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RESEARCH ARTICLE

ANTIFUNGAL EFFECTS OF *TAPINANTHUS GLOBIFERUS* GROWING ON *VITEX DONIANA* AGAINST SOME FUNGAL ISOLATES

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Abstract

Objective: Fungal infections are the major cause of many skin diseases, especially in developing countries. Natural products of medicinal value represent a potential source of chemotherapeutic agents. *Tapinanthus globiferus* has been used extensively in ethnomedicine for the treatment hypertension, ulcer, cancer, diabetes and fungal infections without a scientific basis. This work was aimed at screening the phytochemical constituents and evaluating the antifungal properties of methanol leaf extract its ethyl acetate and *n*-butanol fractions of *T. globiferus* against some clinical fungal isolates including *Candida albicans*, *Trychophyton mentagrophytes*, *Trychophyton rubrum* and *Aspergillus niger* using agar well diffusion and broth micro-dilution techniques.
Methods: Preliminary screening of phytochemical constituents of extract and fractions of *T. globiferus* indicated the presence of carbohydrates, alkaloids, glycosides, tannins, flavonoids, saponins, steroids and triterpenes.
Results: The methanol extract and its fractions demonstrated significant ($p<0.05$) antifungal effect against all the test organisms with mean zone of inhibition ranging from 27.83±0.16-14.46±0.29mm which was higher compared to that of the standard drug, Fluconazole (26.1±0.44 –18.49±0.16 mm). The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of the extract ranged between 6.25–25.0 mg/ml; ethyl acetate fraction had 3.13 – 25.0 mg/ml while *n*-butanol fraction had the least MIC ranging from 0.39-12.5 mg/ml against the test organisms.
Conclusion: Study concluded that T. *globiferus* have good antifungal activity validating the ethnomedicinal claim for the use of the plant in the treating fungal diseases.
Keywords: Antifungal, phytochemical screening, *Tapinanthus globiferus*.

INTRODUCTION

Fungal infection and their complications continue to affect many nations and have claimed the lives of many people especially in Africa. The commonly implicated fungal pathogens includes *Aspergillus*, *Candida Cryptococcus*, *Pneumocystis* spp. Statistics showed that the incident of fungal infection is becoming outrageous each year and claimed the lives of about 1.4 million people worldwide[1]. In 2001, the mortality rate of about 20,000 in sub Saharan African was due to skin diseases[2]. Thus, skin diseases manifest due to lack of supply of potable water, malnutrition and poor environmental sanitation, these factors contribute to the burden of mycotic disease in Africa[3,4,5] and Nigeria inclusive. Medicinal plants have been a rich source of secondary metabolites that are widely used for their therapeutic application; this has been attributed to their affordability, accessibility and lesser side effects[6,7]. About 80% of the world population still relies on plant-based traditional medicines for some aspect of their primary health care. Most often, the search for many potent drug candidates used in modern clinical practice has been achieved via research and development on medicinal plants with therapeutic applications[8]. *Tapinanthus globiferus* (Loranthaceae) is a semi or hemi-parasitic that grows mostly on the branches of different trees including *Citrus*, *Acacia*, *Aloe*, *Vitellaria paradoxa*, *Kola*, *Terminalia* and *Combretum*, as host trees[9,10]. It is widely distributed throughout the tropical and subtropical regions of Western and Eastern African.

The plant is used in ethnomedicine to treat itching[8], tumor[7], ulcers, hypertension, epilepsy, diabetes,

promoting relaxation of muscles before delivery and weakness of vision[11] and it is also used to remove placenta after parturition[12]. Previous phytochemical screening on *T. globiferus* growing on host plants revealed the presence of alkaloid, tannins, saponins, flavonoids, carbohydrate, glycosides, terpenes and steroids[13]. Some pharmacological studies carried out on *T. globiferus* growing on other host species revealed that the plant exhibited, anti-inflammatory, nephro-protective, anti-oxidant activities[14], Antitrypanosomal activity[15] and anticonvulsant activity[16].

Despite its widespread usage, literature search revealed the paucity of research conducted on the plant, hence the need to evaluate the phytochemical constituents and antifungal effect of *T. globeferus* growing on *Vitex doniana* in order to validate the ethnomedicinal claim of its use in the treatment of fungal infections.

MATERIALS AND METHODS

The solvents/reagents used were of analytical grade and were distilled before use, they include methanol, n-butanol, ethyl acetate, chloroform, n-hexane and dimethyl sulfoxide (DMSO; Lobal Chemie Pvt Ltd, India). Sabouruad dextrose agar and broth (Himedia Laboratories Pvt Ltd, India). UV spectrophotometer (Abrera BARCELONA Spain). Ohaus digital weighing balance (Champ 11 CH15R, Ohaus Corporation, Pinebrook NJ, USA), Metler balance (Model P162 supplied by Gallenhamp), 96 well Micro-titre plate, single and multi-channel micropipette (HUAWEI LAB), Vertical automatic electro thermal pressure steam sterilizer (LX-C35L. HEFEI HUATAI Medical Equipment Co. LTD). Microplate Reader (2100-C, Optic Ivyman System) and standard powder of fluconazole (Sigma Aldrich No. F8929, U.S.A.)

Plant sample

Plant sample of *T. globiferus* growing on *Vitex doniana* was collected from Dange Shuni Local Government Area of Sokoto State, Nigeria in December 2016. The plant was identified at the Herbarium Section by Namadi Sanusi of Botany Department, Ahmadu Bello University Zaria, a voucher was deposited (No.900107). The plant material was shed-dried, crushed to powder and kept in a polythene bag for further use.

Preparation of plant material

The powdered leaf of *T. globiferus* (2.0 kg) was exhaustively extracted with 3 L of 90 % methanol for 6 days. The content was filtered using filter paper and the solvent was removed using vacuum rotary evaporator at 40°C to afford crude methanol leaf extract (140 g). Some part of the extract (120 g) was partitioned using different solvents into *n*-butanol, ethyl acetate, chloroform and *n*-hexane fractions.

Preliminary phytochemical Screening

The preliminary screening of phytochemical was performed on the methanol leaf extract *T. globifeus* and its ethyl acetate and *n*-butanol fractions in accordance with the procedures[17,18] to identify the presence of some secondary metabolites.

Antifungal studies

Test organisms

Four clinical fungal isolates obtained from the Clinical Microbiology Department of Usmanu Danfodiyo University Teaching Hospital, Sokoto, includes *Candida albicans*, *Aspergillus niger*, *Trychophyton rubrum* and *Trychophyton mentagrophyte*

Preparation of test organisms

Test organisms were sub-cultured and grown on 10 ml SDA slants, it was eventually stored in the refrigerator at 2-8°C.

Preparation of reference antifungal agent

About 50 mg of fluconazole powder was dissolved in 10 ml dimethyl sulfoxide to prepare a stock concentration of 5 mg/ml, from which 0.05 mg/ml (50 µg/ml) working concentration was also prepared.

Preparation of plant extract/fractions

A 100 mg/ml Stock concentration was prepared when 0.5 g of methanol extract and its fractions (ethyl acetate and *n*-butanol) was dissolved in 5 ml of 10% DMSO and eventually 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) and broth as growth media were weighed and prepared with distilled water according to the manufacturer's specifications. SDA was gently heated to aid its dissolution, it was transferred into an already sterilized Petri dishes, it was allowed to cool and solidify. These were kept aseptically until ready for use.

Determination of the antifungal activity of *T. globiferus*

Standardization and culturing of the fungal isolates

A suspension of solid culture of *Candida albican* (18 h) in Sabo broth was prepared. The standardization was performed according to method by Clinical Laboratory Standard Institute guidelines[19] by inoculating in normal saline and adjusting its turbidity to match that of 0.5 McFarland standard which is equal to 1.0×10^6 CFU/ml. *Aspergillus niger* and *Trichophyton* spp were sub-cultured from (6 days old) SDA slant, the suspension was adjusted to $1.0x10^6$ CFU/ml at 530 nm of a spectrophotometer.

Antifungal screening of *T. globiferus*

The antifungal activity of the plant ant its ethyl acetate and *n*-butanol fractions were carried out according to the method[20]. Sabouraud dextrose agar (SDA) as the media for organism growth was prepared according to instructions by the Manufacturer and was autoclaved at 121°C for 15 min, the media was transferred into sterile dishes and allowed to cool and solidify. A cork borer of 8 mm in diameter was used to punch wells on the plates. 0.1 ml of the inoculum was seeded on the media and cotton swab was used to spray the inoculum on the surface of the media.

About 200 µl of the graded concentration of extract and its fractions was transferred into each well of the micro plate. 0.05 mg/ml Fluconazole which served as positive control, 10% DMSO was also used as negative control, plate was incubated at 27°C for 48-72 h, zone of inhibition was measured using transparent ruler. Each experiment was performed in triplicates.

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) was determined using a 96 wells micro plate as previously described[21]. Liquid media of 100 µl was transferred into each micro well of the micro plate. Extract of 100 µl and its fractions was transferred into well-1 making up 200 µl total volume. Mixture (extract/fractions) and media of 100 µl 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 and inoculum) which served as a positive control. About 100 µl of the fungal inoculum approximately (10^6CFU/ml^{-1}) was transferred into each well except for well-11 of the microplate. The microplate was covered with aluminum foil and allowed to stand for 30 min before incubating at 27°C for 72 h. 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[22].

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 h. The lowest concentration of the extract and fractions that does not yield any fungal growth on the solid medium used was taken as the MFC.

Statistical Analysis

The results obtained were expressed as mean±standard error of mean and it was analyzed for significant using analysis of variance (ANOVA); values were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Preliminary phytochemical screening of the methanol leaf extract and fractions of *T. globiferus* growing on *Vitex doniana* revealed the presence of saponins, tannins, alkaloids, cardiac glycosides, carbohydrates, steroids/triterpenes and flavonoids which varies from the fractions (Table 1). This is in agreement with what was reported[13,16,24,25] on *T. globiferus* growing on other host plants. These phytochemical constituents were reported to be responsible for different pharma-cological and physiological activities of plants[26].

Table 1: Phytochemical screening of the methanol leaf extract, ethyl acetate and *n*-butanol fractions of *T. globiferus*.

Constituents	Test	M.E	EAF	BTF
Carbohydrates	Molisch	+	+	+
Anthraquinones	Bontrager	-	-	-
Steroid/Triterpenes	Liebermann-Burchard	+	-	-
Glycoside	Keller-Killiani	+	+	+
Saponins	Frothing	+	+	+
Tannins	Ferric chloride	+	+	+
	Lead acetate	+	+	+
Flavonoids	Shinoda	+	+	+
	Ferric chloride	+	+	+
Alkaloids	Dragendoff	+	-	-
	Mayer	-	-	-

Key: - = absent; + = present; M.E=methanol extract, EAF=ethyl acetate fraction, BTF=*n*-butanol fraction.

Table 2: Susceptibility test of ME, EAF and BTF of *T. globiferus* against selected fungal species.

Fraction	Conc. (mg/ml)	Test organisms			
		C. candida	*T. mentagrophyte*	*T. rubrum*	*A. niger*
ME	100	18.83±0.44	15.83±0.16	16.50±0.28	14.46±0.29
	50	17.16±0.16	12.83±0.16	13.50±0.28	11.50±0.28
	25	14.16±0.44	11.50±0.28	12.50±0.28	10.33±0.16
	12.5	11.66±0.33	9.63±0.33	10.33±0.16	8.00±0.00
Fluconazole	0.05	25.16±0.44	21.00±0.57	20.16±0.72	18.83±0.44
EAF	100	27.83±0.16	27.16±0.44	27.00±0.57	17.33±0.88
	50	22.33±0.16	24.16±0.16	24.50±0.28	15.50±0.20
	25	18.83±0.44	17.83±0.16	22.00±0.57	13.16±0.44
	12.5	15.00±0.57	8.00±0.00	18.83±0.29	11.50±0.28
Fluconazole	0.05	26.16±0.44	20.00±0.57	22.16±0.72	18.93±0.24
BTF	100	24.50±0.28	15.50±0.28	15.85±0.44	18.83±0.29
	50	23.66±0.33	10.33±0.16	13.33±0.33	17.16±0.16
	25	21.00±0.57	9.38±0.33	11.50±0.28	13.16±0.28
	12.5	19.33±0.33	8.00±0.00	10.33±0.16	11.66±0.33
Fluconazole	0.05	24.16±0.16	22.00±0.53	21.26±0.72	18.49±0.16

Values are mean inhibition zone (mm) ± S.E of three replicates; Key; M.E=methanol extract, EAF=ethyl acetate fraction, BTF=*n*-butanol fraction

The results of antifungal screening indicated that the fungal isolates were significantly inhibited by the methanol extract and its fractions (ethyl acetate and *n*-butanol) and that the activity increases with the increase in the concentration of the extract and fractions (i.e. the activity is dependent on the concentration), ethyl acetate fraction exhibited the highest mean zone of inhibition range of 27.83±0.16–27.00±0.57 mm against all the test organisms except *A. niger* (17.33±0.88 mm); this activity was higher than that of drug (26.1±0.44-18.49±0.16 mm) against the same organism, while methanol leaf extract recorded the least mean zone of inhibition (Table 2). The MIC and MFC of the extract and fractions ranged between 0.39-25 mg/ml (Table 3); *n*-butanol fraction had the lowest MIC at 0.39 mg/ml against *C. albicans*, hence the effect was fungistatic while the ethyl acetate fraction had a MIC and MFC value of 3.13 mg/ml against *T. rubrum*. The lower MIC and MFC values suggest that the fractions have good antifungal activity.

Table 3: MIC and MFC of ME, EAF and BTF of *T. globiferus* against selected fungal species.

	ME		EAF (mg/ml)		BTF	
Organisms	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**
C. albicans	6.25	12.5$^{\eta}$	12.5	12.5*	0.39	0.39*
T. mentagrophyte	12.5	12.5*	25.0	25.0*	12.5	12.5*
T. rubrum	6.25	6.25*	3.13	3.13*	6.25	6.25*
A. niger	25.0	25.0$^{\eta}$	12.5	12.5$^{\eta}$	3.13	3.13*

Key: *= fungicidal effect, η = fungistatic effect; ME=methanol extract, EAF=ethyl acetate fraction, BTF=*n*-butanol fraction

The highest activity observed by the ethyl acetate fraction might be due to the concentration of moderately polar compounds such as flavonoids and their derivatives that have been reported to possess antifungal activity[27]. Of all the fungal isolates used *C. albicans*, *T. mentagrophyte*, and *T. rubrum* were the most susceptible to ethyl acetate fraction. *C. albicans*, *T. mentagrophytes* and *T. rubrum* are implicated in diseases such as candidiasis, *Tineacapitis*, *Tineapedis*, *Tineacorporis*, *Tineabarbae* and T*ineacruris*[28,29]. Interestingly, the n-butanol fraction with zone of inhibition (24.50 mm) when compared to ethyl acetate fraction (27.16 mm), recorded the lowest MIC against *C. albicans* suggesting that the n-butanol fraction might have better antifungal activity at a lower concentration. Fungal species involving *C. albican* and *A. niger* are the major causative agents of infections such as oral candidiasis, oesophageal candidiasis, virginal thrush, lung diseases, and otomycosis[30,31,32]. The fungicidal effect of extract may be as a result of the inhibition of protein synthesis or nucleic acids metabolism of the organisms[33]. Fungicidal effect of the plant extract could also be as a result of the damage it caused to the cell membrane of the organism[34].

CONCLUSIONS

The use of *T. globiferus* as antifungal agent is promising as the methanol leaf extract and its fractions showed excellent antifungal activity against some selected fungal species with *n*-butanol fraction being the most active. This study indicated that *T. globiferus* has demonstrated good antifungal activity validating the ethno medicinal claim for the use of the plant in the treatment of fungal infections.

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AUTHOR'S CONTRIBUTION

Abubakar H: writing original draft, methodology. **Musa AM:** investigation, conceptualization, review. **Abdullahi MI:** writing, review and editing. **Yusuf AJ:** methodology, formal analysis. All authors read and approved the final manuscript for publication.

DATA AVAILABILITY

Data will be made available on request.

CONFLICT OF INTERESTS

None to declare.

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