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Antimycotic Efficacy of Aqueous Extract from *Xylopi aethiopia* Against Some Zoophilic Dermatophytes

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Abstract

Xylopi aethiopia is an aromatic plant popularly used as spice. Its phytochemical constituents were determined and the results observed pharmaceutical constituents such as phenolics (1.51 ± 0.06), flavonoids (0.44 ± 0.75), glycosides (0.42 ± 0.01), saponins (0.22 ± 1.00), tannins (0.62 ± 0.00), steroids (0.14 ± 0.35) and alkaloids (1.94 ± 0.02). Thus, agar-well and disc diffusion methods were used to determine the minimum inhibitory concentration of the aqueous extract from the plant against some zoophilic dermatophytes (*Trichophyton mentagrophytes*, *Microsporum canis* and *Microsporum equinum*) isolated from domestic pets and livestock. The isolates were exposed to different concentrations of the extracts and the control (Itraconazole). Among the isolates, *T. mentagrophytes* was most susceptible to the extract in all the used concentrations with maximum zone inhibition (3.5 mm) at 1.2 mg/mL for agar-well method; while *M. canis* at concentration of 1.2 mg/mL showed maximum inhibition with zone formation of 2.84 mm for disc diffusion method. There was no significant ($p < 0.05$) difference in the results obtained for agar-well and disc diffusion methods and the inhibitory effects of the extract also compared favorably with the control (Itraconazole). Hence, the use of this plant extract in the treatment of these fungal infections in domestic pets and livestock will offer great hope to the rate of constant increase in antibiotic use and resistance observed in most pathogenic microbes.

Keywords: Spice, *Xylopi aethiopia*, Phytochemical, Antimycotic, Dermatophytoses

INTRODUCTION

Dermatophytes are fungi that require keratin for growth. These fungi can cause superficial infections of the skin, hair, and nails. Dermatophytes are spread by direct contact from other people (anthropophilic), animals (zoophilic), and soil (geophilic), as well as indirectly from fomites. Zoophilic dermatophytes are found primarily in animals and cause marked inflammatory reactions in humans who have contact with these infected animals. Infection may also be transmitted via indirect contact with infected animals, such as by their hair (Goldsmith and Fitzpatrick, 2012). These anamorphic (asexual or imperfect fungi) mold genera are: *Microsporum*, *Epidermophyton* and *Trichophyton*.

There are about 40 species in these three genera. Species capable of reproducing sexually belong in the teleomorphic genus *Arthroderma*, of the Ascomycota. The organisms colonize the keratin tissues causing inflammation as the host responds to metabolic byproducts. Colonies of dermatophytes are usually restricted to the non-living cornified layer of the epidermis because of their inability to penetrate viable tissue of an immunocompetent host. Invasion does elicit a host response ranging from mild to severe. Acid proteinases (proteases), elastase, keratinases, and other proteinases reportedly act as virulence factors (Chanda and Baravalia, 2010).

More so, herbal medicine which is also called botanical medicine or phytomedicine refers to the use of any plant's seeds, berries, roots, leaves, bark, or flowers for medicinal purposes (Kuate *et al.*, 2015). Long practiced outside of conventional medicine, herbalism is becoming more main stream as up-to-date analysis and research shows their value in the treatment and prevention of diseases. Indigenous cultures (African and Native American) uses herbs in their healing rituals, while others developed traditional medical systems (Ayurveda and Traditional Chinese Medicine) in which herbal therapies were used systematically (Kuate *et al.*, 2015).

Whole plant extracts have many components that work together to produce therapeutic effects and also to reduce the chances of side effects from any one component (Mhatre *et al.*, 2014). Plant drugs are usually considered to be less toxic and are also freer from side effects than the synthetic ones. In a study, medicinal plants have been reported to be very beneficial in wound care, promoting the rate of wound healing with minimal pain, discomfort, and scarring to the patient (Odimegwu *et al.*, 2008).

However, *Xylopia aethiopica* is an aromatic tree, of the *Annonaceae* family and is native to the lowland rainforest and moist fringe forests in the savanna zones of Africa. The aromatic plant of *X. aethiopica* commonly known as Ethiopia or Negro pepper has been used in Europe, Asia, and Africa as pepper substitute and spice in local cooking (White, 2006). Almost all parts of the plant are used in traditional medicine for managing various ailments including skin infections, candidiasis, dyspepsia, cough and fever (Mishana *et al.*, 2000). In Nigeria, *X. aethiopica* is known as 'Uda' by Igbos, 'Kimba' by Hausas and 'Erunje' by Yorubas. Hence, the present study aimed to evaluate the possible use of extracts from *X. aethiopica* for use as therapeutic agent in the treatment of topical mycotic infections caused by zoophilic dermatophytes isolated from some domestic and livestock animals.

MATERIALS AND METHODS

Collection and Pre-extraction Procedures of the Plant Material

The dried fruits of *Xylopia aethiopica* were purchased from a market in Onitsha, Anambra State, Nigeria. The fruits were cleaned properly and all extraneous materials removed. Cleaning was done by hands. After drying, the plant was powdered and stored in an airtight polypropylene bag till needed for the extraction.

Hydrodistillation of the Plant Material by Clevenger Method

The aqueous extract from the plant materials was achieved by hydrodistillation using 100 g of the sample which was added to 800 ml of distilled water in a 2-liter flask. The setup was placed in a balloon heater for 3 hours. At the end of the distillation, two phases were observed, an aqueous phase (aromatic water) and an organic phase (essential oil), less dense than water. The aqueous extract was collected, dried under anhydrous sodium sulphate and stored in sealed amber bottles at 4°C to avoid photo-oxidation until used for analysis (Majda *et al.*, 2019).

Characterization of the Extract Using Chromatographic Analyses

The chemical composition of the aqueous extract was determined by gas chromatography coupled with mass spectrometry (GC/MS) as described by Majda *et al.*, 2019. The GC analysis was performed using a chromatography equipped with a flame ionization detector (FID) and two capillary columns of different polarities OV type: 101 (25 m x 0.22 mm x 0.25 µm) and Carbowax 20 M (25 m x 0.22 mm x 0.25 µm). The carrier gas was helium with a flow rate of 0.8 ml/min and the oven programming temperature between 50 and 200°C with a gradient of 5°C/min. The Mass spectroscopy (MS) was used in the detection of the constituents and to determine the molecular weight of the compounds and identify the presence of isotopes patterns. MS coupling was performed on a DB1-type fused silica capillary column (25 m x 0.23 mm x 0.25 µm) with helium as a carrier gas and temperature programming identical to that of the GC.

Collection of Sample and Isolation of Test Organisms

A total of 100 samples were collected from domestic pets and farm animals (dog, goat, cattle, swine and rabbit). Scraped hairs from these animals were collected in sterile petri dishes, labeled according to their animal source and aseptically embedded in SDA prepared plates containing 1

% streptomycin. The plates were incubated at 28°C in the dark and observed for one week as described by Reddy *et al.* (2014).

Macroscopic and Microscopic Identification of the Fungal Isolates

The fungal morphology was studied macroscopically by observing the colony characteristics such as pigmentation, colony shape, size and hyphae, and was microscopically examined with the aid of a digital compound microscope, using a lactophenol cotton blue stained slide mounted with portion of the mycelium as described by Gaddeyya *et al.* (2012). The growth on each plate was identified for the specific species using the fungal atlas (Kidd *et al.*, 2016)

Inhibitory Concentration Test

Antimycotic evaluation using agar-well diffusion method

Unto seeded Sabourand Dextrose Agar plates of each of the test isolates, 4 wells were made with sterile cork-borer at equidistant positions and was labeled under the plate and 0.2 mL of the different concentrations (0.1, 0.3, 0.6, 0.9, and 1.2) of the extracts and the control (Itraconazole) were introduced into the different holes using sterile micropipette and allowed to diffuse into the medium for 1 hour at room temperature. The plates were incubated at 37°C for 72 hours. All tests were performed in aseptic conditions and antimycotic activity was expressed as the mean diameter of the clear zone (mm) produced by the extract and the control.

Antimycotic evaluation using disc diffusion method

Filter paper was perforated, sterilized and used as disc. The sterile discs were dipped into the different concentrations (0.1, 0.3, 0.6, 0.9, and 1.2) of the extracts and the control (Itraconazole) respectively and placed at equidistant positions unto the seeded Sabourand Dextrose Agar plates of each of the test organisms and antimycotic activity was expressed as the mean diameter of the clear zone (mm) produced by the extract and the control.

Data Analysis

Data generated were analyzed using one-way analysis of variance and mean separation was done by Duncan's new multiple range test and paired t-tests. Significant difference was accepted at $p < 0.05$.

RESULTS

Phytochemical constituents of *X. aethiopica*

The phytochemical screening of the crude aqueous extract from *Xylopia aethiopica* fruits as shown in Figure 1 contained pharmaceutical constituents such as phenolics (1.51 ± 0.06), flavonoids (0.44 ± 0.75), glycosides (0.42 ± 0.01), saponins (0.22 ± 1.00), tannins (0.62 ± 0.00), steroids (0.14 ± 0.35) and alkaloids (1.94 ± 0.02).

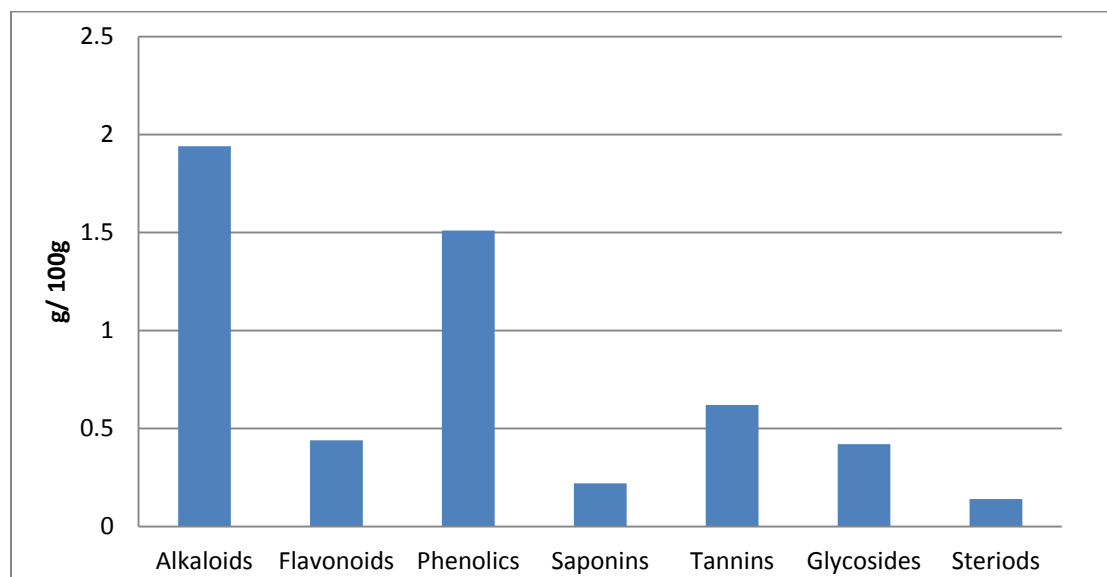


Figure 1: Phytochemical constituents of *X. aethiopica*

Macroscopic and Microscopic Features of the Isolates

The fungal species identified and their sources of isolation were *Trichophyton mentagrophytes* (dog, cattle), *Microsporum canis* var. *distortum* (dog, goat, cattle, swine, rabbit) a dysgonic variant of *M. canis* and *Microsporum canis* var. *equinum* (dog) a genotypic synonym of *M. canis*.

Trichophyton mentagrophytes

Macroscopic Description: The isolated colonies were flat and white to cream in color, with a granular suede-like surface. **Microscopic Description:** The isolate showed large numbers of single-celled microconidia formed in dense clusters. The microconidia were hyaline, smooth-walled, and are spherical in shape. The macroconidia were smooth, spherical, thin-walled, multicelled with spiral hyphae.

Microsporium canis

Macroscopic Description: The isolated colonies of *Microsporium canis* var. *distortum* are flat and spreading, showed pigments of white to cream, with a dense cottony-like surface. While colonies of *Microsporium canis* var. *equinum* were pale buff colored. Microscopic Description: *Microsporium canis* var. *distortum*, had a distinctive distorted macroconidia when viewed under the microscope. While *Microsporium canis* var. *equinum* showed broad, irregular, and spindle-shaped macroconidia with rough thick walls and few septa.

In vitro inhibitory potential of the extract

The results of antimycotic evaluation of the extracts from *X. aethiopica* on *Microsporium canis*, *Trichophyton mentagrophytes* and *Microsporium equinum* are presented in Tables 1, 2 and 3 respectively. For agar-well method, the extract of *X. aethiopica* fruits showed highest antimycotic effect against *T. mentagrophytes* in all the tested concentrations with maximum at 1.2 mg/mL with clear zone of inhibition of 3.5 mm (Table 2). *M. canis* also showed effective inhibition rate with a clear zone of 2.8 mm (Table 1) while *M. equinum* had an inhibition value of 2.7 mm; thus there was no significant difference ($p < 0.05$) in the inhibitory effect of the extract for *M. canis* and *M. equinum* at the highest concentration of 1.2 mg/mL. However, the standard Itraconazole (5 µg) showed significant ($p < 0.05$) clearer zone of inhibition against the entire tested organisms at all concentrations.

Table 1: Antimycotic activity of aqueous extract from *X. aethiopica* on *M. canis*

Conc. (mg/ mL)	Agar-well Method*		Disc Diffusion Method*	
	Extract	Control	Extract	Control
0.1	1.23 ^{bc} ± 1.0	1.04 ^c ± 0.7	1.26 ^d ± 0.1	1.75 ^{cd} ± 0.7
0.3	1.23 ^{bc} ± 0.7	1.52 ^b ± 0.5	1.28 ^d ± 0.5	1.86 ^c ± 0.6
0.6	1.34 ^b ± 0.5	1.57 ^b ± 0.2	2.02 ^c ± 0.4	2.52 ^b ± 0.2
0.9	2.56 ^{ab} ± 0.2	1.57 ^b ± 0.3	2.23 ^b ± 0.7	2.54 ^b ± 0.9
1.2	2.72 ^a ± 0.8	1.91 ^a ± 0.2	2.84 ^a ± 0.5	3.36 ^a ± 1.0

*Zone of inhibition (mm). Values are means ± SD (n = 3). Values on the same column with different superscripts are significantly ($p < 0.05$) different

Table 2: Antimycotic activity of aqueous extract from *X. aethiopica* on *T. mentagrophytes*

Conc. (mg/ mL)	Agar-well Method*		Disc Diffusion Method*	
	Extract	Control	Extract	Control
0.1	1.45 ^d ± 0.3	1.82 ^c ± 1.0	---	1.04 ^d ± 0.2
0.3	2.05 ^c ± 0.6	2.25 ^{bc} ± 0.6	---	1.45 ^c ± 0.3
0.6	2.45 ^{bc} ± 0.7	2.53 ^b ± 0.6	1.93 ^b ± 0.3	2.51 ^b ± 0.8
0.9	2.52 ^b ± 1.0	2.55 ^b ± 0.1	1.98 ^b ± 0.7	2.54 ^b ± 0.4
1.2	3.58 ^a ± 0.5	3.02 ^a ± 0.7	2.11 ^a ± 1.0	2.81 ^a ± 1.0

*Zone of inhibition (mm)

Values are means ± SD (n = 3). Values on the same column with different superscripts are significantly (p < 0.05) different

---: no inhibition

Table 3: Antimycotic activity of aqueous extract from *X. aethiopica* on *M. equinum*

Conc. (mg/ mL)	Agar-well Method*		Disc Diffusion Method*	
	Extract	Control	Extract	Control
0.1	2.05 ^d ± 1.0	2.31 ^c ± 0.2	1.27 ^c ± 0.8	2.36 ^c ± 0.4
0.3	2.47 ^c ± 0.3	2.36 ^c ± 0.5	1.43 ^b ± 0.9	2.55 ^b ± 0.6
0.6	2.48 ^c ± 0.4	2.64 ^b ± 0.3	1.44 ^b ± 0.4	2.58 ^b ± 1.0
0.9	2.69 ^{ab} ± 0.7	2.66 ^b ± 0.2	1.55 ^a ± 0.9	2.82 ^{ab} ± 0.3
1.2	2.75 ^a ± 0.1	2.85 ^a ± 1.0	1.57 ^a ± 0.4	2.96 ^a ± 0.2

*Zone of inhibition (mm)

Values are means ± SD (n = 3). Values on the same column with different superscripts are significantly (p < 0.05) different

DISCUSSION

The presence of these secondary metabolites in *X. aethiopica* has been reported by Asekun and Adeniyi (2004) and demonstrated the plant to have inhibited cell wall formation in fungi leading to death of the organisms. Ogunkunle and Ladejobi (2006) also reported that flavonoids have antimicrobial properties and can coagulate protoplasm of microorganisms. It has also been reported that these compounds are mostly secondary metabolites which are capable of producing definite physiological actions and are the most important bioactive constituents of natural products (Djeridane *et al.*, 2006). The presence of these metabolites suggests great potential of *X. aethiopica* as an active ingredient in the formulation of topical creams for treatment of fungal infections caused by zoophilic dermatophytes.

For disc diffusion method, the extract from *X. aethiopica* showed effective antimycotic effect against *M. canis* at concentration of 1.2 mg/mL with inhibition of 2.84 mm (Table 1). The next best response for inhibition activity was seen against *M. equinum* with highest inhibition of 1.75 mm at 1.2 mg/mL concentration (Table 3). The activity showed least effect on *T. mentagrophytes* with zone diameter of 2.11 mm with no inhibitive effect at 0.1 and 0.3 mg/mL concentrations (Table 2). However, the standard Itraconazole (5 µg) showed significantly ($p < 0.05$) inhibitory effect against the entire tested organism at all concentrations.

In comparison, there was no significant ($p < 0.05$) difference in the results obtained for agar-well and disc diffusion methods and the control (Itraconazole) also compared favorably. Similar findings was made by Chude *et al.* (2018), for *in vitro* activity of *Xylopiya aethiopica* extract against *Monacrosporium bembicodes* isolated from powdered soyabean samples and the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of the extracts exhibited similar activity in their MICs when compared to ketoconazole (control).

CONCLUSION

With the rate of constant increase in antibiotic resistance, there is great need to explore plant-based ingredients in the treatment of microbial infections. The study observed that extract of *Xylopiya aethiopica* has the potential of antimycotic properties for the treatment of diseases caused by dermatophilic fungi. Thus, plants extracts have great potential as antimicrobial

compounds against microorganisms and there is need for further investigation required to isolate, purify and structurally elucidate the active component of this plant. Data from literatures as well as the present study reveal great potential of plants for therapeutic treatment, in spite of limited investigations; such plants can be used in the treatment of infectious diseases caused by resistant microbes, thus minimizing the possible toxic effects. However, the toxicity analysis of the pure compound to determine its therapeutic threshold is crucial and will help in its formulation into crude antibiotic drug or creams.

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