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Phytochemical and Antifungal Studies of *Tapinathus globiferus* Extract and Fractions

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T. globiferus growing on *Tamarindus indica* is a hemi-parasitic plant used in ethomomedicine to treat different ailment including fungal infections. The aim of the study was to conduct phytochemical and antifungal studies of *T. globiferus* growing on *Tamarindus indica*. The plant material was collected, identified and air dried. Powdered plant material was subjected to maceration using methanol to obtained crude methanol leaf extract which was partitioned using *n*-hexane, chloroform ethyl acetate and *n*-butanol. The extract and the fractions obtained were subjected to phytochemical evaluation sing standard protocols. The antifungal studies of the methanol extract and its fractions against *Aspergillus fumugatus*, *Aspergillus niger*, *Trychophytom rubrum* and *Trychophyton mentagrophyte* were investigated using agar well diffusion method at different concentration (100 - 1.25 mg/mL). The phytochemical screening revealed the presence of various secondary metabolites which varies in the extract and fractions. The methanol extract and its fractions showed significant ($p< 0.05$) antifungal activity against all the fungal isolates with the ethyl acetate fraction having the highest zone of inhibition ranging from 10-39 mm then followed by the *n*-butanol fraction with the mean zone of inhibitions from 10-28 mm. The methanol extract and the *n*-hexane fraction recorded lower zone of inhibition from 9-24 mm and 8-26 mm respectively. The standard drug ketoconazole had zone of inhibition from 6-22 mm. The most sensitive organism was *A. niger* while the least sensitive organisms were *T. mentagraphyte* and *A. rubrum* for the ethyl acetate fraction. The study has validated the ethnomedicinal claim for the use of this plant in treatment of the fungal infections.

Keywords: Phytochemical, *Tapinanthus globiferus*, *Tamarindus indica*

1. Introduction

The term resistance is used to describe a relative insensitivity of a microbe to an antimicrobial drug as tested *in vitro* and compared with other isolates of the same species (Juergen and David, 2003). Antifungal resistance is the therapeutic concentration at which the growth of the pathogen is unaffected by antifungal agent. For a variety of reasons, research on antifungal resistance has lagged behind that on antibacterial resistance. The reason for this is that fungal diseases were not recognized as significant pathogens until recently. Antifungal resistance can emerge as a result of the usage of antifungal medications in the treatment of fungal illnesses. Resistance to practically all antifungal medications has been documented in a variety of infections, including Candida and Aspergillus species. The majority of resistance mechanisms have also been elucidated at the molecular level in these pathogens (Clark *et al.*, 1996).

There is a general consensus among researchers, clinicians and companies (pharmaceutical and agrochemical) that new, potent, effective and safe antifungal drugs are needed (Caceres *et al*., 1991). There is a great demand for novel antifungal agents belonging to a wide range of structural classes, selectively acting on new targets with least side effects. The plant (extracts or compounds), that are traditionally used for their antifungal activities might be a potential source for drug development (Caceres *et al*., 1991).

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 skin disease in Africa and Nigeria (Adebola, 2004).

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

A number of compounds isolated from plants such as dimethyl pyrrole, hydroxyl dihydrocornin aglycones, indole derivatives, are reported to have antifungal activities (Tasleem *et al*., 2011).

A number of studies have been reported on the antifungal activity of phenolic compounds from natural sources in recent years. A number of hydroxyl groups on the phenol group are thought to be related to their relative toxicity to microorganisms, with evidence that increased hydroxylation results in increased toxicity (Tasleem *et al*., 2011).

Flavone (i) isolated from *Selaginella tamariscina* exhibited potent antifungal activity against several pathogenic fungal strains and has a very low *hemolytic* effect on human *erythrocytes*. The four compounds eupomatenoid-3 (ii), eupomatenoid-5(iii), conocarpan(iv) and orientin(v) isolated from Piper *solmsianum* exhibited antifungal action against the tested *dermatophytes* tested (Campos *et al.,* 2005).

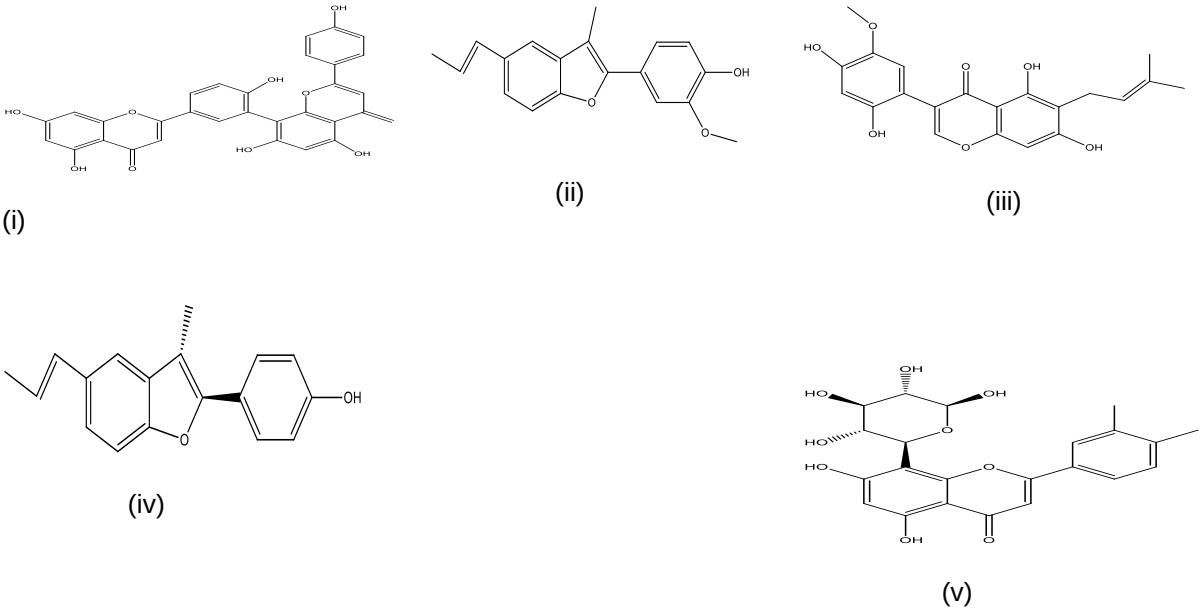

Fig 1: Flavone, Eupomatenoid-3, Eupomatenoid-5, Conocarpan and Orientin (Compose *et al.,* 2005).

Previous phytochemical screening on *T. globiferus* growing on other hosts revealed the presence of alkaloid, tannins, saponins, flavonoids, carbohydrate glycosides and steroid (Abubakar *et al*., 2016). No research has been conducted on the phytochemical studies of *T. globiferus* growing on *Tamarindus indica*.

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 presented was aimed to study

phytochemical and antifungal studies of *Tapinanthus globiferus*, growing on *Tamarindus indica*

2. Materials and Methods

2.1 Sample collection, Identification and drying

The plant material (leaf of *T. globiferus*) was collected from Bodinga L.G.A of Sokoto State 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/0125). The plant material was shed dried, pulverized to powder and stored in a polythene bag before the extraction.

2.2 Extraction and partitioning of plant material (leaf of *T. globiferus*)

Two thousand grams (2000 g) of powdered sample was macerated with 10 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 ^{o}C to obtain crude methanol leaf extract. Some part of the methanol leaf extract (210 g) was suspended in 1000 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.

2.3 Phytochemical screening

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

2.4 Antifungal studies

2.4.1 Test organisms

Four (4) 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 fumugatus, Trychophyton rubrum* and *Trychophyton mentagrophyte*.

2.4.2 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 2 – 8 ^{O}C

2.4.3 Preparation of reference antifungal agents

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

2.4.4 Preparation of crude extract and fractions of T. globiferus

Stock concentrations of 100 mg/cm^3 was prepared with 10 % dimethyl sulfoxide (DMSO) by dissolving 0.5 g (500 mg) each of the crude extract and fractions(*n*-Hexane, 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

2.4.5 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.

2.4.6 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 .

2.4.7 Antifungal screening of T. globiferus

The antifungal activity of crude methanol leaf extract and its fractions (*n*-hexane, 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 cm^3 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/cm^3 ketoconazole (which served as positive control), 10 % (DMSO) used as negative control were dispensed into the wells. The plates were incubated at 27 ^{o}C for 72 hour, the zone of inhibition was measured using transparent ruler. The experiment was carried out in triplicates.

2.5 Statistical Analysis

The results obtained was subjected to the analysis of variance(ANOVA) using SPSS software (Version 22) followed by post hoc test, values were considered significant at $p<0.05$ and data was expressed as mean ± standard deviation

3. Results and Discussion

3.1 Extraction and fractionation

The extraction of 2000 g of *T. globiferus* powder afforded a yield of 300 g of the crude extract and the percent yield from the partitioned fractions are presented in Table1.

3.2 Preliminary phytochemical screening of *Tapinanthus globiferus*

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

3.3 Antifungal studies

The result of Antifungal susceptibility test of methanol, n hexane, ethylacetate and n butanol leaf extract of *T. gloiferus* against some selected fungal species are presented in Table 3, 4, 5 and 6 respectively.

3.4 Extraction and fractionation yield

The extraction of 2000 g of the powdered sample of *T. globiferus* using methanol as extracting solvent yielded 300 g of methanol extract. The fractionation of water soluble portion of methanol leaf extract revealed that *n*-butanol fraction has the highest percentage % yield followed by ethylacetate, chloroform and finally *n*-hexane.

3.5 Phytochemical screening of methanol leaf extract and it fractions

Preliminary phytochemical screening of methanol leaf extract and it fractions(*n*-Hexane, ethylacetate, and *n*-butanol) of *T. globiferus* growing on *Tamarindus indica leaves* revealed the presence of saponins, tannins, alkaloids, cardiac glycoside, steroid, triterpenoids, phenols and flavonoids, ethylacetate and *n*-butanol fractions indicated the presence of similar constituents including flavonoids, alkaloids, tannins, saponins, phenols, steroid and cardiac glycoside while *n*- hexane contained only steroid, and triterpenoids as preseted in (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).

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Table 1: % Yield of partitioned fractions

Solvent	Weight(g)	Yield (%)	Colour
n-hexane	0.703	0.553	Green
Chloroform	0.032	0.015	Light brown
Ethylacetate	12.601	7.067	Brown
n-butanol	57.731	35.820	Redish brown
Water insoluble	37.015	24.677	Green
Methanol	300.0	17.50	Green

Table 2: Preliminary phytochemical constituents of methanol leaf extract and its fractions *T. globiferus*

Constituent	Test	Observation	ME	HF	EF	BF
Flavonoids	Shinoda	Orange color	+	-	+	+
	Sodium hydroxide	Yellow color	+	-	+	+
Alkaloids	Mayer's	A cream ppt	+	-	-	-
	Dragondoff's	Orange 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	+	-	+	+

Key += present - = absent; ME=methanol extract; HF=*n*-hexane fraction; EF=ethylacetate fraction and BF=*n*-butanol fraction.

Table 3: Antifungal susceptibility test of methanol leaf extract of *T. gloiferus* against some selected fungal species.

Test organisms	Zone of inhibition (mm) Concentration(mg/cm^3)			ketoconazole (PC)		DMSO
	100	50	25	12.5	0.05	0.05
A. niger	24.00±1.00*	14.60±1.12*	12.00±1.00*	10.00±1.00a	12.30±0.06	0.00
A. fumugatus	17.60±0.06*	15.00±1.00*	13.00±1.00*	7.00±1.00*	22.30±0.06	0.00
T.mentagraphyte	14.50±1.00*	12.00±1.00*	11.00±01.00*	10.00±1.00*	0.00±0.00	0.00
T. rubrum	14.00±1.00a	12.00±1.00a	11.00±1.00a	9.00±1.00*	12.3±0.06	0.00

Key: = The data mean ± sd * ($p< 0.05$) compared with PC, (ANOVA): a Indicates no statistical significant compared with PC (Positive control).

Table 4: Antifungal Susceptibility test of *n*-hexane fraction *of T. globiferus* against some selected fungal species

Test organisms	Zone of inhibition (mm) Concentration(mg/cm^3)			ketoconazole(PC)		DMSO
Organisms	100	50	25	12.5	0.05	0.05
A. niger	19.00±1.00*	16.00±01.00*	14.00±1.00a	11.00±1.00a	12.30±0.60	0.00
A. fumugatus	20.60±1.00a	19.00±1.00*	16.00±1.00*	13.00±100*	22.30±0.06	0.00
T.mentagraphyte	15.60±0.60*	13.00±1.00*	10.00±1.00*	8.00±1.00*	0.00±0.00	0.00
T. rubrum	26.00±1.00*	18.00±1.00*	15.00±1.00*	14.00±1.00a	12.3±0.06	0.00

Key: = The data mean ± sd * ($p< 0.05$) compared with PC, (ANOVA): a indicates no statistical significant compared with PC (Positive control).

Table 5: Antifungal susceptibility test of ethylacetate fraction *T. globiferus* against some selected fungal species

Test organisms	Zone of inhibition (mm) Concentration(mg/cm^3)			ketoconazole(PC)		DMSO
Organisms	100	50	50	12.5	0.05	0.05
A. niger	39.00±1.00*	22.60±1.00*	18.00±1.00*	13.00±1.00a	12.30±0.60	0.00
A. fumugatus	21.60±1.00a	17.00±1.00*	15.00±1.00*	14.00±1.00*	22.30±0.60	0.00
T.mentagraphyte	20.00±1.00*	18.00±1.00*	13.00±1.00*	10.00±1.00*	0.00±0.00	0.00
T. rubrum	16.00±1.00*	13.00±1.00a	11.00±1.00a	10.00±1.00a	12.3±0.60	0.00

Key: = The data mean ± sd * ($p< 0.05$) compared with PC, (ANOVA): a indicates no statistical significant compared with PC (Positive control).

Table 6: Antifungal susceptibility test of *n*-butanol fraction *T. globiferus* against some selected fungal species

Test organisms	Zone of inhibition (mm) Concentration(mg/cm^3)			ketoconazole (P.C)		DMSO
Organisms	**100**	**50**	**25**	**12.5**	**0.05**	**0.05**
A. niger	28.00±1.00*	17.60±1.00*	14.00±1.00a	11.00±1.00a	12.30±0.60	0.00
A. fumugatus	21.60±1.00a	17.00±1.00*	15.00±1.53*	14.00±1.00*	22.30±0.60	0.00
T.mentagraphyte	13.50±1.00*	12.00±1.00*	11.00±1.00*	10.00±1.00*	0.00±0.00	0.00
T. rubrum	17.00±1.00*	15.00±1.00*	13.00±1.00a	11.00±1.00a	12.3±0.60	0.00

Key: = The data mean ± sd * ($p< 0.05$) compared with PC, (ANOVA): a indicates no statistical significant compared with PC (Positive control).

3.6 Antifungal studies

The susceptibility test of methanol extract and it fractions (*n*-hexane, 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/cm^3) with mean zone of inhibition ranging from 8.00 – 39.00 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 fraction 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* as presented in (Table 4).

Methanol leaf extract showed significant (*p< 0.05*) antifungal activity against *A. niger* at 100 mg/cm^3 which was higher that of ketoconazole at 0.05 mg/cm^3 as presented in (Table 3) . Abubakar *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/cm^3 compared with ketoconazole at 0.05mg/cm^3 (Table 4), similarly, the standard drug, ketoconazole exhibited higher effect against *A. fumugatus* when compared with the fraction at 100 mg/cm^3 although the effect was not statistically significant(Table 6).

Ethylacetate fraction exhibited the highest antifungal activity against *A. niger* while least sensitive organism was *T. rubrum (*Table 5) Abubakar *et al.* (2020) 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 ketoconazole, even though it recorded the mean zone of 21.60 mm against *A. fumugatus* which was lower than that of ketoconazole 22.30 mm but the difference is not statistically significant(Table. 6). 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).

4. Conclusion

Preliminary phytochemical screening of methanol leaf extract and it fractions (*n*-Hexane, ethylacetate, and *n*-butanol) of *T. globiferus* growing on *Tamarindus indica* leaves revealed the presence of saponins, tannins, alkaloids, cardiac glycoside, steroid, triterpenoids, phenols and flavonoids Tapinanthus globiferus growing on *Tamarindus indica* has demonstrated significant antifungal activity validating the ethnomedicinal claim for the use of the plant in the treatment of fungal infections.

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Conflict of interest

The authors declare no conflict of interest.

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