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Antidermatophytic activity of alkaloid and saponin extracts from *Cnidoscopus aconitifolius*.

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ABSTRACT

The antidermatophytic activity of saponin and alkaloid extracts from organs of *Cnidoscopus aconitifolius* against *Trichophyton rubrum*, *Microsporium gypseum*, *Epidermophyton floccosum* and *Trichophyton interdigitale* was studied by the disc diffusion method and the minimum inhibitory concentration (MIC) assessed by the broth microdilution technique. Our findings revealed a wide range of activity against the tested dermatophytes. The saponin root extract exhibited promising activity against all the tested dermatophytes (IZ = 27 – 34mm; MIC = 0.2 – 1.1mg/mL), followed closely by the alkaloid root extract (IZ = 15–30mm; MIC = 0.4–2.3mg/mL). Also, the saponin stem extract was most active against *Microsporium canis* (MIC = 1.7mg/mL) and the saponin leaf extract against *Trichophyton rubrum* (MIC = 1.8mg/mL). Furthermore, the alkaloid stem extract inhibited the growth of *Microsporium canis* (MIC = 0.4mg/mL) while the alkaloid leaf extract inhibited the growth of *Microsporium gypseum* (MIC = 0.9mg/mL). These findings suggest that saponin and alkaloid extracts from organs of *C. aconitifolius* can be used as natural alternatives for the treatment of dermatophycosis caused by these organisms.

Keywords: Antidermatophytic activity, phytochemicals, *Cnidoscopus aconitifolius*, *Trichophyton rubrum*, *Microsporium gypseum*, *Epidermophyton floccosum*, *Trichophyton interdigitale*.

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1. Introduction

Cnidoscoulous aconitifolius is an indigenous West African plant commonly called tree spinach. The plant is a perennial shrub that belongs to the family Euphorbiaceae and is widely distributed in Nigeria. The leaves of tree spinach are edible and contain linamarin with varying commercial and traditional uses [1-3]. Extracts of the plant leaves exert a wide range of biological activities including sedative, antibacterial, antidiabetic, antioxidative, anti-inflammatory, analgesic and hepatoprotective potentials [3-9]. Plant extracts are used to treat fungal infections due to increased concerns over resistance to commercially available antifungal drugs. For instance, fungal infections caused by dermatophytes constitute a serious problem especially in developing countries including Nigeria. The causative organisms which are members of the genera *Trichophyton*, *Microsporum* and *Epidermophyton*, are most prevalent amongst rural dwellers due to poverty, poor hygiene and inadequate health facilities. These infections range from superficial to life threatening conditions, particularly in patients with compromised immune systems [10].

Constraints such as cost, drug interactions, side effects, resistance, etc, may affect the therapeutic efficacy of current antifungal drugs. In order to minimize these challenges, natural products with promising antifungal activities are isolated and used as templates for the development of safer, more potent and less toxic antifungals [11-12]. Despite numerous publications on the antifungal properties of phytochemicals [10-15], information on the dermatophytic activity of *C. aconitifolius* is lacking. Therefore, we report the antidermatophytic activity of saponin and alkaloid extracts from organs of *C. aconitifolius* to provide further insight on the health significance of the plant.

2. Materials and Methods

2.1 Plant material and preparation of extracts

Organs (leaves, stems and root) of *C. aconitifolius* were harvested from a farm in Uyo, Akwa Ibom State, Nigeria in 2016, air dried, pulverized and lyophilised. Saponins were isolated by the modified method of Moghimipour *et al* [16]. Each plant organ (250g) was defatted with hexane, extracted with ethanol (800mL) in a soxhlet apparatus and concentrated *in vacuo*. The residue was suspended in water, then centrifuged at 2500rpm for 45 min, and the supernatant separated and extracted with water saturated with n-butanol.

The butanol phase was concentrated *in vacuo*, redissolved in 50mL methanol and precipitated with excess ether to obtain the crude saponin, which gave a positive frosting test. Alkaloids were isolated by the modified method of Maatalah *et al* [17]. 250g of each plant organ was defatted with hexane, extracted with ethanol (800mL) in a soxhlet apparatus and concentrated *in vacuo*. The concentrated extract was acidified with 0.5M H₂SO₄. The acidic extract was washed with chloroform to remove neutral

components, then basified to pH 9.0 with 10%NH₄OH, then re-extracted with chloroform until the aqueous layer was free of alkaloids. The combined chloroform extracts were evaporated to yield the crude alkaloid extract. This extract tested positive to Dragendorff and Meyer's test.

2.2 Antifungal study

Fungal isolates (*Trichophyton rubrum*, *Microsporum gypseum*, *Epidermophyton floccosum* and *Trichophyton interdigitale*) were obtained from infected patients at the University of Uyo Teaching Hospital, identified through standard methods and maintained by culturing in Sabouraud dextrose agar (SDA) at room temperature until needed. Stock inoculum suspensions were obtained from each strain by scraping the agar surface with sterile saline solution (0.85%) using a sterile loop; the resulting conidial suspension transferred to sterile tubes, left for 15 to 20 minutes to sediment the heavy particles and then adjusted to 0.5 MacFarland scale to obtain a final fungal suspension of 1-5 x 10⁶ cells mL⁻¹.

The antifungal activity of the extracts was evaluated by the disc diffusion assay and the microdilution assay. The disc diffusion assay was performed in accordance with the method of Gomes *et al* [18] with some modification. Briefly, sterile filter paper discs (5 mm) were impregnated with the extracts (2mg mL⁻¹) and controls. The impregnated discs were placed on SDA with chloramphenicol, which had been previously inoculated with 0.1 mL of each fungal isolate, and the plates incubated at 32°C for 5 days. The antifungal activity of each extract was assessed by measuring the zone of inhibition around the disc and compared with the control. ketoconazole (15µg/disc) was used as control. Broth microdilution assay based on the CLSI document M38-A2 with minor modification was used to determine the minimum inhibitory concentration (MIC). A 96-well microtiter plate was used. Briefly, the spore or cell suspension were prepared from recent cultures of each fungal strain on SDA with chloramphenicol and diluted to a final inoculum concentration of 0.4-5 x 10⁴ CFU/mL with RPMI-1640, buffered to pH 7 with MOPS.

A two – fold serial dilution of the extracts was made starting from 15mg/mL for each extract. 100µl of the extracts at varying concentrations and 100 µl of the cell suspensions were added to the wells. Controls were tested along with the samples. The inoculated plates were incubated at 28°C for 4-7 days. The MIC was defined as the lowest concentration showing no visible growth or at least 80% reduction when compared to the control wells. Each test was performed in duplicate [19]. For minimum fungicidal concentration (MFC), 10µL of suspension from the MIC was re-inoculated on SDA and incubated at 28°C for 3-7 days. MFC was defined as the lowest concentration that yielded negative subcultures or only a single colony.

2.3 Statistical Analysis

Results are expressed as mean of duplicate analysis. Data was evaluated using one way analysis of variance (ANOVA). SPSS 14.0 was used for all statistical analysis.

3. Results and Discussion

The emergence of antifungal resistant dermatophyte strains to existing drugs, cost, drug interactions, treatment regimes, toxicity, have prompted research into the development of new platforms for combating these infections in humans. This study was therefore undertaken to evaluate the antidermatophytic activity of saponin and alkaloid extracts from organs of *C. aconitifolius*. Our results indicate the pertinent role of phytochemicals as natural sources of antidermatophytic substances. In the disc diffusion assay, inhibitory zones for saponin extracts ranged from 11 to 34 mm more than 9 to 33 mm for the alkaloid extracts (Figure 1), and indicates a low difference in antifungal property, but this difference was significant at $p < 0.05$. With regards to plant organs, saponin root extract of *C. aconitifolius* was fungicidal against all the test dermatophytes with inhibitory zone (IZ) range of 27 - 34 mm (Figure 1). Saponin stem extract was most active against *M. canis* (IZ = 24 mm) and *T. rubrum* (IZ = 21 mm), while the saponin leaf extract was active against *T. rubrum* (IZ = 20 mm) and *M. gypseum* (IZ = 19 mm). Generally, the inhibitory effect of saponin extracts on the tested dermatophytes followed the trend: root > stem > leaf. This difference may be due to the varying function of the plant parts, chemical composition and amount of these phytochemicals present. Minimum inhibitory concentration (MIC) ranged from 0.2 - 5.6 mg mL⁻¹ for the saponin extracts (Table 1), and correlated positively with the inhibition zones. Saponin root extract

showed the best activity against the test organisms. For instance, *Trichophyton interdigitale* was inhibited at MIC of 0.2 mg mL⁻¹, *Microsporium canis* at MIC of 0.3 mg mL⁻¹, whereas the other dermatophytes were inhibited at MIC of 0.5 - 1.1 mg mL⁻¹. Saponin stem and leaf extracts inhibited the dermatophytes at MIC of 1.7 mg mL⁻¹.

In relation to other studies, saponins are known to exert antifungal activity against a wide range of microorganisms. For instance, saponins, such as hederagenin from stem bark of *Polyscias fulva* inhibited the growth of yeast and dermatophytes with MIC range of 0.78–100 µg mL⁻¹ [20]. Also, spirostanol saponin isolated from the leaves of *Solanum hispidum* inhibited the growth of *T. mentagrophytes* and *T. rubrum* at IC₅₀ of 25 µg mL⁻¹ [21]. In addition, spirostan saponins from *Solanum chrysotrichum* Schlttdl, exerted antimycotic activity against *T. mentagrophytes*, *T. rubrum*, *Aspergillus niger* and *Candida albicans* [22]. Furthermore, other saponins such as triterpene saponins, steroidal saponins or saponin-rich extracts are known to possess antifungal properties [23]. The observed activity of the saponin extracts from organs of *C. aconitifolius* may be due to their ability to interact and bind with sterols in the fungal membrane, causing pore formation, loss of membrane integrity and selective permeability [20].

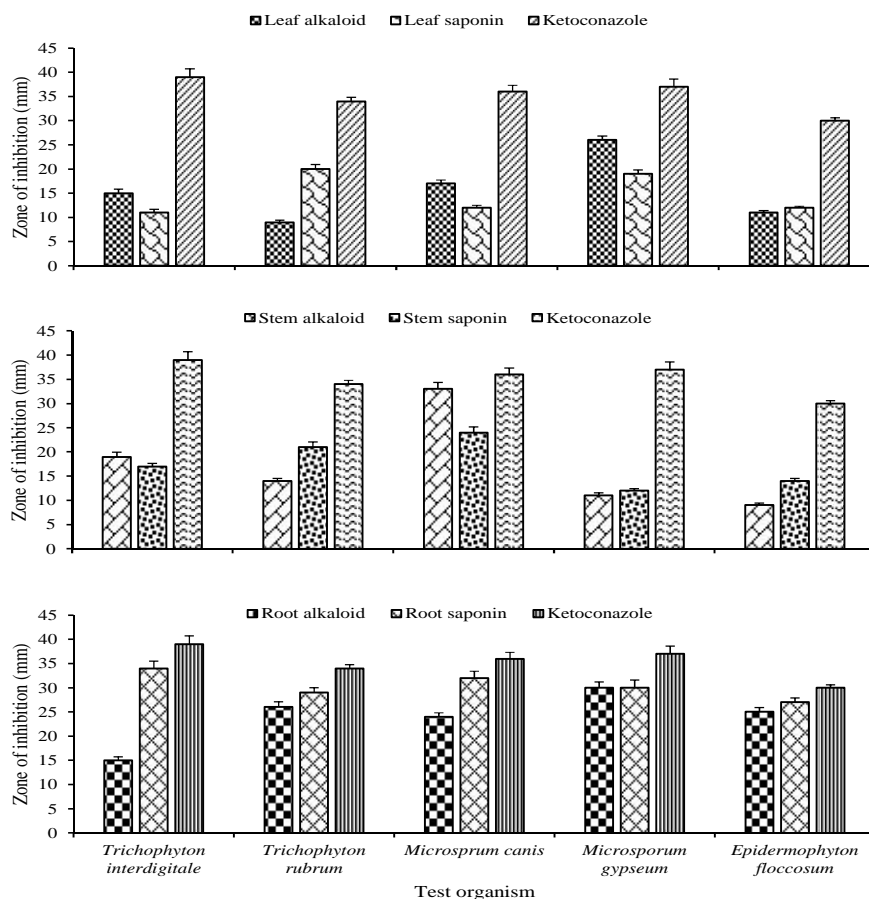


Figure 1: Antifungal activity of the saponin and alkaloid extracts of *C. aconitifolius* on dermatophytes

With regards to the alkaloid extracts from organs of *C. aconitifolius*, the alkaloid root extract exhibited notable activity against *M. gypseum* (MIC = 0.4 mg mL⁻¹), the alkaloid stem extract against *M. canis* (MIC = 0.4 mg mL⁻¹) and the alkaloid leaf extract against *M. gypseum* (MIC = 0.9 mg mL⁻¹). Overall, MIC obtained from the extracts were higher than that of the control and this difference was significant ($p = 0.05$). MFC values (Table 2) were at least one dilution before the MIC values.

Table 1: MIC of alkaloid and saponin extracts of *C. aconitifolius*

Dermatophyte	MIC						
	Alkaloid extracts (mg/mL)			Saponin extracts (mg/mL)			Control (µg/mL)
	Leaves	Stem	Root	Leaves	Stem	Root	Ketoconazole
<i>Trichophyton interdigitale</i>	2.9	2.1	2.3	5.6	3.0	0.2	0.5
<i>Trichophyton rubrum</i>	5.5	3.5	1.1	1.8	2.1	0.9	0.7
<i>Microsporum canis</i>	2.9	0.4	1.7	4.2	1.7	0.3	0.5
<i>Microsporum gypseum</i>	0.9	3.9	0.4	1.9	4.5	0.5	0.5
<i>Epidermophyton floccosum</i>	4.1	5.2	1.2	4.3	3.4	1.1	0.6

Table 2: MFC of alkaloid and saponin extract of *C. aconitifolius*.

Dermatophyte	MFC						
	Alkaloid extracts (mg/mL)			Saponin extracts (mg/mL)			Control (µg/mL)
	Leaves	Stem	Root	Leaves	Stem	Root	Ketoconazole
<i>Trichophyton interdigitale</i>	5.8	4.2	4.6	11.2	6.0	0.4	1.0
<i>Trichophyton rubrum</i>	11.0	7.0	2.2	3.6	4.2	1.8	1.4
<i>Microsporum canis</i>	5.8	0.8	3.4	8.4	3.4	0.6	1.0
<i>Microsporum gypseum</i>	1.8	7.8	0.8	3.8	9.0	1.0	1.0
<i>Epidermophyton floccosum</i>	8.2	10.4	2.4	8.6	6.8	2.2	1.2

Alkaloids or their extracts possess antifungal activity. For example, berberine, an isoquinoline alkaloid exhibited antifungal activity against strains of *C. albicans* and other non – albican species at 200 µg mL⁻¹ [24]. Similarly, other alkaloids such as – carboline, phenyl-ethyl derived alkaloids from *Cyathobasin fruitulosa* and haloxylinas A and B from *Haloxylon saliconium* displayed antifungal potentials by altering the integrity of the plasma and mitochondrial membranes, resulting in DNA damage and cell death [25]. However, our values were slightly higher than reports by Khursheed [26]. This difference may probably be due to solubility, diffusability and the complex chemical nature of the alkaloid extract.

4. Conclusion

In conclusion, we have shown that saponin and alkaloid extracts from organs of *C. aconitifolius* displayed promising activity against dermatophytes, with the best activity exhibited by the root extracts. Based on these findings, the saponin and alkaloid root extracts can serve as a natural alternative for the treatment of fungal infections caused by susceptible dermatophytes. Isolation and identification of the active principles in these extracts is therefore pertinent.

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