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Original Article

Antimycotic Potential of *Calotropis procera* Against *Aspergillus niger* and *Rhizopus stolonifer*

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ABSTRACT

Background: For centuries, there has been a growing fascination with the application of herbs for fungicidal purposes worldwide. This surge in interest could be attributed to the cost-effectiveness and lower toxicities of these herbal remedies compared to the counterpart antifungal drugs. There is substantial scope to explore the curative effects of various plant components, including roots, stems, leaves and fruits of *Calotropis procera*, renowned for its pharmacological activities, encompassing anticancer, antimicrobial, and antioxidant.

Objectives: In the present study, the antimycotic potential of the leaf and root extracts of *C. procera* on the growth of *Aspergillus niger* and *Rhizopus stolonifer* was investigated.

Methods: The antimycotic potential of *C. procera* was established via the agar well diffusion method after initial preliminary phytochemical screening of the extracts.

Results: Alkaloids, flavonoids, saponins, steroids, tannins and terpenoids were present, while glycosides and phenolics were tested negative. The leaf extract inhibited the tested organisms in a concentration-dependent manner where at 100% concentration, both *A. niger* and *R. stolonifer* showed high mycelia inhibition of 81.1% and 79.4%, respectively. Also, the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) values of 31.0% and 48.0% were recorded for *A. niger*, as well as 28.3% and 45.7% were recorded for *R. stolonifer.* The aqueous root extract exhibited mycelia inhibition of 74.4% and 77.8% against *A. niger* and *R. stolonifer* at 100% concentration with respectively MIC and MFC values of 32.7% and 50.3% for *A. niger* and 30.3% and 52.0% for *R. stolonifer*.

Conclusion: The study provides promising evidence of potential medicinal properties associated with *C. procera,* supporting its potential therapeutic applications in traditional medicine.

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Introduction

Microbial infections and antibiotic resistance continue to be a menace and burden on individuals and healthcare systems, transforming into threats to livelihoods and development worldwide, targeting individuals regardless of age [1]. The abuse of antibiotics, entailing misuse and overuse, further complicates the management of antibiotic resistance. Herbal medicines from plant sources have long been considered an alternative to synthetic drugs, partly attributed to their phytoconstituents and synergistic mechanisms of action, which accompany minimal side effects. The application of these plants in managing microbial infections is of particular interest due to the diversity of their mode of action targeting different microbial components and enzymes [2, 3]. The diverse nature of fungal infections involving the Ascomycota and *Basidiomycota phyla* are the main class of pathogenic fungi, with the latter responsible for most of the fungal infections while the former is attributed to invasive meningitis and superficial skin infections [4].

Natural products from different sources are considered the bedrock of modern medicine, serving reservoirs of unlimited compounds that act as drugs of varying efficacy and various pharmacological activities [5, 6]. Furthermore, some of the prescribed medications in modern medicine are originated directly or indirectly from plants [7, 8]. *Calotropis procera* is an Asclepiadaceae found in Asia and Africa. It is characterized by its broadleaf and strong odor, is commonly called Milkweed and is employed in disease management [8]. This plant was reported to show diverse pharmacological properties, including anticancer, analgesic and antimicrobial [9]. Furthermore, this plant has been reported to exert anthelmintic effects in sheep via temporary paralysis of red stomach worms and decreasing percentage egg counts of nematodes inhabiting the gastrointestinal region [10]. Another study reported the plant's anti-odontalgic and anti-syphilitic medicinal use [11]. The anticancer activities of the plant were attributed to its diverse targeting pathways and avoiding apoptotic pathways [12].

Aspergillus niger is responsible for diverse fungal infections in both humans and animals [13]. In humans, it is mainly linked to aspergillosis, a collection of respiratory infections caused by *Aspergillus* species. The infection's severity varies depending on the location and extent, giving rise to conditions like allergic bronchopulmonary aspergillosis, aspergilloma (fungal ball in lung cavities), and invasive pulmonary aspergillosis, which poses a severe and life-threatening risk, especially for individuals with compromised immune systems [11]. Additionally, *A. niger* can cause opportunistic infections in animals, leading to diseases like aspergillosis in avian species and other animals. *Rhizopus stolonifer* is responsible for mucormycosis, also known as zygomycosis. This rare yet severe fungal infection predominantly affects individuals with weakened immune systems, including those with uncontrolled diabetes, organ transplant recipients, or hematologic malignancies [14]. Mucormycosis usually initiates in the sinuses and can extend to the brain or other body regions, potentially leading to life-threatening complications. Swift diagnosis and treatment are essential in effectively managing this invasive fungal infection [15]. In the present study, the antimycotic activity of the fresh leaf and root extract of *C. procera* against *A. niger* and *R. stolonifer* was investigated to justify its applications in traditional and folkloric medicine.

Materials and Methods

Collection of plant sample

The *C. procera* plants were gathered from the surroundings of Modibbo Adama University, Yola. A forest technologist carried out the plant identification from the Forest Technology Department of Adamawa State Polytechnic, where a voucher specimen (No. ASP/FT/23/023) was deposited. The roots and leaves were meticulously washed with tap water to remove impurities. Afterward, the plant parts were subjected to shade drying before being ground into a fine powder using a mechanical grinder. The powdered plant material was sieved through a fine mesh to ensure uniformity. The resulting powder was utilized for extraction using the Soxhlet apparatus, facilitating the retrieval of bioactive compounds from the plant for further investigation and analysis.

Preparation of plant extract

In this study, 50 g of dried leaves and roots of *C. procera* were subjected to extraction using the Soxhlet apparatus with 250 mL of distilled water. After two days, the extracted solution was evaporated using a rotary evaporator, producing crude semi-solid extracts. The extract from the leaves appeared green, while the extract from the roots was dark brown.

Phytochemical screening

The preliminary phytochemical tests were conducted to identify various chemical compounds' presence us-

Table 1. Phytochemical components of leaf and root aqueous extracts of *C. procera*

Variables	Alkaloids	Flavonoid	Glycosides	Phenolic	Saponins	Steroids	Tannins	Terpenoids
Leaf	+++	++	-	-	+	+	+	+
Root	+	++	-	-	+	+	+	+

+Faintly present, ++Moderately present, +++Strongly present, -Absent.

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ing the methods previously described by Evans [16] to detect tannins, flavonoids, saponins, alkaloids, phenols, and glycosides. These tests provide essential insights into the potential bioactive components present in the plant material.

Source of the fungi used

The test organisms used in the study were sourced from the Microbiology Department Laboratory at Modibbo Adama University, Yola, Adamawa State.

Antifungal activity

Different concentrations (100%, 75%, 50% and 25%) of the extracts were tested for their percentage (%) of mycelia inhibition. The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) were determined using 25%, 35%, 45%, and 55% concentrations. The experimental procedure involved preparing potato dextrose agar (PDA) media in petri plates, inoculating them with the fungal species using sterile swabs, and creating wells with a cork borer. The leaf and root extracts were then added to the wells at the specified concentrations, followed by incubation at room temperature for 48 hours before observing the growth inhibition [17]. The wells without the extract were used as a negative control. The percentage of mycelia inhibition was determined as Equation 1:

1.

$$\text{Mycelia inhibition (\%)} = \frac{\text{Mycelia growth of control} - \text{Mycelia growth without extract}}{\text{Mycelia growth of negative control}} \times 100$$

Data analysis

In this study, all experiments were conducted in triplicate. The difference among the groups was evaluated by analysis of variance (ANOVA) followed by Tukey's multiple comparison test at $P<0.05$ significance level using SPSS software, version 22.

Results

Phytochemical components of the crude leaf and root extracts of *C. procera* are presented in Table 1. Phytonutrients in the extracts include alkaloids, flavonoids, saponins, steroids, tannins, and terpenoids, while glycosides and phenolics were tested negative.

The leaf extract inhibited the tested organisms, and the higher the concentration of the extracts, the higher the % mycelia inhibition, as shown in Figure 1. At the extract concentration of 100%, both *A. niger* and *R. stolonifer* showed their highest zone of mycelia inhibition at 81.1% and 79.4%, respectively. MIC and MFC values of 31.0% and 48.0% were recorded for *A. niger*, while 28.3% and 45.7% for *R. stolonifer*, respectively (Figure 2).

The root extract inhibited the tested organisms, as shown in Figure 3. At 100%, the extract demonstrated the highest percentage of mycelia inhibition (74.4%) against *A. niger*, while that of *R. stolonifer* was 77.8%. The MIC and MFC values were 32.7% and 50.3%, respectively, against *A. niger* and 30.3% and 52% for *R. stolonifer*, as shown in Figure 4.

Discussion

Medicinal herbs encompass numerous phytochemicals responsible for addressing diverse ailments. Throughout history, ancient civilizations harnessed the therapeutic potential of these plants, enabling them to treat various diseases [18] effectively. The gifts of nature in the form of medicinal plants with intrinsic healing properties have been widely utilized in numerous countries to alleviate a range of conditions, including muscular spasms and skin diseases [19]. The present investigation revealed numerous phytochemicals in the aqueous extract of *C. procera* leaves and roots, as identified through established detection techniques, demonstrating their therapeutic potential. Using an aqueous solvent for plant extraction aligns with traditional healers and herbalists' practices, who commonly employ water as a readily available solvent for extracting biologically active compounds [20, 21]. The analysis of *C. procera* phytochemical constituents revealed the presence of alkaloids, flavonoids, saponins,

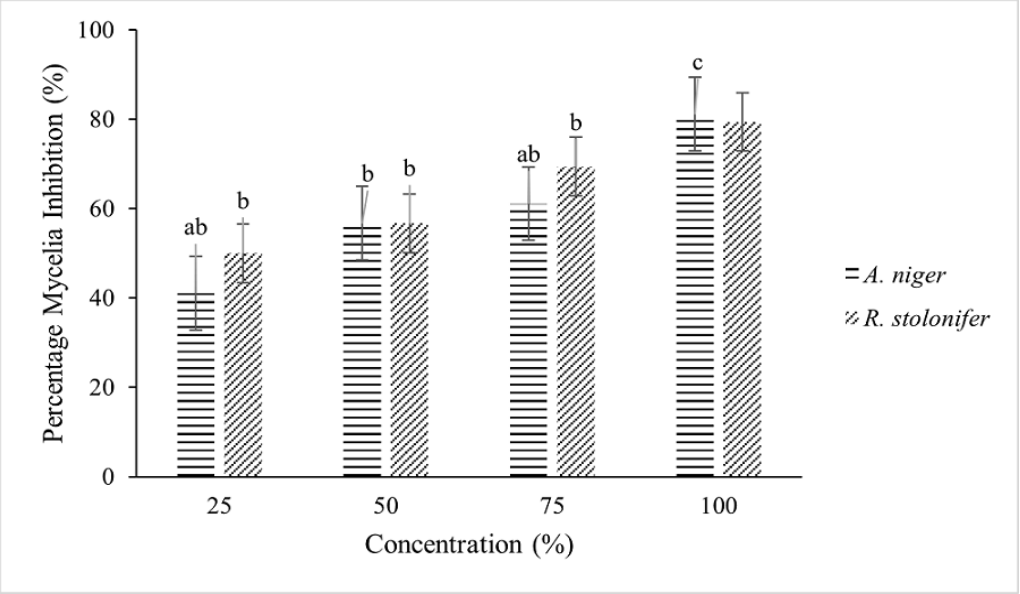

Figure 1. Percentage mycelia inhibition of *C. procera* leaf extract against *A. niger* and *R. stolonifer*

steroids, tannins and terpenoids, while glycosides and phenolics were absent, consistent with findings reported by Sabzal et al. [22].

The aqueous leaf and root extracts of *C. procera* demonstrated notable antifungal activity against *A. niger* and *R. stolonifer*, with varying degrees of growth inhibition. Both leaf and root extracts inhibited the mycelia growth of the fungal isolates, with the leaf extract showing higher efficacy, inhibiting 81.1% and 79.4% of *A. niger* and *R. stolonifer*, respectively. These findings are consistent with previous studies by Hassan et al. [23] and Aliyu et al. [24], which also highlighted the significant antifungal properties of *C. procera* against various fungal species, supporting its potential as a fungistatic or fungicidal agent at relatively low concentrations.

While the antifungal activity of the aqueous leaf and root extracts of *C. procera* has been demonstrated, the specific chemical components responsible for this activity and the underlying mechanisms of action have not been elucidated. It is known that antimycotics, in general, inhibit fungal growth by various mechanisms, such as disrupting fungal membrane permeability, inhibiting

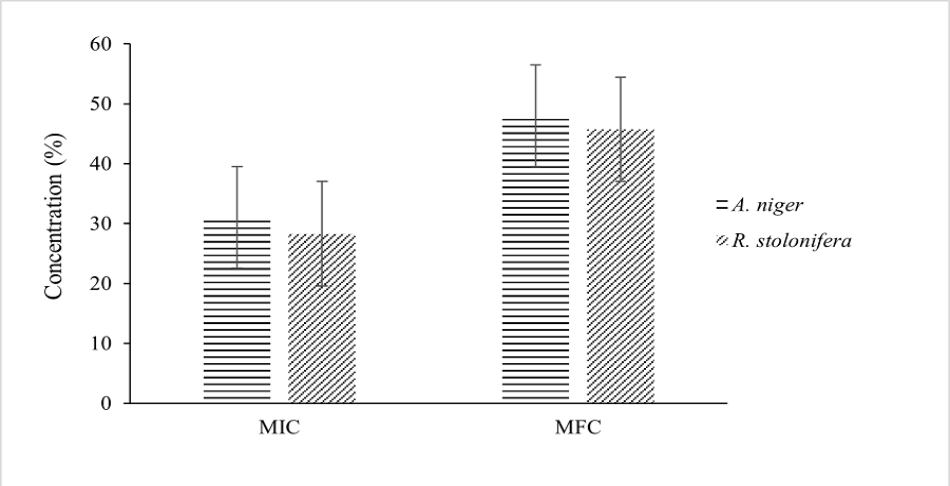

Figure 2. MIC and MFC of aqueous leaf extract of *C. procera* against *A. niger* and *R. stolonifer*

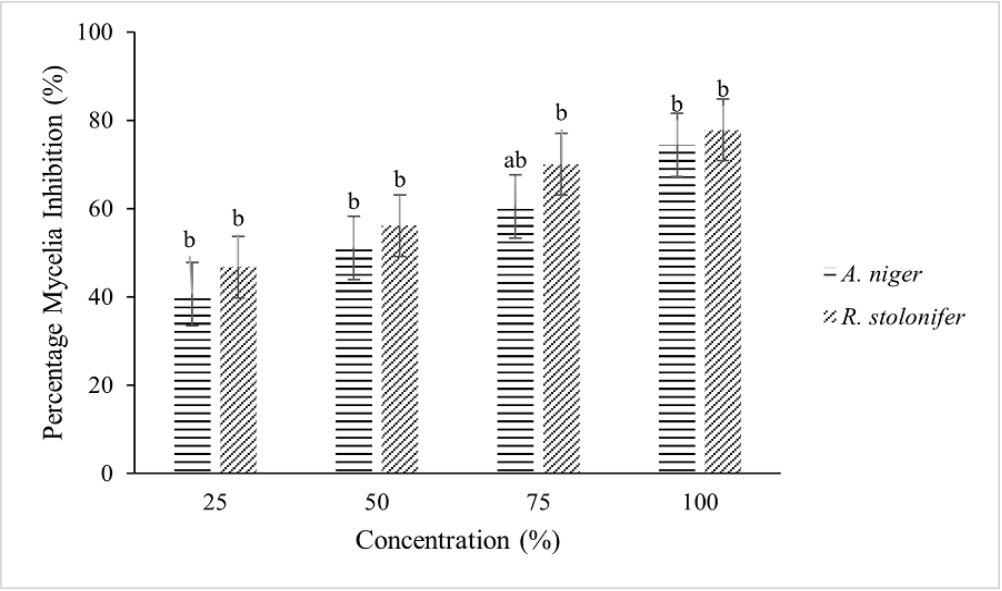

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Figure 3. Percentage mycelia inhibition of *C. procera* root extract against *A. niger* and *R. stolonifer*

sterol synthesis, interfering with nucleic acid synthesis, or inhibiting protein synthesis [24]. Further research is required to uncover the precise bioactive compounds and understand the detailed molecular mechanisms that contribute to the antifungal effects of *C. procera* extracts.

The MIC values of the aqueous leaf extract were notably higher than those of the root extract for both tested organisms, with values of 31% and 28%, respectively. Singh et al. [25] also observed significant antifungal activity in the leaf extract of *C. procera*, with an MIC value of 0.08 mg/mL. The MFC values for the leaf extract were recorded at 48.0 and 45.7 for *A. niger* and *R. stolonifer*, respectively. In contrast, the root extract's MFC values were recorded at 50.3 and 52.0 for *A. niger* and *R. stolonifer*, respectively.

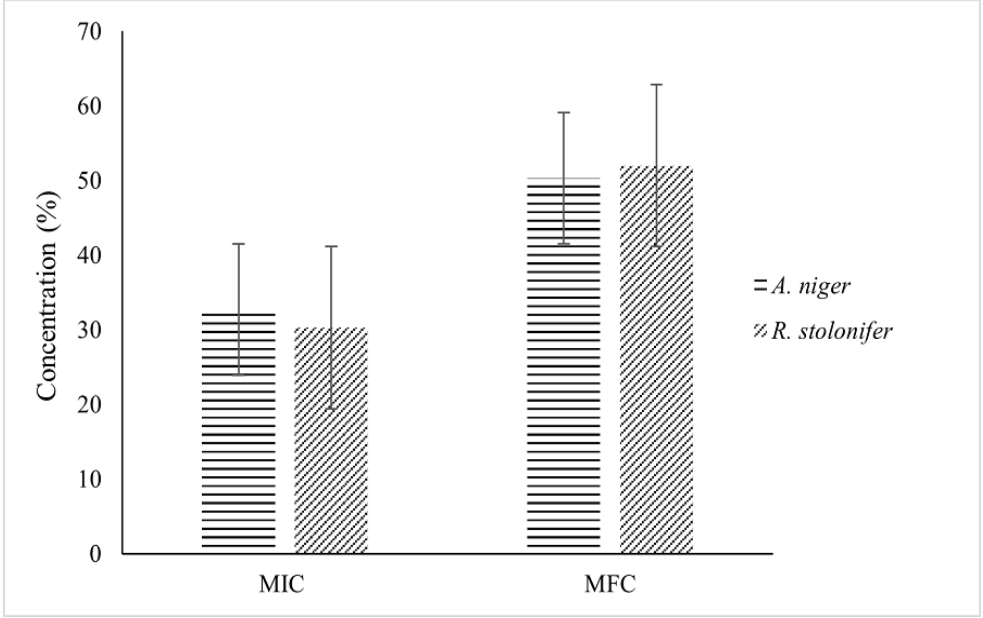

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Figure 4. MIC and MFC of the aqueous root extract of *C. procera* against *A. niger* and *R. stolonifer*

According to Kuta [26], the MIC of *C. procera* on *Epidermophyton flocosum* and *Tricophyton gypseum* was reported as 0.5 mg/mL and 0.9 mg/mL, respectively, while the MFC was 2.0 mg/mL and 4.0 mg/mL, respectively. The variation in MIC values among different fungal species can be attributed to differences in their genetic makeup, drug targets, and resistance mechanisms, such as efflux pumps or alterations in cell wall composition, which influence the susceptibility to antifungal agents.

Conclusion

It was revealed from this study that the leaf and root extracts of *C. procera* inhibited the mycelia growth of *A. niger* and *R. stolonifer*a. The antifungal investigation conducted on *C. procera* leaves and roots obtained in this study supported the traditional use of this plant in folk medicine. The study provides promising evidence of potential medicinal properties associated with *C. procera*, supporting its potential therapeutic applications in traditional medicine practices. Thus, using the aqueous leaf and root extracts of *C. procera* provides an alternative to synthetic chemicals that are expensive and pose potential dangers to farmers, marketers, consumers, and the environment. Further research is warranted to explore the specific bioactive compounds responsible for these observed activities and to ascertain their potential for modern medical applications.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

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Authors' contributions

Conceptualization and supervision: Abdulazeez Mumsiri Abaka and Muhammad Mubarak Dahiru; Methodology, investigation, data collection and analysis: Abdulazeez Mumsiri Abaka, Muhammad Mubarak Dahiru, Ibrahim Ya'u, Saminu Hamman Barau, Aishatu Haruna, and Zainab Abubakar; Writing the original draft: Abdulazeez Mumsiri Abaka and Muhammad Mubarak Dahiru; Review and editing: All authors.

Conflict of interest

The authors declared no conflict of interest.

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