ANTIFUNGAL EFFECTS OF TAPINANTHUS GLOBIFERUS GROWING ON VITEXDONIANA 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 of 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.
screening on *T. globiferus* growing on host plants revealed the presence of alkaloid, tannins, saponins, flavonoids, carbohydrate, glycosides, terpenes and steroids. 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, Antitrypanosomal activity and anticonvulsant activity.

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. globiferus* growing on *Vitexdoniana* 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). Sabouraud dextrose agar and broth (HiMedia Laboratories Pvt Ltd, India). UV spectrophotometer (Aberra BARCELONA Spain). Ohaus digital weighing balance (Champ 11 CH15R, Ohaus Corporation, Pinebrook NJ, USA), Metler balance (Model P162 supplied by Gallenhang), 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 Iyvm System) and standard powder of fluconazole (Sigma AldrichNo. F8929, U.S.A.)

**Plant sample**

Plant sample of *T. globiferus* growing on *Vitexdoniana* 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. globiferus* and its ethyl acetate and n-butanol fractions in accordance with the procedures to identify the presence of some secondary metabolites.

**Antifungal studies**

**Test organisms**

Four clinical fungal isolates obtained from the Clinical Microbiology Department of Usman 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 0.5g 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 albicans* (18 h) in Sabo broth was prepared. The standardization was performed according to method by Clinical Laboratory Standard Institute guidelines 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 *Trychophyton* spp were sub-cultured from (6 days old) SDA slant, the suspension was adjusted to 1.0×10^6 CFU/ml at 530nm 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. 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. 0.1ml 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. Liquid media of 100μl was transferred into
each micro well of the micro plate. Extract of 100μl and its fractions was transferred into well-1 making up 200μl total volume. Mixture (extract/fractions) and media of 100μl 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 (10⁵CFU/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 72h. 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 incubation²².

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

| Constituents     | Test     | ME   | EAF | BTF |
|------------------|----------|------|-----|-----|
| Carbohydrates    | Molisch  | +    | +   | +   |
| Anthraquinones   | Bontrager| -    | -   | -   |
| Steroid/Triterpenes | Liebermann-Burchard | +    | -   | -   |
| Glycoside        | Keller-Killian | +    | +   | +   |
| Saponins         | Frothing | +    | +   | +   |
| Tannins          | Ferric chloride | +    | +   | +   |
| Flavonoids       | Shinoda   | +    | +   | +   |
| Alkaloids        | Dragendoff | +    | -   | -   |

Key: - = absent; + = present; ME=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**

| Test organisms | ME (24.16±0.16 mm) | EAF (19.33±0.33 mm) | BTF (26.16±0.44 mm) |
|----------------|---------------------|---------------------|---------------------|
| *C. candida*   | 15.83±0.33          | 12.26±0.16          | 11.50±0.28          |
| *T. mentagrophyte* | 23.66±0.33          | 20.16±0.57          | 18.83±0.44          |
| *T. rubrum*    | 25.16±0.33          | 22.16±0.72          | 17.33±0.88          |
| *A. niger*     | 18.83±0.57          | 15.00±0.28          | 11.50±0.28          |

**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 *Vitexdoniana* 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 reported¹⁵,¹⁶,²⁴,²⁵ on *T. globiferus* growing on other host plants. These phytochemical constituents were reported to be responsible for different pharmacological and physiological activities of plants²⁶. 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 test organisms; Key: ME=methanol extract, EAF=ethyl acetate fraction, BTF=n-butanol fraction 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.

| Organisms   | MIC (mg/ml) | MFC (mg/ml) | EAF (mg/ml) | BTF (mg/ml) |
|-------------|-------------|-------------|-------------|-------------|
| C. albicans | 6.25        | 12.5*       | 12.5        | 12.5*       |
| T. mentagrophyte | 12.5 | 12.5*       | 25.0        | 25.0*       |
| T. rubrum   | 6.25        | 6.25*       | 3.13        | 3.13*       |
| A. niger    | 25.0        | 25.0*       | 12.5        | 12.5*       |

Key: * = fungicidal effect, = 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. mentagrophyte and T. rubrum are implicated in diseases such as candidiasis, Tinea capitis, Tinea pedis, Tinea corporis, Tinea barbae and Tinea cruris[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, vaginal 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]. Fungalicidal effect of the plant extract could also be as a result of the damage it caused to the cell membrane of the organism[34].

CONCLUSION

The use of Tapinanthus 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.

CONFLICT OF INTERESTS

The authors declare that they have no financial or personal relationships which may have inappropriately influenced them in writing this article.

AUTHOR’S CONTRIBUTION

All authors have worked equally for this work.

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