 filter paper was used.
- Using an incubator set to 50°C for five days, the extract's solvent was eliminated using evaporation. Ultimately, the leftovers were gathered and utilized in the experiment..

#### 2.8.4 Antifungal Susceptibility Testing

#### 2.8.5 Preparation of isolates

- After weighing 2.6g of SDA into a container, 40ml of distilled water was added, and the mixture was autoclaved for 15 minutes at 121°C/15psi to sterilize it. TwentymL of each was then transferred to two Petri dishes and left to harden..
- After solidification, the plates were incubated at 37°C for 30 to 45 minutes to get dry.
- Following the use of lactose phenol cotton blue for species specific identification, a nail scraping sample was cultivated in SDA media, where various dermatophyte types grew. After 72 hours at 32°C, pure isolated colonies of *Trichophyton rubrum* were obtained by subculturing flat, woolly to cottony white colonies.

#### 2.8.6 Preparation of inoculum and stock dilution

- The pure culture for *Trichophyton rubrum* was stirred and aspirated with a syringe to collect fungal spores before being inundated with 5 ml of distillate water to prepare the inoculum. Additionally, the concentration should be between 0.008 to 0.10 when measured

physically to determine the mixture's turbidity at the 0.5 McFarland standard or spectrophotometrically at the absorbance of 625 nm.

- For the antifungal susceptibility testing procedure, a stock solution at a concentration of 100 mg/ml was created by combining 1.5g of the extract with 15ml of distilled water.

**2.8.7 Antifungal sensibility test procedure: agar dilution technique.**

05 (five) different tubes were used:

- The tube 0 was for control tube.
- Inside tube 1, 600ul of working solution was pipetted and 4ml of SDA added and the tube inclined to form a slope.
- Inside tube 2, 300ul of working solution was pipetted and 4ml of SDA added and the tube inclined to form a slope.
- Inside tube 3, pipette 150ul of working solution was pipetted and 4ml of SDA added and the tube inclined to form a slope.
- 75 ul of working solution was pipetted into tube 4, 4 ml of SDA was added, and the tube was tilted to create a slope.
- The tubes were then incubated for 15 minutes at 37°C to remove any remaining moisture. Using a Pasteur pipette, one drop of inoculum was put to each tube on the slope, making sure the inoculum touched the whole surface. The tubes were then sealed and kept at room temperature (25–30°C) for 72 hours, with results being read every 12 hours.

**2.8.8 Phytochemical Screening Procedure**

Phytochemical screening for resins, alkaloids, Saponin, tannins, glycosides and flavonoids was done on the powder spices using conventional methods.

**Test for resins**

0.5g of each powdered plant was mixed with 5 ml of ethanol. In a water bath, the mixture was left to boil for five minutes. After passing the solution through cotton wool, 4 milliliters of 1% aqueous HCl were added to the filtrate. The presence of resins was demonstrated by the production of a thick, sticky precipitate.

**Test for alkaloids**

0.5g of plant powder was combined with 5ml of 2N HCl in a steam bath. The solution was filtered, and 1 ml of each filtrate was mixed with 0.5 ml of wagers reagent. The precipitate's development indicated the existence of alkaloids.

**Test for Saponin**

Water and 0.5 g of spice powder were combined in a test tube. It was thought that the residual froth from heat was early evidence of saponin's presence.

**Test for tannins**

Half a gram of spice powder was combined with ten milliliters of boiling distilled water, and the mixture was filtered. 0.5 milliliters of 6% ferric chloride were added to the filtrate. A deep green hue

suggested the presence of tannins. A bluish hue indicated the presence of tannins after the second portion of the filtrate was treated with iodine solution.

**Test for glycosides**

0.5g of spice powder was combined with 10ml of boiling distilled water. Two milliliters of the filtrate were hydrolyzed by a few drops of strong HCl after filtering, and the mixture became alkaline by adding a few drops of ammonia solution. Five drops of this solution were boiled in two milliliters of Benedict's qualitative reagent to identify glycosides; this produced a reddish-brown precipitate.

**Test for flavonoids**

After dissolving 0.5g of powdered spices in 2ml of diluted NaOH solution and adding a few drops of strong H2SO4, a brown precipitate appeared that did not alter after two minutes, suggesting the lack of flavonoids. The solution will turn colorless if flavonoids are present.

**3.0 Results**

**3.1 Result of phytochemical analysis of the different spices**

**Table 1:** Result of phytochemical analysis of the different spices

Seed powder	Phytochemicals					
	Resins	saponins	Alkaloids	Tannins	Glycosides	Flavonoids
<i>Afrostryrax lepidophyllus</i>	+++	+	+++	-	-	+++
<i>Xylopi aethiopica</i>	+++	+	+++	+++	-	+
<i>Monodera myristica</i>	-	+	-	-	-	+

- (-)=absent
- (+)= present
- (+++)= highly positive

**3.2 Result of antifungal susceptibility and evaluation of the Minimum Inhibition Concentration(MIC).**

**Table 2:** Evaluation of antifungal activity and minimum inhibition concentration(MIC) on *Afrostryrax lepidophyllus*, *Monodora myristica* and *Xylopi aethiopica* on *T. rubum*

Test Organism	Minimum Inhibitory concentration(mg)	
	Aqueous extract	Ethanollic extract
<i>Afrostryrax lepidophyllus</i>	25.00	100.00
<i>Monodora myristica.</i>	100.00	0.00
<i>Xylopi aethiopica</i>	100.00	0.00

#### 4.0 Discussion

Results of the phytochemicals screening Components (Saponin, resins, alkaloids, tannins, glycosides, flavonoids) of spices during this study indicate that, *XylopiA AethiopicA* contain all of the six secondary metabolites studied except glycosides, antifungal susceptibility testing on *Trichophyton rubrum* revealed sensitive to the aqueous extraction of *XylopiA AethiopicA* at an MIC=100mg/ml at room temperature for 48hours .

On the other hand, phytochemical screening of *Afrostryrax lepidophyllus* revealed that the spice is highly rich in resins, alkaloids and flavonoids but saponins present in few amount. The result of antifungal susceptibility testing on *Trichophyton rubrum* with the aqueous extract of *Afrostryrax lepidophyllus* revealed it to be sensitive with an MIC of 25mg/ml. while alcoholic extract of *Afrostryrax lepidophyllus* revealed sensitive at an MIC of 100mg/ml.

The phytochemicals screening of *Monodora myristica* showed the presence in little amount of saponin and flavonoids only, the antifungal sensibility testing on *Trichophyton rubrum* using aqueous extract also showed sensitivity at an MIC of 100mg/ml

#### 4.1 Limitation

During handling process of this study many problems have been meet like graning process to have a powder formed, also spices are very rare in the market so those who was there are very expensive.

#### 4.2 Delimitation

The study was restricted only to some selected spices mostly sold and often use in Mezam division particularly in Mankon. The reseach have been only limited in this area because lack of ressources, time limited and difficulties to move in other division cause by the crisis.

### 5.0 Conclusion

The objective of the study on the evaluation on the phytochemical and antifungal properties of *Afrastyrax lepidophyllus*, *XylopiA aethiopicA* and *Monodora myristica* on *Trichophyton rubrum*, revealed sensitivity to the aqueous extracts of all the spices. The ethanolic extract of *A lepidophyllus* also revealed sensitivivity to *T.rubrum*. This proves that all the spices have antifungal properties and as such can be used as alternative treatment for fungal skin infection cause by *T. rubrum*.

#### 5.1 Recommendations

**We recommend:**

##### - To the Ministry of Public Health

That the government through the Ministry of Scientific Research, the Ministry of Higher Education, should initiate research reward programs aimed at promoting initiative and the use of local spices in the improvement of the health sector that can help reduce our dependency on the imported goods and promote consumption of our local plant. The results support the significant role played by spices as one of the alternative antifungal treatment for skin fungal infection particularly for *Trichophyton rubrum*.

##### - To the research commities

That these plant extracts should be used to test for the susceptibility of other groups of dermatophytes and others microganism as bacteria.

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