JBP
Journal of Biochemicals and Phytomedicine
eISSN: 2958-8561

Phytochemical Screening and Antifungal Studies of *Tapinanthus Globiferus* Growing on *Balanites Aegyptica*

Mukhtar Tukur[1]*, Mansur Y. Ibrahim[1], Hassan Abubakar[1]

[1]Department of Chemistry, Sokoto State University, Sokoto, Nigeria

ARTICLE INFO

Article Type:
Research

Article History:
Recived: 1 Jul 2023
Revised: 12 Oct 2023
Accepted: 27 Oct 2023
Available online: 25 Dec 2023

Keywords:
Phytochemical screening,
fungal pathogens,
Tapinanthus globiferu,
Itraconazol

* ***Corresponding author:***
E-mail: tukurmukhtar@ssu.edu.ng

ABSTRACT

Introduction: Tapinanthus globiferus, a hemi-parasitic plant, is employed in ethnomedicine for treating various ailments, including fungal infections. This study aimed to investigate the phytochemical and antifungal properties of T. globiferus growing on Balanites aegyptiaca.

Methods: The antifungal efficacy of the methanol leaf extract of T. globiferus and its fractions was tested against four selected human fungal pathogens including Aspergillus fumigatus, Aspergillus niger, Trichophyton rubrum, and Trichophyton mentagrophytes. Antifungal efficacy was conducted using agar well diffusion at various concentrations (100 - 12.5 mg/mL).

Results: The extract and its fractions showed significant fungal activity ($p < 0.05$) against all pathogens. The ethyl acetate fraction displayed the highest zone of inhibition, ranging from 10.00 to 29.00 mm, followed by the n-butanol fraction with zones of inhibition ranging from 10.00 to 25.00 mm. The methanol leaf extract, n-hexane, and chloroform fractions exhibited lower mean zones of inhibition: 7.00-19.00 mm, 8.00-21.00 mm, and 5.00-14.00 mm, respectively. The standard drug Iitraconazole had a mean zone of inhibition ranging from 6.00 to 22.30 mm. The Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of the methanol extract and its fractions varied, with ranges for the methanol extract (5.25 -25.5 mg/mL), MFC (5.22-25.5 mg/mL), n-hexane MIC (3.13–12.5 mg/mL), MFC (3.13-24.0 mg/mL), chloroform MIC (3.5-20.0 mg/mL), MFC (3.5-20.0 mg/mL), ethyl acetate MIC (2.13-25.0 mg/mL), MFC (2.13-25.0 mg/mL), and n-butanol MIC (4.4-12.6 mg/mL), MFC (4.4-12.6 mg/mL).

Conclusion: A. niger was the most sensitive organism, while T. mentagrophytes and T. rubrum were the least sensitive to the ethyl acetate fraction. This study supports the ethnomedicinal use of the plant in treating fungal infections.

Please cite this paper as:
Tukur M, Ibrahim MY, Abubakar H. Phytochemical screening and antifungal studies of *Tapinanthus Globiferus* growing on *Balanites Aegyptica*. Journal of Biochemicals and Phytomedicine. 2023; 2(2): 75–81. doi: 10.34172/jbp.2023.15.

Intorduction

Natural products are chemical compounds or substances isolated from living organism. The chemistry of natural product includes their biosynthesis, extraction, identification, quantification, structural elucidation, chemical reactions, physical, and chemical properties. They are produced by the pathway of primary or secondary metabolism. Metabolism is defined as series of enzyme catalyzed biochemical reaction or

transformation occurring within the cells of an organism which are mainly required for its growth, development and for proper response to its environment (Nwokeji *et al*., 2016).

Mistletoes are group of multipurpose plants used by traditional herbalists to treat different diseases in both urban and rural communities especially in the sub-Saharan Africa (Adesina *et al*, 2013). Mistletoe is commonly known as bird lime, all heal, devil's fuge (Adesina *et al*, 2013). It is a general term for woody shoot parasites in several plant families especially *Loranthaceae* and *Viscaceae.* Most genera of African mistletoes belong to the family *Loranthaceae*. Mistletoes of the *Loranthaceae* and *Viscaceae* are *hemiparasitic* plants and their preparations in the form of extracts, infusions, tinctures or tea bags are widely used in various cultures in almost every continent to treat or manage various health problems including hypertension, *diabetes mellitus*, inflammatory conditions, irregular menstruations, menopause, epilepsy, arthritis, cancer, etc (Adesina *et al*., 2013). Mistletoe is commonly found growing on tree crops like cocoa, kolanut and coffee. Mistletoe can also grow on citrus trees like orange (*Citrus sp*.) and guava (*Psidium guajava* L.) (Ogunmefun *et al.*, 2015).

Tapinanthus globiferus (Figure 1) is a woody, spreading shrub with blackish, smooth stems made rough by the presence of lenticels. The leaves are opposite but sometimes alternate. The leaf length varies from 7-20 cm while the leaf width could be 3-15 cm. The leaves are thick, ovate, obtuse, rounded to cuneate at base. Petiole length up to 2 cm long and grooved axially, nerves pinnate with barely prominent and irregular lateral nerves. The inflorescence is a sub-sessile fascicle with up to 6 flowers. The flower is bisexual with a red corolla tube up to 2 cm long and a swollen base that is greenish in color. Calyx forming a short tube enclosing the corolla tube. The stamens are five alternating with the petals and partly fused to the petals. The unattached portion of the filament curls up as soon as the petal lobes open. The fruits are one-seeded, globose and green when immature (Bassey, 2012).

Figure 1: *Tapinanthus globiferus* in its natural habitat

A fungus (plural: fungi or funguses) is any member of the group of eukaryotic organisms that include microorganisms such as yeasts and molds, as well as the more familiar mushrooms. These organisms are classified as a kingdom, fungi, which is separate from the other eukaryotic life kingdoms of plants and animals. There are many species of fungi that causes skin infections in man. These are mainly *Dermatophytes* (*Trichophyton*, *Epidermophyton* and *Microsporum* specie), *Malassezia furfur Candida*, *Aspergillus, Trichothecium roseum, Cladosporium*, *Fusarium, Curvularia*, *Penicillium*, *Epidermetaphyton*, *Drechslera* and *Alternaria* specie also cause skin, hair or nail infections (Gupta and Gupta, 2013). Skin diseases and their complications are a significant burden on the health system of many nations. The most frequent fungal pathogens being *Candida*, *Aspergillus*, *Pneumocystis*, and *Cryptococcus*. Lack of portable water supply are also the contributing factor to the burden of skin disease in Africa and Nigeria (Adebola, 2004).

Despite the fact that extensive research dedicated to the development of new therapeutic strategies, there are only a limited number of available drugs to fight against fungal infections (Patrick *et al*., 2012). Some of the antifungal agents used currently in clinical practice for treatment of tropical and systemic fungal infections include: Nystitin, Natamycin, Ketoconazole, Itraconazole, Fluconazole, Voriconaze, Butoconazole, Terconazole, Posaconazole and Ciclopirox. Others includes: Griseofulvin, Undecylenic acid Caspofungin.

Itraconazole is a triazole antifungal agent that is widely used for the prevention and treatment of fungal infection. it is an orally bioavailable agent with broad spectrum antifungal activity. Itraconazole remains a useful drug for the management of allergic and invasive mycoses worldwide. It is taken orally in capsule form to treat fungal infections that start in the lungs and spread throughout the body. Itraconazole can also be used to treat fungal infections of the nails, although it is important to point out that treatment of nail fungal infections does not result in healthier looking nails. Normal nail appearance will occur only with new growth, which can take up to six months for full nail growth. Oral solutions of this antifungal agent can be used to treat oral *candidiasis*. Patients on proton pump inhibitors should take itraconazole with a cola soft drink to aid in bioavailability (Simon *et al*, 2015).

Adverse effects include: diarrhea, constipation, gas, stomach pain, heartburn, sore or bleeding gums, sores in and around the mouth, headache, dizziness, sweating, muscle pain, decreased sexual desire or ability, nervousness, depression and runny nose. More severe side effects can include: excessive tiredness, loss of appetite, upset stomach, vomiting, tingling or numbness in the extremities, fever, chills, rash, hives and difficulty breathing or swallowing (Simon *et al*, 2015).

Pharmacologically, *T. globiferus* exhibit nephro-protective, anti-inflammatory and anti-oxidative properties (Adekunle *et al*.,2012). Extract of *T. globiferus* exhibited antitrypanosomal activity by suppressing *parasiteamia* development (Abedo *et al*., 2013). Residual aqueous extract of *T. globiferus* growing on *Ficus glumbosa* possess bioactive constituents with anticonvulsant activity in mice and chick (Abubakar *et al*., 2016). *T. globiferus* extract exhibited antitrypanosomal activity (Abedo *et al*., 2013).

T. globiferus growing on *Ficus glumosa* leaf exhibited antioxidant antikindling effect (Abubakar *et al.*, 2018). *T. sdodoneifolius* has been reported to have anxiolytic and antidepressant effects with a sedative side effect (Foyet *et al.*, 2014) and cardiovascular effects (Sylvin *et al*., 2005). *T. sessilifolius* has been reported to exhibit

antimalarial activity (Okpako and Ajaiyeoba, 2004), anti-inflammatory and anti-oxidant properties (Adekunle *et al*., 2012).

The aim of this research is to carry out phytochemical and antifungal studies on the leaf of *Tapinanthus globiferus* growing on *Balanites aegyptiaca* and to Evaluate the effect of methanol leaf extract of *T. globiferus* and its fractions (n-hexane, chroloform, ethylacetate, and n- butanol) against some fungal isolates using agar well diffusion method.

Materials and Methods

Sample Collection and Identification

The plant material (*T. globiferus*) was collected from Gunburawa village of Wamakko L.G.A in Usmanu Danfodiyo University Sokoto, Nigeria. The sample was identified by Abdulazeez Salihu at the Herbarium Unit of Biological Sciences, Usmanu Danfodiyo University Sokoto by comparing with specimen number (UDUS/ANS/0135).

Sample Preparation and Extraction

The plant material was shed dried, pulverized to powder and stored in a polythene bag prior to extraction. One thousand two hundred grams (1200 g) of the powdered sample was macerated with 5 L of methanol with occasional agitation for 3 days, the extract was filtered and the solvent was evaporated with the aid of rotary evaporator at 40 ºC to obtain crude methanol leaf extract of *T. globiferus* (210 g). Some part of the methanol leaf extract (150 g) was suspended in 700 mL of distilled water which was then filtered and partitioned with solvent of increasing polarity to obtain *n*-hexane (HF), chloroform (CF), ethylacetate (EF) and *n*-butanol (BF) fractions.

Phytochemical Screening

Various chemical tests were conducted on the methanol extract and fractions to identify the presence of secondary metabolite such as alkaloids, flavonoids, tannins, saponins, terpenoids, phenols and steroid according to the method described by Evans (2002).

Antifungal Studies

Four different fungal isolates obtained from the Department of Clinical Microbiology Usmanu Danfodiyo University Teaching Hospital, Sokoto were used for the study. They include *Aspergillus niger*, *Aspergillus fumugtus Trychophyton rubrum* and *Trychophytom mentagrophyte*

Preparation of Test Organisms

Test organisms were sub cultured and grown on 10 mL Sabouraud dextrose agar slants and was eventually kept in the refrigerator at 8 ^{O}C.

Preparation of Reference Antifungal Agents

Stock solutions of itraconazole (5 mg/mL) was prepared by dissolving 50 mg of the powder in 10 m L of distilled water from which 0.05 mg/mL (50 µg/mL) working concentration was prepared.

Preparation of Crude Extract and Fractions of T. globiferus

Stock concentrations of 100 mg/mL was prepared with 10 % dimethyl sulfoxide (DMSO) by dissolving 0.5 g (500 mg) each of the crude extract and fractions(*n*-Hexane, chloroform ethylacetate, and *n*-butanol) in 5 mL of 10 % DMSO and two-fold serial dilution was carried out to obtain three more concentrations of 50, 25 and 12.5 mg/mL.

Preparation of Culture media

The Sabouraud Dextrose agar (SDA) as growth media were weighed and prepared with distilled water according to the manufacturer's instructions. SDA was gently heated to aid its dissolution, it was dispensed into sterile petri dishes and allowed to cool and solidify.

Cultivation and Standardization of Test Fungi

Culture of the *Aspergillus fumugatus, A. niger, Trichophyton mentagrophyte* and *trichophytum rubrum* were suspended into sterile Sabouraud Dextrose liquid medium. They were standardized by inoculating in normal saline to compare their turbidity to 0.5 McFarland standards which is approximately 1.0×10^6 Cfu/mL.

Antifungal Screening of T. globiferus

The antifungal activity of crude methanol leaf extract and its fractions (*n*-hexane, chloroform ethyl acetate and *n*-butanol) was determined through susceptibility test using agar well diffusion method (Olowosulu *et al.*, 2005). Wells were bored into the solidified SDA plates using a sterile cork borer of 6 mm in diameter. 0.1 mL of the inoculum was seeded and spread evenly over the surface of the sterilized media using a sterile cotton swab stick. The wells were filled separately with 200 µL solution of the graded concentration of extract and fractions.0.05 mg/mL itraconazole (which served as positive control), 10 % (DMSO) used as negative control were dispensed into the wells. The plates were incubated at 27 ºC for 72 hour, the zone of inhibition was measured using transparent ruler. The experiment was carried out in triplicates.

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) was determined using a 96 wells micro plate Liquid media of 100 µL was transferred into each micro well of the micro plate. The extract of 100 µL and its fractions was transferred into well-1 making up 200 µL. 100 µL of the mixture (extract and fractions) and media was taken from well-1 to well-2 and serially diluted (2-fold) up to well-10 where 100 µL finally discarded from the last well, well 11 (extract blank) served as negative control and well-12 (media + inoculum) which served as a positive control. About 100 µL of the fungal inoculum approximately (10^6 CFU/ mL-1) was transferred into each well except for well-11 of the microplate. The microplate was covered with aluminum foil and allowed stand for 30 min before incubating at 27°C for for 72

hrs. The experiment was performed in triplicate. The MIC of the extract/fraction is the lowest concentration that caused growth inhibition of more than 90% after 48 h of incubation.

Determination of Minimum Fungicidal Concentration (MFC)

Twenty (20 µL) of each well which exhibited no visible or apparent growth after MIC determination was sub-cultured onto the solid media (SDA) and was incubated at 27°C for 48 hrs. The lowest concentration of the extract and it fractions that does not yield any fungal growth on the solid medium used was taken as MFC.

Statistical Analysis

The results obtained was subjected to the analysis of variance (ANOVA) using SPSS software followed by post hoc test, values were considered significant at $p<0.05$ and data was expressed as mean ± standard deviation

Results

The extraction of 1200 g of *T. globiferus* afforded a yield of 210 g of the crude extract and the percent yield from the partitioned fractions are presented in Table 1.

Preliminary phytochemical screening of the methanol leaf extract and its fractions (*n*-hexane, chloroform, ethylacetate and *n*-butanol) revealed the presence of flavonoids, tannins, saponins, cardiac glycosides, steroids/terpenes, phenol and alkaloid (Table 2).

Susceptibility test result showed that the methanol leaf extract and its fractions (*n*- hexane, chloroform, ethyl acetate and *n*-butanol) exhibited significant ($p< 0.05$) antifungal activity at different concentration (100-12.5 mg/mL) with mean zone of inhibition ranging from 5.00-29.00 mm. The methanol extract had mean zone of inhibition ranging from 9.00-19.00 mm while *n*-hexane, chloroform, ethyl acetate and *n*-butanol fractions had mean zone of inhibitions ranged from 8.00-21.60 mm, 5.00-14.00 mm, 10.00-29.00 mm and 10.00-25.00 mm, respectively (Tables 3 to 8).

Table 1: Yield of partitioned fractions

Solvents	Weight (g)	Yield (%)	Colour
n-hexane	0.803	0.553	Green
Chloroform	0.022	0.015	Light brown
Ethylacetate	10.601	7.067	Brown
n-butanol	57.731	35.820	Redish brown
Water insoluble	37.015	24.677	Green
Methanol	210.00	17.50	Green

Table 2: Preliminary phytochemical screening of extract and its fractions of *Tapinanthus globiferus*

Constituents	Test	Observation	ME	HF	CF	EF	BF
Flavonoids	Shinoda	Orange color	+	-	+	+	+
	Sodium hydroxide	Yellow color	+	-	+	+	+
Alkaloids	Mayer's	A cream ppt	+	-	+	-	-
	Dragondoff's	rose red ppt	+	-	-	+	+
Saponin	Frothing	Formation froth	+	-	+	+	+
Tannins	Lead sub-acetate	Cream color ppt	+	-	+	+	+
Triterpenoids/steroids	Salkowki's	Red brown color	+	+	-	-	-
	Lieberman	Purple color	+	+	-	-	-
Phenols	Ferric chloride	A dark green color	+	-	+	+	+
Cardiac glycoside	Keller-Kiliani	A brown ring at interface	+	-	+	+	+

+: Present; -: Absent; ME: Methanol extract; HF: n-hexane fraction; CF: Chloroform; EF: Ethylacetate fraction; BF: n-butanol fraction.

Table 3: Antifungal susceptibility test of methanol leaf extract of *Tapinanthus globiferus*

Test organisms	Zone of inhibition (mm) Itraconazole (mg/mL)					
	100	50	25	12.5	0.05	0.05#
Aspergillus niger	19.00±1.00*	14.60±1.12*	12.00±1.00*	10.00±1.00a	12.30±0.06	0.00
Aspergillus fumugatus	17.60±0.06*	15.00±1.00*	7.00±1.00*	13.00±1.00*	22.30±0.06	0.00
Tapinanthus mentagraphte	14.50±1.00*	12.00±1.00*	11.00±01.00*	10.00±1.00*	6.00±0.00	0.00
Tapinanthus rubrum	14.00±1.00a	12.00±1.00a	11.00±1.00a	9.00±1.00*	12.3±0.06	0.00

Each value represents Mean ± SD; *: $p< 0.05$ compared with positive control; #: DMSO group

Table 4: Antifungal Susceptibility test of n-hexane fraction of *Tapinanthus globiferus*

Test organisms	Zone of inhibition (mm) Itraconazole (mg/mL)					
	100	50	25	12.5	0.05	0.05#
Aspergillus niger	19.00±1.00*	16.00±01.00*	14.00±1.00a	11.00±1.00a	12.30±0.60	0.00
Aspergillus fumugatus	20.60±1.00a	19.00±1.00*	16.00±1.00*	13.00±100*	22.30±0.06	0.00
Tapinanthus mentagraphte	15.60±0.60*	13.00±1.00*	10.00±1.00*	8.00±1.00*	0.00±0.00	0.00
Tapinanthus rubrum	21.00±1.00*	18.00±1.00*	15.00±1.00*	14.00±1.00a	12.3±0.06	0.00

Each value represents Mean ± SD; *: $p< 0.05$ compared with positive control; #: DMSO group

Table 5: Antifungal Susceptibility test chloroform fraction of *Tapinanthus globiferus*

Test organisms	Zone of inhibition (mm) Itraconazole (mg/mL)					
	100	50	25	12.5	0.05	0.05#
Aspergillus niger	11.00±1.00*	7.60±1.00*	13.00±1.00a	6.00±1.00a	10.20±0.40	0.00
Aspergillus fumugatus	8.60±1.00a	9.00±1.00*	8.00±1.53*	8.00±1.00*	22.30±0.40	0.00
Tapinanthus mentagraphte	9.50±1.00*	8.00±1.00*	7.00±1.00*	5.00±1.00*	0.00±0.00	0.00
Tapinanthus rubrum	14.00±1.00*	10.00±1.00*	10.00±1.00a	7.00±1.00a	12.3±0.40	0.00

Each value represents Mean ± SD; *: $p< 0.05$ compared with positive control; #: DMSO group

Table 6: Antifungal susceptibility test of ethylacetate fraction *Tapinanthus globiferus*

Test organisms	Zone of inhibition (mm) Itraconazole (mg/mL)					
	100	50	25	12.5	0.05	0.05#
Aspergillus niger	29.00±1.00*	22.60±1.00*	18.00±1.00*	13.00±1.00a	12.30±0.60	0.00
Aspergillus fumugatus	21.60±1.00a	17.00±1.00*	15.00±1.00*	14.00±1.00*	22.30±0.60	0.00
Tapinanthus mentagraphte	20.00±1.00*	18.00±1.00*	13.00±1.00*	10.00±1.00*	0.00±0.00	0.00
Tapinanthus rubrum	16.00±1.00*	13.00±1.00a	11.00±1.00a	10.00±1.00a	12.3±0.60	0.00

Each value represents Mean ± SD; *: $p< 0.05$ compared with positive control; #: DMSO group

Table 7: Antifungal susceptibility test of n-butanol fraction *Tapinanthus globiferus*

Test organisms	Zone of inhibition (mm) Itraconazole (mg/mL)					
	100	50	25	12.5	0.05	0.05#
Aspergillus niger	25.00±1.00*	17.60±1.00*	14.00±1.00a	11.00±1.00a	12.30±0.60	0.00
Aspergillus fumugatus	21.60±1.00a	17.00±1.00*	15.00±1.53*	14.00±1.00*	22.30±0.60	0.00
Tapinanthus mentagraphte	13.50±1.00*	12.00±1.00*	11.00±1.00*	10.00±1.00*	0.00±0.00	0.00
Tapinanthus rubrum	17.00±1.00*	15.00±1.00*	13.00±1.00a	11.00±1.00a	12.3±0.60	0.00

Each value represents Mean ± SD; *: $p< 0.05$ compared with positive control; #: DMSO group

Table 8: MIC and MFC of extract and fractions of *Tapinanthus gloiferus*

	ME		HF		CF (mg/mL)		EF		BF	
Test organism	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**
Aspergillus niger	5.26	14.5*	14.5	14.5	3.5	3.5*	12.5	12.5*	0.40	0.40ɳ
Aspergillus fumugatus	14.5	14.5ɳ	24.0	24.0*	20.0	20.0	25.0	25.0*	12.6	12.6*
Tapinanthus mentagraphte	5.25	5.22*	3.13	3.13	11.1	11.1	2.13	2.13*	5.25	5.25*
Tapinanthus rubrum	25.5	25.5	12.5	12.5	12.6	12.6	12.7	12.7ɳ	3.17	3.17

*: Fungicidal; ɳ: Fungistatic; ME: Methanol extract; HF: *n*-hexane fraction; CF: Chloroform; EF: Ethylacetate fraction; BF: *n*-butanol fraction.

Discussion

Preliminary phytochemical screening of methanol leaf extract and it fractions(*n*-Hexane, chloroform, ethylacetate, and *n*-butanol) of *T. globiferus* growing on *Balanites aegyptiaca leaves* revealed the presence of saponins, tannins, alkaloids, cardiac glycoside, steroid, triterpenoids, phenols and flavonoids while ethylacetate and *n*-butanol fractions indicated the presence of similar constituents including flavonoids, alkaloids, tannins, saponins, phenols, steroid and cardiac glycoside and *n*-hexane contained only steroid, and triterpenoids, (Table 2).The presence of these phytochemical compounds in *T. globiferus* growing on other plants have been reported (Emaikwu *et al*.,2019). These secondary metabolites are thought to be responsible for the pharmacological activities of plant extract (Emaikwu *et al*., 2019).

The susceptibility test of methanol extract and it fractions (*n*-hexane, chloroform, ethylacetate and *n*-butanol) exhibited varying antifungal activity against

the test organisms and the activity was concentration dependent. The methanol leaf extract and it fractions exhibited significant ($p<0.05$) antifungal activity at the graded concentration (100 – 12.5 mg/mL) with mean zone of inhibition ranging from 5.00–29.00 mm against the test organisms (*A. niger*, *A. fumugatus*, *T. mentagraphyte* and *T. rubrum*). *Ethylacetate* fraction showed the highest mean zone of inhibition against *A. niger* while *n*-hexane and chloroform fractions exhibited the least mean zone against *T. mentagraphyte*. The standard drug showed the mean zone of inhibition range (12.30–22.30 mm) against all the test organisms*;* the drug showed the highest mean zone of inhibition against *A. fumugatus* and there was no activity against *T. mentagraphyte* (table 3-7).

Methanol leaf extract showed significant ($p< 0.05$) antifungal activity against *A. niger* at 100 mg/mL which was higher that of itraconazole at 0.05 mg/mL (Table 3). Hassan *et al.* (2020) reported a lower mean zone of inhibition against the same organisms for *T. globiferus* growing on the other host suggesting that methanol leaf extract may be used for the management of fungal infections caused by *A. niger*. Similarly, the methanol lef extract of *T. globiferus* growing on other host have been reported to exhibit significant ($p< 0.05$) antifungal activity (Harborne *et al*., 1993).

n-Hexane fraction indicated a higher antifungal activity against *T. rubrum* at 100 mg/mL compared with itraconazole at 0.05 mg/mL (Table 4), similarly, the standard drug, itraconazole exhibited higher effect against *A. fumugatus* when compared with the fraction at 100 mg/mL although the effect was not statistically significant(Table 4).

Chloroform fraction exhibited a higher antifungal activity against *T. rubrum* at 100 mg/mL and lowest at 12.5 mg/mL. the itraconazole showed no activity against *T. rubrum* at 0.05 mg/mL (Table 5).

Ethylacetate fraction exhibited the highest antifungal activity against *A. niger* while least sensitive organism was *T. rubrum (*Table 6) mukhtar *et al.* (2022) reported a higher mean zone of inhibition against *T.rubrum* and lower zone of inhibition was reported for *A.niger* The highest activity observed by the ethylacetate fraction might be due to the concentration of moderately polar compounds such as flavonoids and their derivatives that have been reported to possess antifungal effect (Harborne *et al*., 1993). Ethylacetate fraction is a very good antifungal agent for the treatment of different fungal infections such as *Onychomycosis*, *oral candidiasis*, *oesophageal candidiasis* and *viginal thrush* (Harborne *et al*., 1993).

n-butanol fraction showed antifungal activity against *A. niger* when compared to itraconazole, even though it recorded the mean zone of 21.60 mm against *A. fumugatus* which was lower than that of itraconazole 22.30 mm but the difference is not statistically significant (Table 7). However of all the fungal isolates used *A. niger* was the most susceptible to ethylacetate fraction and *A. niger* is the most dangerous of all the many common fungal isolate causing *tinea cruris*, *oral trush* and *balanoposthitis* (Xia *et al*., 2019). The MIC and MFC of the methanol extract and its fractions ranged from 0.4-25.5 mg/mL (Table 8). n-butanol fraction showed the lowest MIC 0.4 mg/mL against *A. niger* while the ethyl acetate fraction had a MIC and MFC value of 3.13 mg/mL against *T. mentagraphte*. The lower MIC and MFC values suggest that the fractions have good antifungal activity.

Conclusions

Preliminary phytochemical screening of the methanol leaf extract and its fractions (*n*-hexane, chloroform, ethylacetate and *n*-butanol) using standard procedure revealed the presence secondary of metabolites. The susceptibility test of methanol extract and it fractions (n-hexane, chloroform, ethylacetate and n-butanol) using agar well diffusion exhibited varying antifungal activity against the test organisms and the activity was concentration dependent. A *niger* was the most sensitive organism, while *T. mentagrophytes* and *T. rubrum* were the least sensitive to the ethyl acetate fraction. n-butanol fraction showed the lowest MIC 0.4 mg/mL against *A. niger* while the ethyl acetate fraction had a MIC and MFC value of 3.13 mg/mL against *T. mentagraphte*. The lower MIC and MFC values suggest that the fractions have good antifungal activity. This study supports the ethnomedicinal use of the plant in treating of fungal infections.

Declarations

Conflict of interest

There is no conflict of interest among the authors.

Acknowledgement

The authors acknowledgment the lab technologist department of Clinical microbiology Usmanu Danfodiyo University Sokoto.

Consent for publications

The author approved the manuscript for publication.

Funding/support

This work is an institution-based research and was supported by the Nigerian Tertiary Education Trust Fund (TETFund). Research Projects (RP) Intervention via the Institutional Based Research Fund (IBRF) with references number TETF/DR&D/CE/UNIV/SOKOTO/IBR/2021/VOL.1

Authors' contributions

MT contributed in conceptualization, methodology, software, validation, formal analysis, investigation, resources, data curation, original draft preparation, review and editing, visualization, supervision, and project administration. MYI contributed in conceptualization, methodology, investigation, resources, review, and editing. HA contributed in conceptualization, original draft preparation, review, and editing.

Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors.

References

Abedo JA, Jonah OA, Abdullahi RS, Mazadu MR, Idris HY, Muhammed H. Evaluation of trypanosomal activity of *Tapinanthus globiferus* and *Gongronema latifolium* on *Trypanosoma congolense*. Biological Science Research. 2013; 10:8-20.

Abubakar A, Yunus AT, Abubakar MR, Ugwar JO, Muhammad AA. Antioxidant and antikindling effect of *T. globiferus* growing on *Ficus glumosa* in pentylenetetzole-induced kindled rat. African Journal of Biochemistry. 2018; 17 (4):73-80. doi:10.5897/AJB2017.16048.

Abubakar K, Adebisi IM, Ugwah JC. Phytochemical screening and anticonvulsant activity of the residual aqueous fraction of *Tapinanthus globiferus* growing on *Ficus glums*. Herbal Medicine. 2016; 2: 2. doi: 10.21767/2472-0151.100013.

Adebola O. Prevalence of skin diseases in Ibadan, Nigeria. International Journal of Dermatolology. 2004; 43: 3136. doi.org/10.1111/j.1365-4632.2004.01967.

Adekunle AS, Oyewo BE, Afolabi OK. Therapeutic efficacy of *Tapinanthus globiferus* on acetaminophen induced nephrotoxicity, inflammatory reactions and oxidative stress in albino rats. International Research Journal of Biochemistry and Bioinformatics. 2012; 2(2): 041-5.

Adesina SK, Illoh HC, Johnny II, Jacob IE. African mistletoe (loranthaceae); Ethnoparmacology, chemistry and medicinal values: An update. African Journal of Traditional, Complementary and Alternative Medicine. 2013; 10(4): 161-70. doi:10.4314/ajtcam.v10i4.26.

Bassey ME. Phytochemical investigation of *Tapinanthus globiferus* from two hosts and the taxonomic implication. International Journal of Chemical Environmental and Pharmaceutical Research. 2012; 3(2): 174-7.

Emaikwu V, Nduke IG, Iyun ORA, Anyam JV. Preliminary phytochemical and antimicrobial activity screening of crude extract of bird lime (*T. globiferus*). Journal of Applied Science Environmental. 2019; 23 (2): 305-308. doi: 10.14314/Jasem.v23i2.16.

Evans WC. Threase and Evan. Pharmacology, 15th Edition, WB, 2002.

Harquin Simplice F, David Emery T, Hervé Hervé NA. Enhancing spatial memory: anxiolytic and antidepressant effects of *Tapinanthus dodoneifolius* (DC) Danser in mice. Neurology Research International. 2014; 5; 2014. doi.org/10.1155/2014/974308**.**

Gupta S, Gupta BL. Evaluation of the incidences of dermatophillic infection in Rajastahan: Case studies from Rajasthan, India. International Journal of Medical Science. 2013; 5: 229-32. doi.org/10.5897/JMMS2013.0929.

Harborne JB, Greenham J, Williams CA, Eagles J Markham KR. Phytochemistry. 1993; 34: 21.

Leung S, Poulakos MN, Machin J. Posaconazole: an update of its clinical use. Pharmacy. 2015; 3(4):210-68. doi:10.3390/pharmacy3040210.

Mukhtar T, Hassan A, Amina JY. Phytochemical and antifungal studies of *Tapinanthus globiferus* extract and fractions. Caliphate Journal of Science & Technology. 2022; 4(1):71-7. doi: 10.4314/cajost.v4i1.9.

Nwokeji PA, Enodiana OI, Ezenweani SR, Osaro-Itota O, Akatah HA. The chemistry of natural product: Plant secondary metabolites. International Journal of Technology Enhancements and Emerging Engineering Research. 2016; 4(8):2347-4289.

Ogunmefun IOT, Olatunji OT, Adarabioyo MI. Ethnomedicinal survey on the uses of mistletoe in south-western Nigeria. European Journal of Medicinal Plants. 2015; 8(4):224-30. doi: 10.9734/EJMP/2015/11048.

Okpako LC, Ajaiyeoba EO. *In vitro* and *in vivo* antimalarial studies of *Striga hermonthica* and *Tapinanthus sessilifolius* extracts. African Journal of Medicine and Medical Sciences. 2004; 33 (1):73-5. PMID: 15490799.

Ouédraogo S, Traoré A, Somé N, Lompo M, Guissoe PI, Scott C, et al. Cardiovascular properties of aqueous extract from *Tapinanthus dodoneifolius* DC danser. African Journal of Traditional, Complementary and Alternative Medicines. 2005;2(1):25-30.

Patrick V, Selene F, Alix TC. Antifungal resistance and new strategies to control fungal infections. International Journal of Microbiology. 2012; 6(2): 2-6. doi: 10.1155/2012/713687.

Xia DA, Yayoi N, Duerma T, Kyaw ZH, Osamu Y, Eishim M. Antifungal activity and mechanism of action of Ou-gon (Scuttellaria root extract) components against pathogenic fungi. Journal of Scientific Reports. 2019; 9 (1):1-10. doi:10.1038/s41598-3891-w.