



Anticandidal Effect of Extracts of Wild Polypore, *Trametes elegans*, on *Candida* Species Isolated from Pregnant Women in Selected Hospitals in Southwest Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author ATA carried out the research, wrote the protocol and wrote the manuscript. Author VOO designed and supervised the study. Authors TAO and SIA managed the literature searches and statistical analyses. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Considering the significance of candidiasis among pregnant women, the study was designed to investigate the anticandidal effect of extracts of *Trametes elegans* (Spreng: Fr.) Fr. (fam.: Polyporaceae) against *Candida* species isolated from pregnant women and to screen for the phytochemical constituents of the crude extract of *T. elegans*.

Place and Duration of Study: *Candida* species were isolated from 132 high vaginal swabs (HVS) collected from pregnant women attending ten selected hospitals in Ondo, Osun and Oyo States, Nigeria.

Methods: Extracts of *Trametes elegans* was prepared with methanol, acetone and n-hexane. Phytochemical screening of the macrofungus extracts were thereafter performed qualitatively and quantitatively. The isolated *Candida* species were subjected to antifungal assay to determine the anticandidal efficacy of the macrofungus extracts.

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Results: A total of 67 *Candida* isolates were obtained from the patients. The prevalent organisms were *C. albicans* (49/67, 73.13%), *C. glabrata* (9/67, 13.43%), *C. krusei* (6/67, 8.96%) and *C. tropicalis* (3/67, 4.48%). Methanol gave the highest yield (3.4 mg/g), while n-hexane gave the least (0.7 mg/g). All the extracts contained saponin, tannin, steroid, terpenoid and cardiac glycosides, while flavonoid was only found in acetone extracts. Saponin was highest (31.77 mg/g) while flavonoid content was least (1.65 mg/g). Zones of inhibition ranging from 5.00 to 30.00 mm, 4.00 to 15.67 mm and 4.33 to 17.67 mm were produced by methanol, acetone and n-hexane extracts respectively. Methanol extract of *T. elegans* produced the highest anticandidal activity with an inhibition zone of 30.00 mm against isolate A3 (*C. albicans*.) The least inhibition zone (4.00 mm) was recorded with acetone extract of *T. elegans* against isolate A2.

Conclusion: The high anticandidal activity exhibited by mushroom extracts suggests that bioactive compounds from these mushrooms could be developed into antifungal agents for the treatment of candidiasis.

Keywords: *Candida*; mycoses; phytochemistry; *Trametes elegans*.

1. INTRODUCTION

Candidiasis, thrush, or yeast infection is a fungal infection (mycosis) of any species from the genus *Candida*, *Candida albicans* is a member of the normal human microbiome in most individuals. *Candida albicans* resides as a lifelong, harmless commensal [1].

Once the organism crosses into the bloodstream of the patient it is readily able to invade and flourish in major organs such as the liver, kidney, heart, and brain. Infection of these major organs can very easily become life-threatening [1]. *Candida* infections of the latter category are also referred to as invasive candidiasis, and usually occur in immunocompromised persons, such as people suffering from viral infections and cancer patients [1].

Fungi belonging to the genus *Candida* are part of the harmless flora of the female genitals [2]. However their overgrowth as opportunistic pathogens in the vulva and/or vagina can lead to vulvovaginal candidiasis [3]. More than 75% of women are affected by vulvovaginal candidiasis at least once in their lifetime [4]. Vulvovaginal candidiasis is most common during pregnancy and may result into complications such as premature rupture of membranes, preterm delivery, chorioamnionitis, congenital cutaneous candidiasis etc. [5].

Mushrooms contains bioactive compounds that have been reported to be used in the treatment of microbial infections [6]. *Trametes* is a genus of fungi that is characterized by a pileate basidiocarp, di- to trimitic hyphal systems, smooth non-dextrinoid spores and a hymenium usually without true hymenial cystidia.

The genus is widely spread and includes about fifty species. The main characters of *Trametes elegans* are an ochraceous brown basidiocarp, cyanophilous skeletal hyphae and often ochraceous colour of binding and skeletal hyphae. Emergence of numerous antibiotic resistant microorganisms of which *Candida albicans* is not an exemption constitutes a major public health problem. This has led to a search for effective antimicrobial substances from sources such as plants and macrofungi. Wild macrofungi such as *Trametes* species had been found to have bioactive compounds with potent antimicrobial property. Considering the importance of candidiasis amongst pregnant women and the enormous potential of *Trametes elegans* (Spreng: Fr.) Fr. (fam.: Polyporaceae) as sources of antimicrobial agents, the present study was designed to evaluate the antifungal activity of extracts of *T. elegans* against *Candida* species isolated from pregnant women attending hospitals in Ondo, Osun and Oyo State, Nigeria and to screen for the phytochemical constituents of the crude extract of *T. elegans*.

2. MATERIALS AND METHODS

2.1 Clinical Sample Collection

High vagina swabs (HVS) were collected from 132 pregnant women attending ten selected hospitals in Ondo, Osun and Oyo States, Nigeria. Pregnant women were at varying gestational periods (3-8 months).

2.2 Cultivation and *Candida* Isolation

High Vaginal Swabs were cultivated on Sabouraud dextrose agar at 37°C and incubated

for 24 - 48 hours. The cultures were sub-cultured severally to get pure cultures. An agar slant technique was employed in the preservation of cultures. This was done by preparing double strength of Sabouraud dextrose agar in McCartney bottles; it was allowed to cool and was set in a sloping position. Sterilized inoculating loop was used to transfer colony to the surface of the slope agar slant, it was then incubated at 37°C for 24 - 48 hours after which they were refrigerated at 4°C until when required.

2.3 Candida Species Identification

Candida species were identified based on morphological characteristics and biochemical tests.

2.3.1 Morphological characteristics

Appearance of the colony of each isolates on SDA media was studied and recorded. Each isolate was Gram stained and examined microscopically with the aid of a light microscope. When viewed, *Candida* isolates showed oval budding yeast cells. KOH Mount was then performed; Smears were prepared for each sample by adding HVS to a drop of 10% KOH on a clean, grease-free slide and placing a cover slip over it. The preparations were slightly warmed to digest the materials and examined under the microscope for yeast cells, pseudohyphae [7].

2.3.2 Biochemical characterization

Biochemical characterization was performed using germ tube test, chlamydo-spores formation test, sugar assimilation test and CHROMagar test.

2.3.2.1 Germ tube test

Five millilitre of human serum was dispensed into a small test tube. Yeast colony was then inoculated from the culture plate into the test tube. The tube was then incubated at 37°C for 2-3 hours. Using a Pasteur pipette, a drop of the serum yeast culture was transferred to a glass slide and a drop of lactophenol cotton blue was added to the preparation to stain the yeast cells and cover with a cover glass. The preparation was then viewed under the microscope for sprouting yeast cells that is tube-like outgrowths from the cells known as germ tubes. When the yeast cells do not show sprouting, it was recorded as yeast other than *Candida albicans* [7].

2.3.2.2 Chlamydo-spores formation test

All *Candida* isolates were tested for the production of chlamydo-spores in corn meal agar with Tween 80. The isolates were inoculated in corn meal agar which involves streaking and stabbing the media with a 48 hours old yeast colony and covered with sterile cover slip and incubated at 25°C for 72 hours. Chlamydo-spore production was examined after staining with lactophenol cotton blue [6]. The isolates were categorised as chlamydo-spore positive and negative.

2.3.2.3 Sugar utilization test

Sugar indicator broth was prepared using peptone water medium containing 0.5% fermentable sugars and 0.01% phenol red to note the colour change. Ten millilitres (10ml) of sugar indicator broth was dispensed into the test tubes each and Durham's tube was inverted carefully into each test tube to trap the gas if produced. The test tube was sterilized in the autoclave at 121°C for 15 mins. After sterilization, culture of the isolates was introduced aseptically into the test tubes; a test tube was assigned to each labelled isolates. A test tube without inoculation serves as control. The whole set up was incubated for 2-7 days at 37°C and observed daily for colour change to yellow which indicate acid production while gas production was indicated by displacement of the medium in the Durham tube [7]. The sugars used include Glucose, Sucrose, Lactose, Galactose, Maltose, Trehalose and Xylose.

2.3.2.4 CHROMagar test

CHROMagar was prepared according to manufacturer specification. *Candida* isolates produces varying colors when grown on CHROMagar. *C. albicans* produces green colonies, while *C. glabrata* produces pale purple colonies whereas *C. krusei* produces pink colonies and *C. tropicalis* produces dark blue colonies [7].

2.4 Collection of Macrofungus

Fresh fruit bodies of *Trametes elegans* were handpicked from rotten woods in the wild. The fruiting bodies were kept dry by wrapping in polythene paper containing silica gel. The polythene bags were labeled for easy identification. The morphological characteristics of these macrofungi were also recorded in their natural habitats.

2.5 Extraction Screening of Macrofungus

Extracts of the wild polypore *T. elegans* collected from Ipogun, Ondo State were prepared as described by Harborne [8]. After collection, the mushrooms were homogenized into fine powder. Powder of *Trametes* species (400 g) were weighed into four Litres of different solvents (in ratio 1:10) and left for 72 hours with frequent stirring. The solvents used were n-hexane, acetone and methanol. Each mixture was then sieved using muslin cloth. It was then filtered using Whatman filter paper No 1. The filtrates were collected in a sterile beaker and concentrated using rotary evaporator (Resona, Germany). Before using any of the extracts, they were reconstituted with 30% dimethylsulphoxide (DMSO) and sterilized by filtration using Millipore membrane filter (0.2 micron meter). The weight of the dried extract were measured and reported as percentage recovery.

2.6 Phytochemical Screening of Macrofungus

Each of the mushroom extracts (1 g) was carefully weighed into 250 mL conical flask and 25 ml of distilled water was added. It was mixed and stopped with rubber bung, then placed in water bath for 2 hours at 37°C, after which it was removed to cool. The content was filtered with the use of Whatman filter paper No 1 and the filtrate was kept for analysis. Phytochemical screening of the macrofungus extract was thereafter performed using the method described by Harborne [8].

2.7 Anticandidal Screening of Macrofungus

Isolates were subjected to antifungal assay to determine the anticandidal efficacy of *T. elegans* extracts. This was done using agar well diffusion method as described by Esimore and Adikwu [9]. The *Candida* isolates were grown on Sabouraud Dextrose Agar (SDA) and standardized to 0.5 McFarland standards. About 20 ml of Sabouraud Dextrose Agar was poured aseptically into sterile Petri dish and allowed to gel. *Candida* culture (0.1 ml) was placed on the Petri dish and was evenly spread. With the aid of sterile cork borer, 10 mm in diameter, wells were bored on solidified agar medium. Each extract (100 mg/ml) was prepared and 0.5 ml of the prepared extract was then introduced into well appropriately labelled. It was then incubated at 37°C for 24- 48 hours. After incubation, diameter zone of

inhibition were measured and recorded. 30% dimethylsulphoxide (DMSO) and antifungal agent was introduced into different well on each plate and this serves as negative and positive control respectively. The minimum inhibitory concentration (MIC) was carried out using the tube dilution techniques as described by Doughari et al. [10] Varying concentrations of the extract (3.125, 12.50, 25 and 50 mg/ml) were prepared into test tubes using 20% tween 20 as reconstituting solvent. SDA broth (2ml) which was prepared according to manufacturer specification was added to 0.5 ml of varying concentrations of the extracts. *Candida* isolates previously diluted to 0.5 McFarland turbidimetric standard was introduced to the tubes. 20% tween 20 was introduced into different test tube and this serves as control.

A tube containing only broth was seeded with *Candida* isolates as described above to serve as positive control while a tube that was not inoculated serve as negative control. All the broth cultures were then incubated at 37°C and incubated for 24- 48 hours. After incubation the tubes were then examined for *Candida* growth by observing for turbidity using photo spectrometer. The minimum inhibitory concentration of the extracts was estimated for the *Candida* isolates in triplicates.

2.8 Statistical Analyses

Data obtained were analyzed by one way analysis of variance and means were compared by Duncan New Multiple Range Tests (SPSS 16.0 version) where necessary. Differences were considered significant at $p < 0.05$.

3. RESULTS AND DISCUSSION

A total of 67 *Candida* species namely *C. albicans* (49/67, 73.13%), *C. glabrata* (9/67, 13.43%), *C. krusei* (6/67, 8.96%) and *C. tropicalis* (3/67, 4.48%) were isolated from the HVS samples with *C. albicans* having the highest occurrence (Table 2). HVS samples in the study conducted by Menza et al. [11] showed similar *Candida* species with the exception of *C. parapsilosis*.

Table 1 shows the Extraction yield of *T. elegans*, among the extraction solvents; methanol was the most efficient with a yield of 3.4 mg/g, while n-hexane had the least extraction capacity with a yield of 0.7 mg/g. The result from Brahma et al. [12] findings showed that alcoholic solvent such

as methanol to be more efficient in extraction of phytochemicals.

Table 1. Extraction yield of *T. elegans*

| Solvent | Yield (mg/g) |
|----------|--------------|
| Methanol | 3.4 |
| Acetone | 1.8 |
| n-Hexane | 0.7 |

Table 2. Distribution of *Candida* species isolated from 10 selected hospitals in southwest Nigeria

| Isolate | Number of isolates (%) |
|---------------------------|------------------------|
| <i>Candida albicans</i> | 49 (73.13) |
| <i>Candida glabrata</i> | 9 (13.43) |
| <i>Candida krusei</i> | 6 (8.96) |
| <i>Candida tropicalis</i> | 3 (4.48) |
| Total | 67 (100) |

All the extracts contained saponin, tannin, steroid, terpenoid and cardiac glycosides, while flavonoid was only found in the acetone extracts (Table 3). Fig. 1 shows that the phytochemical with the highest value was saponin (31.77 mg/g) while flavonoid content of the acetone extract had the least value (1.65 mg/g).

Phytochemicals such as saponins, tannins, flavonoids and terpenoids present in the extracts have been documented to possess antifungal properties [7,11,13,14]. For instance, the antifungal activity of saponins has been attributed to their ability to interact with fungal membrane sterols [5]. Tannins are potent inhibitors of microbial extracellular enzymes [3]. They can interfere with microbial metabolism through the inhibition of oxidative

phosphorylation and are also capable of depriving microbes of substrate and metal ion uptake required for growth [15]. Also, terpenes, methyl chavicol and linalool isolated from *Ocimum sanctum* affected ergosterol synthesis and caused membrane damage in *Candida* species [16,17].

Zones of inhibition ranging from 5.00 to 30.00 mm, 4.00 to 15.67 mm and 4.33 to 17.67 mm were produced by the methanol, acetone and n-hexane extracts respectively (Tables 4 and 5). The methanol extract of *T. elegans* produced the highest anticandidal activity with an inhibitory zone of 30.00 mm.

The MIC values indicate that the *Trametes* species extracts inhibited the growth of *Candida* species from different hospitals at different concentrations (Table 6). The concentrations ranges from 25 mg/ml to 50mg/ml. Methanol extract of *Trametes elegans* has the lowest MIC (25 mg/ml) while n hexane extract of *Trametes elegans* has the highest MIC (50 mg/ml).

At the same concentration, commercial antifungals were more effective against the *Candida* species when compared to *Trametes* species extracts. This may be as a result of refined materials used in the production of antifungals. Oladunmoye [18] reported that antibiotics have high degree of purity; conventional antibiotics and other pharmaceutical products are usually prepared from synthetic materials by means of reproducible manufacturing techniques and procedures expressing purity and high fractionation which certainly will enhance antimicrobial effect than crude extracts.

Table 3. Qualitative phytochemical constituents in extracts of *Trametes elegans*

| Phytochemical | Extracts | | |
|---------------------------|-----------|---------|----------|
| | n- Hexane | Acetone | Methanol |
| Saponin | + | + | + |
| Tannin | + | + | + |
| Phlobatannin | - | - | - |
| Flavonoid | - | + | - |
| Steroid | + | + | + |
| Terpenoid | + | + | + |
| Alkaloid | - | - | - |
| Anthraquinone | - | - | - |
| Cardiac glycosides | | | |
| Legal test | + | + | + |
| Keller kiliani test | + | + | + |
| Salkowski test | + | + | + |
| Lieberman test | + | + | + |

Keys: +: Present, -: Absent

Table 4. Anticandidal activity of methanol and acetone extracts of *Trametes elegans* (100 mg/mL) on *Candida* isolates

| Hospitals | Zones of inhibition on <i>Candida</i> isolates (mm) | | | | | | | | |
|-----------|---|---------------|--------------|----------------|-------------|-------------|-------------|-------------|-------------|
| | A1 | A2 | A3 | A4 | A5 | B | C1 | C2 | D |
| | Methanol | | | Extract | | | | | |
| JH | 28.00±1.158c | 10.00±0.58a | 30.00±1.158b | 29.00±0.58b | NS | NS | NS | NS | NS |
| SSHA | 17.33±0.67d | 13.33±0.88b | 13.67±0.88a | 0.00±0.00a | 12.33±0.88b | 15.00±0.58c | 0.00±0.00a | 15.67±0.88a | 0.00±0.00a |
| MCA | 15.67±0.88d | 5.00±0.58b | 9.67±0.88c | 0.00±0.00a | 18.33±0.88e | 0.00±0.00a | 19.67±0.33e | NS | NS |
| SHON | 16.67±0.88d | 7.67±0.00b | 15.00±0.58c | 0.00±0.00a | 15.33±0.88c | NS | NS | NS | NS |
| MCON | 17.67±0.88e | 0.00±0.00a | 7.33±0.88b | 16.00±0.58e | 0.00±0.00a | 14.00±0.58d | 10.67±0.88c | NS | NS |
| SHOW | 17.67±0.67d | 14.67±0.67c | 5.00±0.88a | 5.67±0.88a | 10.00±0.58b | NS | NS | NS | NS |
| FMC | 17.00±0.58d | 5.33±0.00b | 7.33±2.85c | 14.00±1.15e | 0.00±0.00a | 0.00±0.00a | 14.67±0.33c | NS | NS |
| WGH | 14.00±0.58c | 6.00±0.58a | 0.00±0.00a | 13.33±0.88b | 10.00±0.58d | NS | NS | NS | NS |
| OAUTHC | 8.00±0.67b | 0.00±0.00a | 10.33±0.88c | 0.00±0.00a | 11.66±0.88d | 0.00±0.00a | 0.00±0.00a | 9.33±0.88b | 8.00±0.58a |
| UCH | 10.00±0.88c | 7.00±1.15.00a | 10.33±0.33c | 0.00±0.00a | 12.33±0.88d | 7.00±1.15a | 0.00±0.00a | 6.67±1.15b | 0.00±0.00a |
| | Acetone | | | Extract | | | | | |
| JH | 13.67±0.88d | 8.00±0.58a | 10.00±0.58b | 12.33±0.88c | NS | NS | NS | NS | NS |
| SSHA | 13.00±0.58b | 10.00±0.88c | 11.33±0.88d | 8.00±0.58b | 10.67±0.88b | 11.67±0.88d | 0.00±0.00a | 12.00±0.58d | 0.00±0.00a |
| MCA | 10.00±0.58c | 5.00±0.58b | 0.00±0.00a | 0.00±0.00a | 12.67±0.88d | 0.00±0.00a | 15.67±0.33e | NS | NS |
| SHON | 12.33±0.88d | 0.00±0.00a | 10.00±0.58c | 7.00±0.58b | 12.33±0.88d | NS | NS | NS | NS |
| MCON | 8.00±0.58c | 0.00±0.00a | 0.00±0.00a | 11.33±0.88e | 6.00±0.58b | 10.00±0.58e | 9.00±0.58d | NS | NS |
| SHOW | 13.00±0.58c | 14.67±0.67d | 0.00±0.00a | 5.33±0.33a | 9.67±0.58b | NS | NS | NS | NS |
| FMC | 7.00±0.58d | 4.00±0.00b | 0.00±0.00a | 15.00±0.58e | 5.33±0.88c | 0.00±0.00a | 10.33±0.88e | NS | NS |
| WGH | 10.00±0.58c | 0.00±0.00a | 5.67±0.33b | 10.00±1.15c | 12.67±0.88d | NS | NS | NS | NS |
| OAUTHC | 10.00±0.58c | 0.00±0.00a | 8.33±0.88b | 0.00±0.00a | 9.67±0.33c | 0.00±0.00a | 0.00±0.00a | 0.00±0.00a | 0.00±0.00a |
| UCH | 10.33±0.33c | 0.00±0.00a | 10.33±0.33c | 0.00±0.00a | 8.33±0.67b | 0.00±0.00a | 10.00±0.58c | 0.00±0.00a | 10.00±0.58c |

Values are presented as mean ± S.E (n=3). Values with the same superscripts letter(s) along the same row are not significantly different (P≤ 0.05).

Keys: JH: Jobateh hospital, Akure ; SSHA: State Specialist Hospital, Akure ; MCA: Mother and Child Hospital, Akure; SHOW: State hospital, Owo; FMC: Federal Medi-cal Centre, Owo; MCON: Mother and child Hospital, Ondo; SHON: State Hospital, Ondo; WGH: Wesley Guild Hospital, Ilesha; UCH: University College Hospital, Ibadan; OAUTHC: Obafemi Awolowo University Teaching Hospital Complex; NS: No isolate; A1 to A5: *Candida albicans*; B: *Candida krusei*; C1 & C2: *Candida glabrata*; D: *Candida tropicalis*

Table 5. Anticandidal activity of n-hexane extract of *Trametes elegans* (100 mg/mL) and griseofulvin (50 mg/mL) on *Candida* isolates

| Hospitals | Zones of inhibition on <i>Candida</i> isolates (mm) | | | | | | | | |
|-----------|---|-------------|-------------|-------------------|-------------|-------------|-------------|------------|-------------|
| | A1 | A2 | A3 | A4 | A5 | B | C1 | C2 | D |
| | n-Hexane | | | Extract | | | | | |
| JH | 17.67±0.88d | 0.00±0.00a | 10.67±0.33c | 8.00±0.58b | NS | NS | NS | NS | NS |
| SSHA | 11.67±0.33e | 11.67±0.88e | 9.00±0.58d | 0.00±0.00a | 5.67±0.88b | 10.00±0.88a | 8.67±0.00c | 0.00±0.00a | 0.00±0.00a |
| MCA | 8.00±0.58e | 0.00±0.00a | 5.33±0.88c | 6.00±0.58d | 4.33±0.88b | 0.00±0.00a | 14.33±0.33f | NS | NS |
| SHON | 10.00±0.58c | 0.00±0.00a | 7.00±0.58b | 0.00±0.00a | 10.67±0.88c | NS | NS | NS | NS |
| MCON | 10.67±0.58b | 0.00±0.00a | 0.00±0.00a | 7.00±0.58b | 0.00±0.00a | 12.00±0.58c | 0.00±0.00a | NS | NS |
| SHOW | 17.67±0.57d | 14.33±0.33c | 0.00±0.00a | 0.00±0.00a | 9.33±0.58b | NS | NS | NS | NS |
| FMC | 17.00±0.58d | 0.00±0.00a | 0.00±0.00a | 11.00±1.15e | 0.00±0.00a | 0.00±0.00a | 8.33±0.33b | NS | NS |
| WGH | 14.00±0.58d | 0.00±0.00a | 0.00±0.00a | 11.33±0.88b | 12.00±0.58c | NS | NS | NS | NS |
| OAUTHC | 8.00±0.67c | 7.33±0.88b | 9.67±0.88d | 0.00±0.00a | 7.33±0.88b | 0.00±0.00a | 0.00±0.00a | 7.33±0.88b | 0.00±0.00a |
| UCH | 10.00±0.58d | 0.00±0.00a | 6.00±0.33b | 0.00±0.00a | 8.00±0.88c | 0.00±0.00a | 6.00±0.58b | 0.00±0.00a | 0.00±0.00a |
| | Griseofulvin | | | (50 mg/mL) | | | | | |
| JH | 35.67±0.88d | 15.00±0.58a | 22.33±0.58b | 28.33±0.88c | NS | NS | NS | NS | NS |
| SSHA | 20.00±0.58f | 11.67±0.88d | 18.33±0.88a | 8.67±0.58c | 13.67±0.88e | 21.67±0.88f | 5.33±0.88b | 0.00±0.00a | 0.00±0.00a |
| MCA | 19.67±0.58d | 13.00±0.58b | 7.67±0.00a | 7.67±0.88a | 18.00±0.88d | 12.67±0.88b | 13.61±0.33c | NS | NS |
| SHON | 11.00±0.88b | 13.33±0.00c | 14.33±0.58d | 7.00±0.58a | 12.33±0.88c | NS | NS | NS | NS |
| MCON | 15.33±0.58e | 2.67±0.58a | 8.33±0.00c | 14.67±0.88e | 7.67±0.58a | 6.33±0.58b | 9.67±0.58d | NS | NS |
| SHOW | 11.33±0.58b | 14.67±0.67d | 5.33±0.00a | 15.33±0.33d | 12.67±0.58c | NS | NS | NS | NS |
| FMC | 22.33±0.58f | 7.33±0.88c | 7.00±0.00c | 17.00±0.58e | 5.67±0.88b | 0.00±0.00a | 14.33±0.88d | NS | NS |
| WGH | 12.67±0.58c | 7.67±0.58a | 7.67±0.33a | 10.33±1.15b | 13.00±0.88d | NS | NS | NS | NS |
| OAUTHC | 10.67±0.58c | 1.67±0.88b | 12.67±0.88d | 0.00±0.00a | 8.33±0.33c | 0.00±0.00a | 10.67±0.67a | 0.00±0.00a | 10.67±0.00a |
| UCH | 11.33±0.33e | 10.00±0.00e | 8.67±0.33c | 2.67±0.00b | 7.67±0.67c | 10.00±0.48e | 10.00±0.58e | 0.00±0.00a | 0.00±0.00a |

Values are presented as mean ± S.E (n=3). Values with the same superscripts letter(s) along the same row are not significantly different (P≤ 0.05).

Keys: JH: Jobateh hospital, Akure ; SSHA: State Specialist Hospital, Akure ; MCA: Mother and Child Hospital, Akure; SHOW: State hospital, Owo; FMC: Federal Medi-cal Centre, Owo; MCON: Mother and child Hospital, Ondo; SHON: State Hospital, Ondo; WGH: Wesley Guild Hospital, Ilesha; UCH: University College Hospital, Ibadan; OAUTHC: Obafemi Awolowo University Teaching Hospital Complex; NS: No isolate; A1 to A5: *Candida albicans*; B: *Candida krusei*; C1 & C2: *Candida glabrata*; D: *Candida tropicalis*

Table 6. The mean MIC of extracts of *Trametes elegans* (mg/mL) on *Candida isolate*

| Hospitals | MIC values of extracts (mg/mL) on <i>Candida isolates</i> | | | | | | | | | | | | | | | | | | | | | | | | | | |
|-----------|---|----|----|----|----|----|----|----|----|--------------------------------------|----|----|----|----|----|----|----|----|---------------------------------------|----|----|----|----|----|----|----|----|
| | Methanol extract of <i>T. elegans</i> | | | | | | | | | Acetone extract of <i>T. elegans</i> | | | | | | | | | n-Hexane extract of <i>T. elegans</i> | | | | | | | | |
| | A1 | A2 | A3 | A4 | A5 | B | C1 | C2 | D | A1 | A2 | A3 | A4 | A5 | B | C1 | C2 | D | A1 | A2 | A3 | A4 | A5 | B | C1 | C2 | D |
| JH | 25 | 50 | NI | 25 | 50 | NS | NS | NS | NS | 50 | 50 | NS | 50 | NS | NS | NS | NS | NS | 50 | NI | NI | 50 | NS | NS | NS | NS | NS |
| SSHA | 50 | NI | 50 | NI | 50 | 25 | NI | NI | NI | 50 | NI | 50 | NI | 50 | 50 | NI | NI | NI | 50 | NI | 50 | NI | NI | NI | NI | 50 | NI |
| MCA | 50 | NI | 50 | NI | NI | NI | 25 | NS | NS | 50 | NI | NI | NI | 50 | NI | 50 | NS | NS | NI | NI | NI | NI | NI | NI | 50 | NS | NS |
| SHON | 25 | 50 | NI | 25 | NI | NS | NS | NS | NS | 50 | NI | NI | 50 | 25 | NS | NS | NS | NS | 50 | NI | 50 | NI | NI | NS | NS | NS | NS |
| MCON | 50 | NI | 50 | 25 | NI | 25 | NI | NS | NS | 50 | NI | NI | 50 | NI | 50 | NI | NS | NS | 50 | NI | NI | NI | NI | 50 | NI | NS | NS |
| SHOW | 25 | 25 | 50 | NI | NI | NS | NS | NS | NS | 50 | 50 | NI | 50 | NI | NS | NS | NS | NS | 50 | 50 | NI | NI | NI | NS | NS | NS | NS |
| FMC | 50 | NI | NI | 50 | NI | NI | 25 | NS | NS | NI | NI | NI | 50 | NI | NI | 50 | NS | NS | 50 | NI | NI | 50 | NI | NI | NI | NS | NS |
| WGH | 50 | 50 | NI | 25 | 25 | NS | NS | NS | NS | 50 | NI | NI | 50 | 50 | NS | NS | NS | NS | NI | NI | NI | 50 | 50 | NS | NS | NS | NS |
| OAUTHC | NI | NI | NI | 50 | 50 | NI | NI | 50 | 50 | NI | NI | NI | 50 | 50 | NI | 50 | NI | NI | NI | 50 | NI | NI | 50 | NI | NI | NI | NI |
| UCH | 50 | 50 | NI | NI | 25 | 50 | NI | NI | NI | 50 | NI | 50 | NI | NI | NI | 50 | NI | 50 | 50 | NI | 50 | NI | 50 | NI | NI | NI | NI |

Keys: JH: Jobateh hospital, Akure ; SSHA: State Specialist Hospital, Akure ; MCA: Mother and Child Hospital, Akure; SHOW: State hospital, Owo; FMC: Federal Medi-cal Centre, Owo; MCON: Mother and child Hospital, Ondo; SHON: State Hospital, Ondo; WGH: Wesley Guild Hospital, Ilesha; UCH: University College Hospital, Ibadan; OAUTHC: Obafemi Awolowo University Teaching Hospital Complex; NS: No isolate; NI: No inhibition; A1 to A5: *Candida albicans*; B: *Candida krusei*; C1 & C2: *Candida glabrata*; D: *Candida tropicalis*

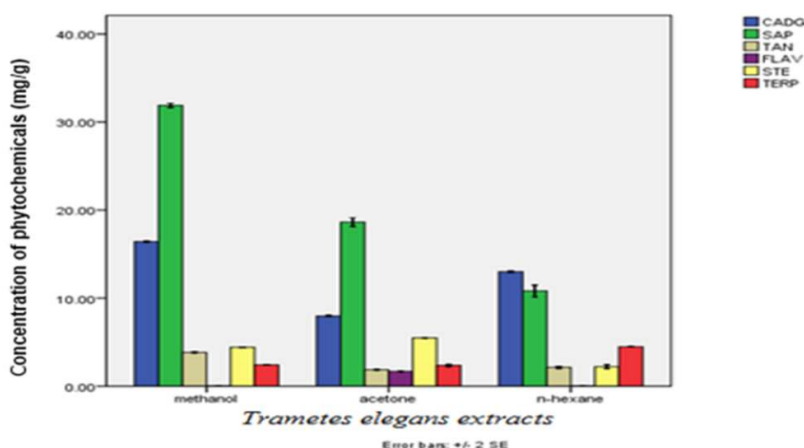


Fig. 1. Quantitative phytochemical constituents of *T. elegans* extracts

Keys: CADG- Cardiac glycosides, FLAV- Flavonoid, SAP- Saponin, TERP- Terpenoids

4. CONCLUSION

The study confirms the inhibitory effect of *Trametes elegans* extracts against isolated *Candida* species.

Our findings have revealed that, extracts of *T. elegans* can serve as potential source of new classes of bioactive compounds effective in combating candidiasis which poses a great threat to women. Since mushrooms are available and readily accessible, the use of *T. elegans* will serve as a cost effective alternative in the treatment of candidiasis.

Further studies should be carried out on the purification, isolation and characterization of the bioactive compounds present in the macrofungus extracts. Also further pharmacological studies that will make the mushroom an excellent future pharmaceutical candidate should be carried out.

ETHICAL APPROVAL

All authors hereby declare that all experiment have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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