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Research Article

Examining the use of African mistletoe (*Tapinanthus globiferus* (A. *digitata*) from Dutsin-Ma to control human fungal infections

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Abstract — Numerous skin conditions are mostly brought on by fungi, particularly in underdeveloped nations. Chemotherapies may be derived from natural compounds with therapeutic efficacy. Without any supporting evidence, *Tapinanthus globiferus* has been widely used in ethnomedicine to cure fungal infections, diabetes, cancer, ulcers, and hypertension. The purpose of this study was to use agar well diffusion techniques to screen the phytochemical constituents and assess the antifungal properties of *T. globiferus's* aqueous methanolic leaf extract against a few clinical fungal isolates, such as *Alternaria nees*, *Microsphaeropsis arundis*, and *Aspergillus niger*. The African mistletoe *Tapinanthus globiferous* is a parasitic plant that grows on several plants, including citrus, *Videx doniana*, *Adensonia digitatata*, *Piliostigma thonningii* etc. Saponins, tannins, cardiac glycosides, steroids, terpenoid, and flavonoids were detected in the Aqeous methanolic leaf extract of *T. globiferus* growing on *Vitex doniana*, *Adensonia digitata*, *Vitellaria paradoxa*, and *Piliostigma thomningiii*, but terpenoid was not present in the *T. globiferous* growing on *Vitellaria paradoxa*.. The presence of this antifungal activity of *T. globiferous* is not as a result of a single phytochemical. The MIC of T. *globiferous* growing on the host plants was determined using disc diffusion method. The antifungal activity of *T. globiferous* growing on the respective host plants increase with increase in concentration. At some concentration the activity of the extract is higher than that of Fluconazole. All the fungal isolates are susceptible to *T. globiferous* except *Microsphaeropsis arundis*. Since the aqueous methanolic leaf extract of *T. globiferus* exhibits strong antifungal activity against a few chosen fungus species and clearly supports the plant's ethnomedical use in treating fungal infections, the use of this plant as an antifungal drug seems encouraging.

Keywords— Fungi, *T. globiferus A. digitate*, Phytochemical screening, Extract, Antifungal screening

1. Introduction

Microorganisms known as fungi are distinguished by the presence of chitin in their cell walls. While some fungus is edible, others can be extremely harmful and cause diseases that could be fatal. Commonly referred to as mycoses, fungal infections can spread easily. Many people have died as a result of fungal infections and their complications, which continue to burden the health systems of many countries. This is particularly true in Africa, where the most common fungal pathogens are Aspergillus, Candida, Pneumocystis, and Cryptococcus species.[1]

According to statistics, fungal infections are killing off around 1.4 million people annually and are becoming more and more common.[1]The death rate in 2001 was approximately 20,000.

African Mistletoe (*Tapinanthus globiferus*) called 'Afomo' in Yoruba, 'Kauchi' in Hausa and 'Apari' in Igbo, is a traditional medicinal plant of Hausa land in Northern Nigeria[2].

Alkaloids, tannins, saponins, flavonoids, carbohydrates, glycosides, terpenes, and steroids were found in *T. globiferus* growing on host plants according to a previous phytochemical screening[3]. According to several pharmacological investigations, *T. globiferus* growing on different host species showed anti-inflammatory, nephroprotective, anti-oxidant, antitrypanosomal, and anticonvulsant properties[4,5].

It is possible for medicinal plant poisoning to result from misidentification, improper preparation, or ineffective administration and dosage. Numerous harmful and fatal consequences have been documented by health centres and emergency departments with the usage of herbal products[6].

2. Related work

Although ethnobotanically, plants are used for hunting, clothing, food, medicine, shelter, and religious rites, their main application is in medicine[17]. Studies in ethnobotany record indigenous knowledge regarding the applications of plants. The entire community and industry can benefit from the vast knowledge that the local people possess about cultivating, harvesting, and using medicinal plants[18]. Traditional medicine is widely practiced in Pakistan, China, India, Japan, Sri Lanka, Nigeria, and Thailand [18].

A previous study by Satish et al. 2007 potentially tested the aqueous extract of 52 plants from different families for their antifungal potential against eight important species of *Aspergillus* such as *Aspergillus candidus, Aspergillus columnaris, Aspergillus flavipes, Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus ochraceus, and Aspergillus tamarii.* Among fifty-two plants tested, aqueous extract of *Acacia nilotica, Achras zapota, Datura stramonium, Emblica officinalis, Eucalyptus globules, Lawsonia inermis, Mimusops elengi, Peltophorum pterocarpum, Polyalthia longifolia, Prosopis juliflora, Punica granatum and Sygigium cumini* have recorded significant antifungal activity against one or the other Aspergillus species tested.

3. Experimental Method

A sample of *T. globiferus* plants growing on *Adansonia digitata* was gathered on August 7, 2023, from the Dutsin-Ma Local Government Area in Katsina State, Nigeria. Malam Abubakar Lawal Abba of the Federal University Katsina's Biological Science Department discovered the plant at the Herbarium Section, and voucher specimens (FUDMA 2328, FUDMA 2329, and FUDMA 2330) were placed. The gathered plant material was shed-dried, ground into a powder, and preserved for later use in a polythene bag.

Each 50g powdered leaf of *T. globiferus* was thoroughly extracted over the course of six days using 1000ml of 80% methanol. To obtain crude methanolic leaf extract of *T. globiferus*, the content was filtered using filter paper, the solvent was eliminated using a vacuum rotary evaporator, and the mixture was then put to a water bath.

3.1 Preliminary Phytochemical Screening

The preliminary screening of phytochemical was performed on the Aqueous methanolic leaf extract *T. globifeus* in accordance with the procedures to identify the presence of some secondary metabolites[8].

3.2 Antifungal studies

About 50 mg of fluconazole powder was dissolved in 1 ml distilled water to prepare a stock concentration of 50 mg/ml. Aspergillus niger, Mucor circinelloides, and Alternaria nees were isolated from different people within Dutsin-Ma Metropolis. Skin Scrapings of the people were collected and stabbed on a Sabouraud dextrose agar (SDA), incubated at room temperature, and grown fungi were sub-cultured to obtain pure culture. Macroscopic examination of color and texture together with Microscopic examination of spore were performed for proper Identification of fungal species.

3.3 Evaluation of *T. globiferus* antifungal activities

The fungal isolates were suspended in a solid culture. As per the guidelines provided by the Clinical Laboratory Standard Institute, the standardization process was carried out by inoculating in normal saline and modifying its turbidity to match the 0.5 McFarland standard, which is equivalent to 1.0×10^6 CFU/ml[11]. Six-day-old SDA plates were used to make the suspension, which was then calibrated to $1.0x10^6$ CFU/ml at 501 nm using a spectrophotometer.

3.4 Screening for antifungals in *T. globiferus*

The manufacturer's instructions were followed to make Sabouraud dextrose agar (SDA) as the medium for organism development. The media was autoclaved at 121°C for 15 minutes, then it was put into sterile plates and allowed to cool and solidify. Using a 6 mm diameter poncher, filter paper was punched to create a disk that was submerged in the extract solution. The media was then seeded with 0.1 ml of the inoculum, and the inoculum was sprayed on the surface using a cotton swab. Using sterile forceps, the soaked disks were removed and put on the agar that had been seeded. The plate was incubated at 50 mg/ml of fluconazole, which was used as the positive control, and sterile distilled water, which was used as the negative control.

3.5 The minimal inhibitory concentration (MIC)

Using sterile plain containers, the minimum inhibitory concentration (MIC) was ascertained as previously mentioned[11].One milliliter of liquid medium was added to each plain container. One milliliter of extract at various concentrations was poured into simple containers holding two milliliters of liquid medium in total. As a negative control, a simple container with only liquid media was used. With the exception of the final container, 0.1 ml of the fungal inoculum (10^6 CFU/ml-1) was added to each plain container. For 48 hours, the unadorned receptacles were kept at room temperature. The minimum concentration of the extract that resulted in growth inhibition exceeding 90% following a 48hours incubation period is known as the extract's MIC[12].

3.6 Minimum fungicidal concentration (MFC)

Following MIC determination, 0.1 ml of each container that showed no discernible or apparent growth was subcultured onto solid media (SDA) and incubated for 48 hours at room temperature. The MFC was determined to be the lowest concentration of the extract that does not result in any fungal growth on the solid medium.

4. Results and Discussion

Table 1: Phytochemical screening of the AME of *T. globiferus* from *Adansonia digitata* plants

Phytochemical Constituents	Method	*Adensonia digitata*

Tannins	$FeCl_3$	+
Alkaloids	Mayer's	-
Saponins	Foaming	++
Steroids	Salkowski	+
Flavonoids	Schinoder	+++
Cardiac glycosides	Kella-Kiliani	+
Terpenoids	Salkowski	++
Anthraquinones	Borntrager	++

Key: - = Absent; + = Low level; ++ = moderate level; +++ = high level; AME =Aqueous Methanolic Extract.

The table shows the phytochemical constituents of *Adensonia digitate*, were the results shows high presence of flavonoid, followed by saponins, terpenoids and anthraquinones and little presence of tannins, cardiac glycosides and no alkaloids.

The methanol leaf extract of *T. globiferus* growing on *Adensonia digitata* underwent preliminary phytochemical screening, which identified the presence of flavonoids, anthraquinones, steroids, tannins, saponins, and cardiac glycosides. This is consistent with reports3,5,14, and 15 on *T. globiferus* colonizing host plants. It has been revealed that these phytochemical components are in charge of certain pharmacological and physiological processes in plants[16].

Table 2: Susceptibility of selected fungal species to AME of *T. globiferous* from *Adensonia digitata*

Host plant	Conc. (mg/ml)	*Aspergillus Niger*	*Alternaria nees*	*Mucor circinelloides*
Adensonia digitata	500	42.00±1.41	16.00±1.41	28.50±2.12
	250	26.50±0.71	15.00±1.41	25.00±0.71
	125	25.00±1.41	0	24.50±2.12
	62.5	23.23±1.06	0	18.50±0.71
Fluconazole	50	21.50±2.21	37.00±0.00	19.50±0.71

Key: Values are mean inhibition zone (mm) ± S.E of two replicates; AME = Aqueous Methanolic Extract; S.E = Standard Error; Conc. = Concentration.

Table 2 shows the antifungal susceptibility of some selected fungal species *T. globiferous* from *Adensonia digitate* at various concentrations using single commercially produced antifungal drug; Fluconazole where at 500mg/ml of the drug shows high susceptibility of the drug and 62.5 show least zone of inhibition.

The antifungal screening findings showed that the methanol extract strongly inhibited the fungal isolates and that the activity increased as the extract's concentration increased (i.e., the activity is concentration-dependent), at 500 mg/ml. The largest mean zone of inhibition of 42±1.41mm was seen in the AME *of T. globiferous* growing on *Adesonia digitata* when compared to *A. niger*.

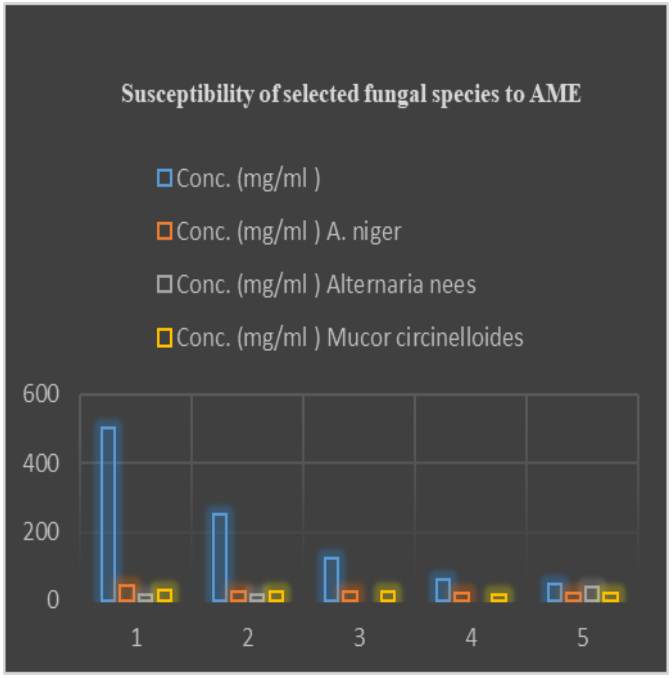

Figure 1 showing Susceptibility of selected fungal species to AME of *T. globiferous* from *Adensonia digitate*

Table 3: AME of T. gloubiferous from various host plants at the Minimum Inhibitory Concentration (MIC) against particular fungal species Test organisms MIC (mg/ml)

Host plant	***Aspergillus niger***	***Alternaria nees***	***Mucor circinelloides***
Adensonia digitate	125	125	125

Table 3 shows AME of *T. globiferous* from various host plants at the Minimum Inhibitory Concentration (MIC) against particular fungal species Test organisms, out of the prepared concentrations 125 mg/ml found to be the minimum inhibitory concentration value.

When tested against all of the isolated *T. globiferous* fungi growing on *Adensonia digitata*, the extracts' mean minimum inhibitory concentration (MIC) was 125 mg/ml. As a result, the outcome was fungistatic.

Table 4: Minimum Fungicidal/ Fungistatic Concentration of AME of *T. gloubiferous* from different host plant against selected fungal species

	Test organisms		
Host plant	***Aspergillus niger***	***Alternaria nees***	***Mucor circinelloides***
Adesonia digitate	500[#]	0	125[*]

Key: *=fungistatic

Table 4 shows Minimum Fungicidal/ Fungistatic Concentration of AME of T. gloubiferous from different host plant against selected fungal species were at 500mg/ml the fungi was completely killed due to the absence of growth unlike other concentrations which happens to have fungal colonies. AME of T. globiferous growing on Adensonia digitata has no fungicidal activities against Alternaria nees;

however, AME of T. globiferous had an MFC value of 500 mg/ml and these concentrations have a fungicidal activity against A. niger. The MFC values of the extracts range from 0 mg/ml, 125 mg/ml, and 500 mg/ml, respectively.

According to Deeni and Sadiq 2002, Adensonia digitata's strong antifungal activity is most likely due to the type of host plant. The kind of host tree appears to have a significant impact on the chemical components (particularly alkaloids) and mineral content of the corresponding mistletoes.

5. Conclusion and Future Scope

Out of the Plant sample of *T. globiferus* growing on *Vitex doniana, Piliostigma thonningii, Vitellaria paradoxa* and *Adansonia digitata* that have been collected from Musawa Local Government Area of Katsina State, the plants possesses all the components required for medicinal purposes and based on the Preliminary phytochemical screening of the methanol leaf extract of *T. globiferus* growing on *Vitex doniana, Adensonia digitata, Vitellaria paradoxa* and *Piliostigma thomningii* revealed the presence of saponins, tannins, cardiac glycosides, steroids, Terpenoid, Anthraquinones and flavonoids while terpenoid is absent in the *T. globiferous* growing on *vitellaria paradoxa*, antifungal screening indicated that the fungal isolates were significantly inhibited by the methanol extract and the activity increases with the increase in the concentration of the extract. Patients infected with fungi can use *T. globiferous* for the treatment as tested in this research and suggested on the antifungal effect of *T. globiferous*, as there are little researched conducted.

Data Availability
All the necessary data have been captured here with the exception of preliminary data which will be available via the corresponding author email when contacted.

Conflict of Interest
There is no conflict of interest

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Authors' Contributions
The first Authors carried out majority of the experimental analysis while second author dwelled on protocol development, researched literature, the manuscript and majority of the write up. However, the third author obtained ethical clearance and fund of the research.

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