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## Research Article

# Cells Adhesion Inhibitory Activity of Identified Compounds from *Taminalia catappa* Against Clinical Isolate of *Trichosporon asahi*

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### ABSTRACT

*Trichosporon* infection represents an emerging and still underestimated type of fungal infection that can lead to severe medical manifestations. It has been found to be associated with a high mortality rate worldwide, especially among immunocompromised patients. *Trichosporon asahi* rapidly forms biofilm which are cells composed of extracellular matrix. This biofilm formation by cells adherence to surfaces is a mechanism of antibiotic resistance employed by *T. asahi*. This research work is aimed at determining the adhesion inhibitory activity of identified compounds from *T. catappa* against clinical isolate of *Trichosporon asahi*. Microdilution method was used to form adhered cells on the microtitre wells and Column chromatography was used to fractionate the n-butanol extract of *T. catappa*. The wells containing nitrogen-based broth were inoculated with cell suspension of *Trichosporon asahi* isolate and treated with fractions of N-butanol extracts of *T. catappa*. Fraction (F8) significantly inhibited the formation of adhered cells compared to other fractions, which is reflected in the concentration of the adhered cells formed. GC-MS of the F8 revealed various compounds with methyl ester having 99% purity. F8 fraction of *T. catappa* contains compounds most especially methyl ester with 99% purity that disrupts the formation of adhered cells therefore reducing antibiotics resistance.

**Keywords:** Adhered Cells; Biofilms; Column Chromatography; Microdilution; *Trichosporon asahi*

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### INTRODUCTION

There is high increase in the prevalence of fungal infections over the past 30 years resulting as one of the major causes of death in immune compromised (Li *et al.*, 2020). In addition to common fungal infections such as candidiasis and aspergillosis, infections by non-candida yeast like *Trichosporon* are rising in immuno compromised individuals with deteriorating conditions and high mortality (Mehta *et al.*, 2021). Among *Trichosporon* genus, *T. asahii* is the species most frequently isolated from clinical samples of blood and urine, thus most research efforts are directed towards understanding its virulence. It has been found that both *T. asahii*, and with *T. inkin*, are excellent biofilm producers.

*Trichosporon* spp. belongs to the phylum Basidiomycota. It is a yeast-like fungus macroscopically characterized by forming radially symmetrical colonies; these colonies may be white or yellowish, with a smooth texture, creamy, cerebriform, powdery or moist. Microscopically, *Trichosporon* spp. forms hyaline septate hyphae, with abundant arthroconidia and blastoconidia (Colombo *et al.*, 2011).

When phenotypic switching occurs *in vivo*, they involve modifications in the expression of virulence factors, or alterations in the microorganism-host cell interactions, thus provoking an increment in the pathogenicity and evasion of the immune response (Jain *et al.*, 2006).

There is a number of virulence factors that facilitate *Trichosporon* spp. invasion, including phenotyping plasticity and the production of metabolites such as calcineurin or extracellular lytic enzymes (Matsumoto *et al.*, 2022; de-Andrade *et al.*, 2023). However, the most widely documented virulence factor of *Trichosporon* spp. is the ability to adhere to implanted medical devices and subsequent producing of biofilms (Shu-chen *et al.*, 2007). Hyphae and arthroconidia are important in the formation of biofilm by *T. asahii* (Kurakado *et al.*, 2021). Biofilm formation is affected by environmental factors, such as temperature, pH, or nutrient availability (Tan *et al.*, 2016).

Adhesion and biofilm formation by yeast causes significant damage to biological system. They are considered virulence factors that facilitate host invasion. Fungal cells may adhere to both biotic and abiotic surfaces, posing a threat of infection and biofilm development (Amann *et al.*, 2025). Medical and industrial materials (such as glass, stainless steel or silicone) may become contaminated with adherent fungal cells of environmental origin, which, upon contact with immunocompromised people, could serve as infective agent (Alonso *et al.*, 2023). Thus, studies towards adhesion and biofilm development are of great importance.

The leaves of *T. catappa* contains flavonoids, tannins, saponins and phytosterols (Jain *et al.*, 2009). The leaves and the barks are used in herbal medicines for treatment of liver diseases. Phytochemicals such as flavonoids, alkaloids, terpenoids, tannins, berberines, quinines, and emetins synthesized by plants are widely used in the treatment of diseases (Hülya and Zeyneddin, 2024). *Terminalia catappa* has been investigated in various pharmaceutical studies as it contains a variety of chemical components (Pandya *et al.*, 2013). *T. catappa* L. leaf extracts exhibit biological activities, including antioxidant (punicalagin, punicalin, terfluvina A and B, chebulic acid, benzoic acid, cumaric, and its derivatives) (Kinshita *et al.*, 2007), antidiabetic ( $\beta$ -carotene) (Anand *et al.*, 2015), anticancer (punicalagin) (Naitik *et al.*, 2012), antiviral. The bark, leaves and fruit of *T. catappa* were used in different countries like India, Malaysia and Philippines to cure dermatitis and also for hemostatic and antipyretic purposes (citation). In hepatoma and hepatitis treatment, the leaves of *T. catappa* have been widely used in Taiwan by

shredding and drying (Gayathri *et al.*, 2019). In Northern Nigeria traditional use of the dried fallen leaves of *Terminalia catappa* is in the treatment of typhoid fever and fungal infections (Sule *et al.*, 2006). The aim of this work is to evaluate the cells Adhesion inhibitory activity of characterized compounds from N-butanol extract of *Taminalia catappa* against *Clinical Isolates of Trychosporon asahi*.

## **MATERIALS AND METHODS**

### **Plant collection and Identification**

The leaf of *T. catappa* was harvested fresh from a residential garden in Ungwar Rimi, Kaduna North Local Government Area Kaduna-Nigeria. The leaf was authenticated at the herbarium section of the Department of Biological Sciences, Kaduna State University, Kaduna and the voucher number (KASU/BSH/043) was deposited. Clinical isolates of fungi were collected from blood samples diagnosed with candidiasis at Microbiology Laboratory Barau Dikko Teaching Hospital, Kaduna. *Trichosporon asahi* was identified in the clinical blood samples using the ITS region and amplified using two (2) primers which are: Forward primer ITS1 5'-TCCGTAGGTGAACCTGCGG-3' and reverse primer ITS4: 5'-TCCTCCGCTTATTGATATGC-3') by polymerase chain reaction (Maimuna *et al.*, 2025).

### **Preparation of Crude Extracts and Fractions of N-butanol extract of *Taminalia catappa***

The extraction of phytochemicals from crude extracts of N-butanol extract of *Taminalia catappa* was determined in accordance with the method described by Handa *et al.* (2008). The leaf of *T. catappa* was air dried and pulverized into powdered form. Exactly 200 g of the pulverized sample was added into a conical flask, 1.5 L of n-butanol was added to the pulverized samples and allowed to stay for 48 hours. After 48 hours, the mixture was filtered using Whatman no. 1 filter paper. The supernatant was decanted into a cleaned beaker and then allowed to evaporate. The crude extract was fractionated using Column chromatography as described by Sembiring *et al.* (2018) using two (2) solvent systems. The solvents used were N-Hexane, Chloroform ethylacetate and N-Butanol in different ratios with increasing polarity starting from absolute n-hexane (100%), n-hexane : chloroform (4:1, 3:2, 2:3 and 1:4), ethylacetate : n-butanol (4:1, 3:2, 2:3 and 1:4) and absolute n-butanol (100%). Exactly 62 fractions each having 20

ml were collected. Thin layer chromatography (TLC) was conducted on each fraction using TLC plates on each of the 62 fractions collected. Fractions with the same retention factor were pooled together in the same beaker to make eight (8) fractions (F1-F8). The fractions were tested for cells adhesion inhibitory activity against clinical isolates of *Trychosporon asahi*. Formular for Retention factor (Rf) = Distance travelled by solute / Distance travelled by solvent

#### **Cells Adhesion Inhibitory Activity of Fractions of N-butanol Extract of *Taminalia catappa* against Clinical Isolates of *Trychosporon asahi*.**

Adhered cells of *trychosporon asahi* isolates was formed by microdilution method in accordance with the method described by Ramage *et al.* (2005). Exactly 100  $\mu$ l of *T. asahi* suspension (0.5 McFarland standard which is equivalent to  $1 \times 10^8$  cfu/ml). The cell concentration was diluted to give ranges of cell concentration ( $5 \times 10^7$  cfu/ml,  $25 \times 10^6$ cfu/ml  $12.5 \times 10^6$  cfu/ml and  $6.25 \times 10^6$  cfu/ml) and standard curve was plotted. Exactly 100  $\mu$ l of the cell suspension ( $5 \times 10^7$  cfu/ml) was inoculated into individual wells of polystyrene 96 well plates (flat bottom) containing 100  $\mu$ l of Nitrogen based broth containing, 0.05 M glucose and 100  $\mu$ l of each of the pooled fractions in their respective wells. The plates were incubated at 37°C for 90 minutes (adhesion period). Caspafungin and Voriconazole were added separately into Positive control wells without the isolate suspension while the negative control wells contain only the cell suspension and nitrogen-based broth. The supernatant which contains planktonic cells and liquid medium was then discarded and wells were gently washed twice with phosphate buffered saline (PBS) to get rid of any non-adherent cells and then 100  $\mu$ l of Nitrogen based broth was added into all wells and incubated for 48 hours. After the required incubation time for each well, an aliquot of 100  $\mu$ l of 2% crystal violet was added to each well and incubated for 20 minutes at 37°C. Then, 150  $\mu$ l 95% ethanol was added to dissolve the dyed adhered cells and 100  $\mu$ l of each mixture was transferred to a new 96 well microtitre plate. The absorbance for each well was determined using microplate reader at 630nm. The resulting absorbance value is directly proportional to the number of adherent cells, which can then be extrapolated from a standard curve to get the absolute concentration of the adhered cells.

#### **Calculations**

Linear regression analysis

$$Y = MX + C$$

Y = Absorbance

M = Slope of the Line

X = Concentration of the adhered cells

C = Intercept on Y axis

$$X = \frac{Y - C}{M}$$

#### **Characterization of the most Potent Fraction of *T. catappa* against Adhered Cells of *T. asahi* Fraction using Gas Chromatography**

The identification of compounds present in the most potent fraction of *T. catappa* was characterized using Gas Chromatography Mass Spectrometry (GC-MS). GC-MS analysis was performed using the equipment Thermo GC-Trace Ultra Version. Sample was analyzed using agilent technologies 7890A GC and 5977B MSD with experimental conditions of GC-MS system 5.0, Thermo MS DSQ II. The equipment has a DB 35 – MS Capillary Standard non-polar column with dimensions of 30 mm  $\times$  0.25 mm ID  $\times$  0.25  $\mu$ m film. The carrier gas used was Helium with a flow rate of 1.0 ml/min. The injector was operated at 250 °C and the oven temperature was programmed as follows: 60 °C for 15 min, then gradually increased to 280 °C at 3 min. The identification of components was based on Willey and NIST libraries as well as comparison of their retention indices. The constituents were identified after comparison with those available in the computer library (NIST and Willey) attached to the GC-MS instrument. Results obtained were tabulated. In the gas chromatography part, temperature programme (oven temperature) was 40°C raised to 250°C at 5°C / min and injection volume was 1 $\mu$ l. Samples dissolved in methanol were run fully scan at a range of 40--650 m/z and the results were compared by using Nist Mass Spectra library search programme.

#### **Data Analysis**

Results were expressed as  $\pm$ SD. Test were performed in triplicate. Significant differences were analyzed between groups using one way analysis of variance (ANOVA). Post Hoc mean separations were performed by Turker Kramer multiple tests at  $p < 0.05$ .

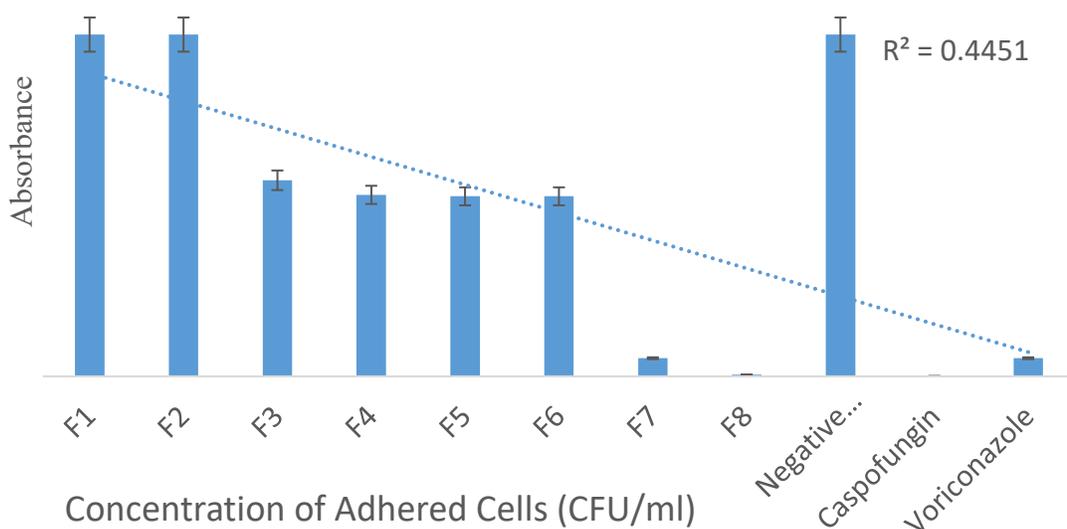
#### **RESULTS AND DISCUSSION**

Figure 1 showed the inhibitory activity of fractions of N-butanol extracts of *T. catappa* and standard drugs on adhered cells formed by clinical isolates of *trichosporon asahi*. The Negative control well gave

the highest concentration of adhered cells which was significantly ( $p < 0.05$ ) different from the wells treated with F7 and F8 but, no significant ( $p > 0.05$ ) difference with wells treated with F1 and F2. Caspofungin significantly inhibited the formation of adhered cells while voriconazole's inhibition of the adhered cells was not significant from the level of inhibition of the adhered cells by F7 lower inhibition of the adhered cells when compared to F8, Fraction 8 gave the highest inhibition of the adhered cells among the fractions and so chosen as the most potent fraction of the N-butanol extract of *Taminalia catappa* Leaf.

Characterization of F8 by GCMS revealed 42 compounds out of which 12 of them have quality above 70%. The 12 compounds are Hexadecanoic acid (83 %), Pentadecanoic acid (98%), Phthalic acid (86%), ethyl ester (95 %), 9,12-Octadecadienoic acid (99%), Methyl stearate (97%), 9-Hexadecyn-1-ol (81 %), E-11-Hexadecenoic acid (70 %), Squalene (90 %), Hexadecane (92 %) and Palmitoleic acid (81 %) (Table 1). Hexadecanoic acid is one of the major compounds with 83% purity. It was shown to have antifungal properties in research conducted by Agoramoorthy *et al.*, 2007. A saturated fatty acid (Hexadecenoic acid

or palmitic acid) abundant compound in the extracts of *T. catappa* with antifungal activities against mycelial growth and spore production (Ahsan, 2017). Inhibition of mycelial growth is most likely responsible for the reduction in adhered cells by the *T. asahi* in treated wells. Palmitoleic acid, hexadecane and phthalic acid have been reported to disrupt cell membrane and reduce nutrient uptake by also disrupting the integrity of the cell membrane and reduction in energy utilization, respectively (Prasath *et al.*, 2020). F8 has the most reduced adhered cells and therefore most potent. This is most likely due to the presence of 99% methyl ester which is reported by Chang *et al.* (2024) to disrupt adhered cells formed by fungi by inhibiting fungal growth, inducing oxidative stress and also inhibiting cell wall synthesis. This mechanism of action is in comparison with the standard drugs used in this work Caspofungin and voriconazole. Echinocandin antifungal agents, such as caspofungin (CAS), damage fungal cell walls by inhibiting beta-1,3-D-glucan synthase, and azole antifungal agents, such as voriconazole (VRC), are known to inhibit the synthesis of cell membranes (Lee *et al.*, 2017).



**Figure 1: Inhibitory Effects of Fractions of N- butanol Extracts of *Taminalia catappa* against formation of Adhered Cells by Clinical Isolates of *Trychosporon asahi***

**Table 1: Major Compounds Identified from the Most Potent Fraction (F8) of *T. catappa* against Adhered Cells of Clinical Isolates of *T. asahi*.using GC-MS**

PK	AREA PCT	LIBRARY/1D	REF	CAS	% Purity
1	0.402	Hexadecanoic acid, 15-methyl-, methyl ester	144336	006929-04-0	83
2	17.47	Pentadecanoic acid, 14-methyl-, methyl ester	130841	005129-60-2	98
3	1.006	Phthalic acid, 5-methylhex-2-yl butyl ester	178818	1000371-09-0	86
4	1.425	Hexadecanoic acid, ethyl ester	144304	000628-97-7	95
5	3.61	9,12-Octadecadienoic acid, methyl ester	153873	002462-85-3	99
6	14.42	12-Octadecenoic acid, methyl ester	155730	056554-46-2	99
7	1.719	Methyl stearate	157881	000112-61-8	97
8	0.345	9-Hexadecyn-1-ol	100555	1000342-40-3	81
9	1.229	E-11-Hexadecenoic acid, ethyl ester	142114	1000245-71-9	70
14	4.098	Squalene	243219	000111-02-4	90
18	0.857	Hexadecane, 1-(ethenyloxy)-	128822	000822-28-6	92
30	23	Palmitoleic acid	115312	000373-49-9	81

## CONCLUSION

Fraction (F8) of the N-butanol extract of *Terminalia catappa* significantly inhibited adhered cells formation and therefore most potent. This is most likely due to the presence of 99% methyl ester in the most potent fraction of n-butanol extract of *T. catappa* which disrupts adhered cells formed by fungi by inhibiting fungal growth, inducing oxidatative stress and also inhibiting cell wall synthesis.

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