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Comparison of anti-aspergillar activity of extracts of *Tectona grandis* Linn according to two antifungal susceptibility testing

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ABSTRACT

Plants used as antimicrobials in the treatment of infectious diseases in folk medicine are regularly evaluated in different Laboratories. And, the processes of evaluation of antifungal susceptibility are often different to one laboratory to another. This study was undertaken in the goal to compare parameters obtained (MIC and MFC) with two antifungal susceptibility testing. The first one is Agar slant double dilution tubes method (M₁) and the second is disc diffusion method /broth dilution according document CLSI M38-A2 supplemented to 2% glucose (M₂). Ethanol (EtOH 70%), Methanol (MeOH) and n-Hexane (n-H_{exTG}) extracts of leaves of *Tectona grandis* have been tested in comparison to Itraconazole against two clinical strains of *Aspergillus fumigatus* 896/AB and *Aspergillus flavus* 1006/OM isolated to HIV patients. Results showed that on *A. flavus*, agar slant method (M₁) gave MIC ranging between 200 µg/mL and 800 µg/mL while disc diffusion meth/broth dilution (M₂) showed growth inhibition between 100 µg/mL and 400 µg/mL. On *A. fumigatus*, inhibition was observed between 200µg/mL and 400 µg/mL with M₂ in contrary M₁ gave MIC located between 400 µg/mL and 800 µg/mL. For each strain tested, according method M₂, MIC and MFC are identical and with method M₁, these parameters were different. Evaluation of Itraconazole on each strain gave with M₂, MIC= 50 µg/mL while inhibition has been showed at 100 µg/mL and 200µg/mL with M₁. Also considering extracts, hydroalcoholic extract of *T. grandis* (EtOH_{TG}) showed antifungal susceptibility testing less 2 to 4 efficiency than Itraconazole. Disc diffusion method/broth dilution (M₂) according CLSI M38-A₂ to evaluate *Tectona grandis* antifungal susceptibility is the process of evaluation which allowed having lowest antifungal parameters (MIC and MFC) on *A. flavus* and *A. fumigatus*.

Keywords: Antifungal susceptibility testing, Extract, Slant method.

INTRODUCTION

Fungal participation in the aetiology of infections has increased considerably [1]. However, as medical technology has improved, the survival of patients with severe and life-threatening illnesses has led to a rapid increase in the immunosuppressed population. These changes are correlated with a substantial increase in the rate of invasive fungal infections (IFIs) like Aspergillosis. Moreover, not only drug-resistant strains are emerging and the number of infections by intrinsically drug-resistant species are developed, which limit their utilization as medicines [2,3]. In the goal to find solutions to these multiple drug-resistant, antifungal activities of medicinal plants were explored. From time immemorial, many plants have been used by man as a source of treatment of various disease conditions, particularly at the community level. These plants are referred to as alternative or complementary medicines [4,5]. The World Health Organization (WHO) estimated that 80% of the world population relied on medicinal plants for their health care needs. Using medicinal plants represent an alternative medicine that is economical, accessible, and applicable to various pathologies, particularly in developing countries [6]. Studies have intensively investigated plant species with medicinal properties to assess the feasibility, sustainability and affordability of the use of natural drugs [7,8]. Plants produce a variety of medicinal components, sources of antimicrobial drugs that can inhibit the growth of pathogens [9,10]. Compounds, essential oils and extracts, have revealed their inhibitory activity on the in vitro growth of some nosocomial infection caused by fungi [11].

In ethnomedicine, *Tectona grandis* is a renowned plant for its antifungal properties. Indeed, *T. grandis* Linn. is commonly known as teak tree belongs to family verbenaceae and is one of the most important heart wood of the world over. *T. grandis* leaves extract are widely used in the folklore for the treatment of various kinds of wound, especially burn wound [12;13;14]. In addition, a study conducted by Shalini, 2007 [15] on a crude methanol extract of *Tectona grandis* showed at 1000 µg/mL inhibition on the in vitro growth of *Alternaria cajani*, *Curvularia lunata*, *Fusarium Sp* and *helminthosporium sp*. There are more than two decades that number of studies have been conducted in Laboratory to evaluate antimicrobial

activity of extracts and essential oils of medicinal plants [16,17]. Unfortunately, methods to determine whether a natural product is an antifungal were different from one laboratory to another laboratory. Among these methods, there may be mentioned, slant double dilution method, microdilution or macrodilution or broth or agar diffusion disc, according to Clinical and Laboratory Standards Institute (CLSI) document M27-A3, M38-A and M2-A8 [18,19,20].

Thus, this study sought to compare results obtained with two methods of antifungal susceptibility testing with *Tectona grandis* extract against pathogenic human fungi (*Aspergillus flavus* and *Aspergillus fumigatus*). Agar Slant double dilution method described by Kra and al., 2002 [21]; and the second method is that indicated by CLSI in document M38-A2 [22,23].

MATERIALS AND METHODS

Collection, identification and preservation of the plant sample

Leaves of plant material of *T. grandis* were collected at Agboville town, Region of Agn by, C te d'Ivoire in December 2013, and authenticated by Professor Ake Assi Laurent, National Floristic Center (NFC), Felix Houphou t Boigny University-Abidjan (C te d'Ivoire). The leaves were removed; air dried, powdered, labeled and stored in the air tight container before extraction.

Strains tested

Strains of *Aspergillus flavus* 1006/OM and *Aspergillus fumigatus* 896/AB were provided by the department of Mycology of the Faculty of Medical Sciences Felix Houphou t Boigny University. These strains were isolated from patients with HIV Service of Infectious Diseases, CHU Treichville in C te d'Ivoire.

Extraction

Powdered leaves (500 g) mixed in a quantity of each solvent (1L), were continuously extracted separately with Ethanol 70%, Methanol and n-Hexane by maceration for 48 hours. This operation was repeated three times for each extraction. The extract was filtered and the filtrate dried under vacuum with a rotary evaporator at 45 C to obtain a brownish product of *Tectona grandis* leaves extract. The different extract was coded following:

Ethanol 70% (Hydroalcohol) extract of *Tectona grandis*: EtOH_{TG}

Methanol extract of *Tectona grandis*: MeOH_{TG}

n-Hexane extract of *Tectona grandis*: n-He_{TG}

They were stored in a refrigerator pending further investigations.

Antimicrobial susceptibility testing and determination of minimum inhibitory concentration (MIC)

Agar slant double dilution method (M₁)

Antifungal susceptibility testing were carried out on culture medium Sabouraud prepared according manufacturer's instructions. Hydroalcoholic (EtOH 70%), Methanol (MeOH) and Hexane (n-Hexane) extracts were incorporated separately to the medium following method of double dilution tubes on sloping Agar. For each strain per test, eleven (11) tubes were used including nine (09) test tubes containing plant's extract and two (02) control tubes, one without plant's extract as growth control of fungi and the other without fungi and plant's extract as a control of sterility of culture medium. Extracts concentrations range in the tubes go from 3.125  g/mL to 800  g/mL with geometrical connection in order of 1/2. All eleven (11) tubes were autoclaved at 121 C, 1 bar for 15 minutes and then the tubes were inclined to room temperature of the laboratory to allow their cooling and solidification of the agar. Inoculum was

obtained by sampling with a loop diameter of 2 mm with a sterile single colony which was homogenized in 10 mL of distilled water giving a suspension containing 10⁶ cells/mL. Then, 1 mL of this suspension was transferred to 9 mL of sterile distilled water giving a suspension 10⁻¹. Fungi culture on agar slant previously prepared was made by inoculation of 1000 cells of the strain of *A. fumigatus* and *A. flavus* corresponding 10  L standardized inoculum 10⁻¹ suspension containing 10⁵ cells/mL. For each test, the load of inoculum was verified by a series of secondary dilutions. The cultures thus produced were incubated at 30 C for 48 hours. After this incubation time, MIC (Minimum Inhibitory Concentration) was determined by the lowest concentration of extract that inhibits visual growth of fungi in an agar dilution susceptibility test. In addition, new sterile Sabouraud agar was used to reseed a sampling tube showing MIC values in the goal to determine MFC (Minimum Fungicidal Concentration). The content of the MIC in the serial dilution were then cultured on to the prepared media, they were incubated at 30 C for 48 hours. MFC was observed for colonial growth on the new sterile Sabouraud agar, MFC is the tube with lowest concentrations of the extract in serial dilution with the growth of one colony. There were performed five replicates for each extract concentration and control against the fungi.

Antifungal susceptibility testing CLSI Method (M₂)

Antifungal activity of extracts of *T. grandis* was evaluated according to the method described CLSI in two steps:

- Disc diffusion method to determine susceptibility

- Broth dilution method to determine the Minimum Inhibitory Concentrations (MIC)

Disc diffusion method-according document CLSI M38-A2 supplemented to 2% glucose (M₂)

Antifungal activities of *T. grandis* crude hydroalcoholic, methanol and n-hexane extracts was determined using each extract (8 mg) was measured and dissolved in 10 mL of DMSO to obtain the concentrations of 800  g/mL. It was initial concentrations used to check anti-aspergillar activities. The growth medium used for the fungi Mueller-Hinton Agar. The medium was prepared according to the manufacturer's instructions and sterilized at 121 C for 15 minutes. The sterilized medium was then poured into sterile Petri dishes, and the plates were covered, allowed to cool and solidify. Extracts were separately screened using the plate diffusion method CLSI (2009) [24]. The sterilized medium was seeded with 0.1 mL (100  L) of standard inocula of test moulds. Inoculum equivalent to 0.5 McFarland is prepared from a suspension obtained from cultures seven days Agar Potato Dextrose at 30 C. The DO was 0.08 to 0.10 at 625 nm, which corresponds to an equivalent of 0.4-5 inoculum 10⁶ conidia/mL. The inoculum was spread evenly over surface of the medium by the use of sterile swab. 6 mm discs prepared extemporaneously are sterilized by autoclaving, and then impregnated with 20  L of DMSO. Plates are incubated at 30 C for 45 hours. Fungal activity was determined by measuring the diameter of the inhibition zones around the disks. The absence of fungi growth results in a translucent halo around the disc, the diameter of which is measured with a transparent ruler and the result recorded in millimeters [25]. There were five replicates for both extract concentration and control against fungi. Evaluation of the MIC was evaluated by broth dilution method using Roswell Park Memorial Institute medium (RPMI) [26].

Broth dilution Method (Determination of Minimum Inhibitory Concentration)

The 96 well microplate allow to determine the Minimum Inhibitory Concentrations (MIC) of plant extracts. For each line of microplate, in the 12 wells, we introduced 100  L RPMI 1640 except well N 1, where we introduced only 200  L of tested extract at 800  g/mL. Wells N 11 and N 12, served respectively to control of fungi growth

and control of sterility of culture medium. Then we take away 100 μL of the plant extract in first well to the second well, and 100 μL to the second well to the 3rd well, 100 μL in the 3rd well to the 4th well using a micropipette. Thus, Extracts concentrations range in the wells goes from 3.125 $\mu\text{g}/\text{mL}$ to 800 $\mu\text{g}/\text{mL}$ with geometrical connection in order of 1/2. And then, we added up 100 μL of 1.5×10^6 CFU of the inoculum (DO ranging between 0.12 to 0.15 at 530 nm) in 96 wells except those as served to control of medium sterility. Reading is performed by eye knowing that MIC is the lowest concentration of the test substance for which no visual disturbance was observed in the well. We used as reference antifungal Itraconazole [27].

Determination of Minimum Fungicidal Concentration (MFC)

MFC was carried out to check whether the test fungi were killed or their growth inhibited according method described by Canton and *al.*, 2003[28]. Mueller-Hinton Agar was prepared according to the manufacturer's instructions, sterilized at 121°C for 15 min, poured into sterilized Petri dishes, the plate were allowed to cool and solidify. For each test, the two wells containing plant's extract with concentrations strictly superior or equal to CMI determined, will serve to determine MFC. For these, 20 μL of content for each well was transferred to Petri dishes containing extemporary prepared media. They were incubated at 30°C for 48 hours. After that, the plates were observed for colonial growth, the MFC is the plates with lowest concentrations of the extract in serial dilution with only one colony growth, thus determined. There were performed five replicates for both extract concentration and control against the fungi.

Phytochemical screening

Phytochemical analysis of hydroalcoholic, methanol and n-hexane leaves extract of *T. grandis* was performed using different chemical tests to check the presence of active metabolites such as sterols/terpens, alkaloids, flavonoid, saponins, anthracens derivatives, glycosids, reducing compounds, anthocyanins and tannins in the plant by standard methods [29;30;31].

Test for sterols/terpens

Liebermann-Buchard test: Anhydrous acetic acid (1 mL) was added to 1 mL of chloroform and cooled to 0°C then a drop of concentrated sulphuric acid was added to the cooled mixture followed by the leaves extract. The resultant mixture was observed for blue, green, red or orange color that changes with time.

Salkowski test: A little quantity of the leaves extract was dissolved in 1 mL of chloroform, thereafter; 1 mL of concentrated sulfuric acid was added down the test tube to form two phases. Formation of red or yellow coloration was taken as an indication for the presence of sterols.

Test for flavonoids

Shinoda test: To each extract solution of the leaves extract, 3 pieces of magnesium chips were added followed by a few drops of concentrated hydrochloric acid. Appearance of an orange, pink or red to purple color indicates the presence of flavonoids.

Sulphuric acid test: leaves extract (0.5 g) was dissolved in concentrated sulfuric acid and notable color change was observed (if any).

Ferric chloride test: Stem extract (0.5 g) was boiled in distilled water and filtered to 2 mL of the filtrate, two drops of freshly prepared ferric chloride solution was added; green, blue or violet coloration indicated the presence of phenolic hydroxyl group.

Sodium hydroxide test: each extract (0.5 g) was dissolve in 2 mL of 10% aqueous sodium hydroxide solution and filtered to give yellow color, a change in color from yellow to colorless on addition of dilute hydrochloric acid indicated the presence of flavonoids.

Test for alkaloids

Extract (0.5 g) was stirred with 5 mL of 1% aqueous hydrochloric acid on a water bath and filtered. 3 mL of the filtrate was divided into three. To the first 1 mL of freshly prepared Dragendoff's reagent was added and observed for formation of orange to brownish precipitate. To the second, 1 mL of Mayer's reagent was added and observed for formation of white to yellowish or cream colored precipitate. To the third 1 mL of Wagner's reagent was added to give a brown or reddish or reddish brown precipitate.

Test for tannins

Ferric chloride test: A small quantity of the extract was boiled in water and filtered. Two drops of ferric chloride was added to the filtrate, the formation of a blue-black, or green precipitate was considered for the presence of tannins.

Test for saponins

Frothing test: The leaves extract (0.5 g) was shaken with water in a test tube. Frothing which persisted for 15 min indicates the presence of saponins.

Tests for anthracens derivatives

Microscopic observation: Microscopic observation of *T. grandis* powders supplemented with 10% NaOH. The presence of red coloration indicates presence of anthracene derivatives.

Test for coumarins

Ammonia solution test: 5 ml of extract of three extracts are evaporated to dryness. The residue thus obtained is taken up in hot water. A volume of this aqueous phase is added with an ammonia solution (NH₄OH) 10% and another volume is kept as control. The appearance of fluorescence in observation under UV indicates the presence of coumarins.

Test for reducing compounds

Fehling test for reducing compounds: Their detection involves treating 1 ml of the extract with 2 mL of distilled water and 2 mL of Fehling's solution (1 mL of Fehling's solution A + 1 mL of Fehling's solution B) and then the tubes are heated in a water bath at 40°C. Positive a test is revealed by the formation of a brick-red precipitate.

Test for anthocyanins

Acid sulphuric test: A 1 mL portion of the infused; 5 mL of sulfuric acid (H₂SO₄) at 10% is added, then ammonium hydroxide (NH₄OH) to 25 %. If the color is not acidification increases, then turns blue violet in basic medium, it can be concluded the presence of anthocyanins.

Statistical analysis

The zones of inhibition demonstrated from this experiment were presented as mean \pm SD (standard deviation) of the five replicates for both extract concentration and control against the fungi. The comparisons between the control group and the test groups (i.e. antimicrobial activities of the leaves extracts against test fungi) were performed by one-way analysis of variance (ANOVA)

with non-parametric post-hoc Dunnett's test using GraphPad Prism (version 6.0). P-values < 0.05 were considered statistically significant.

RESULTS

Table 1: Results of percentages yields for maceration of crude extract from *T. grandis*

Extracts	Quantity of powdered leaves (g) in maceration	Quantity of extract after three times cycle (g)	Percentages (%)
EtOH _{TG}	500	75.9	15.18
MeOH _{TG}	500	46.25	9.25
n-Hex _{TG}	500	13.75	2.75

Antimicrobials assay

Results of antimicrobials activity have been reported in Fig 1, 2, 3 and 4. Among the 3 tested extracts, only extract EToH of *T. grandis* showed better antifungal activity with values of parameters antifungal lowest MIC and MFC ranging 100 µg / mL to 200 µL/mg on *A. flavus* and 200 µg/mL to 400 µg/mL on *A. fumigatus*. For other extracts (MeOH and n-HEX), the values of MIC/MFC are between 200 µg / mL and 800 µg/mL. All Extracts gave a clear and effective

Percentage of the plant extract

Percentages yield of plant extract after extraction by Ethanol 70%, Methanol and n-Hexane using maceration were calculated to respectively 15.18%; 9.25% and 2.75% (Table 1). They were extracted from 500 g of the powdered leaves.

inhibition on the growth of two strains. Zones of inhibition (ZI) for the crude extract measured in millimeter were found to be 20 – 29 mm on *A. flavus* and 20-26 mm on *A. fumigatus* (Table 2). More so, it was found that considering extracts, the zones of inhibition were highest with EToH_{TG}; maximal ZI was 29 mm for *A. flavus* and the lowest diameter were obtained with n-Hex_{TG}, minimal ZI was 20 mm on both *A. fumigatus* and *A. flavus*. The interesting parameters (MIC/MFC/ZI) have been obtained by CLSI method.

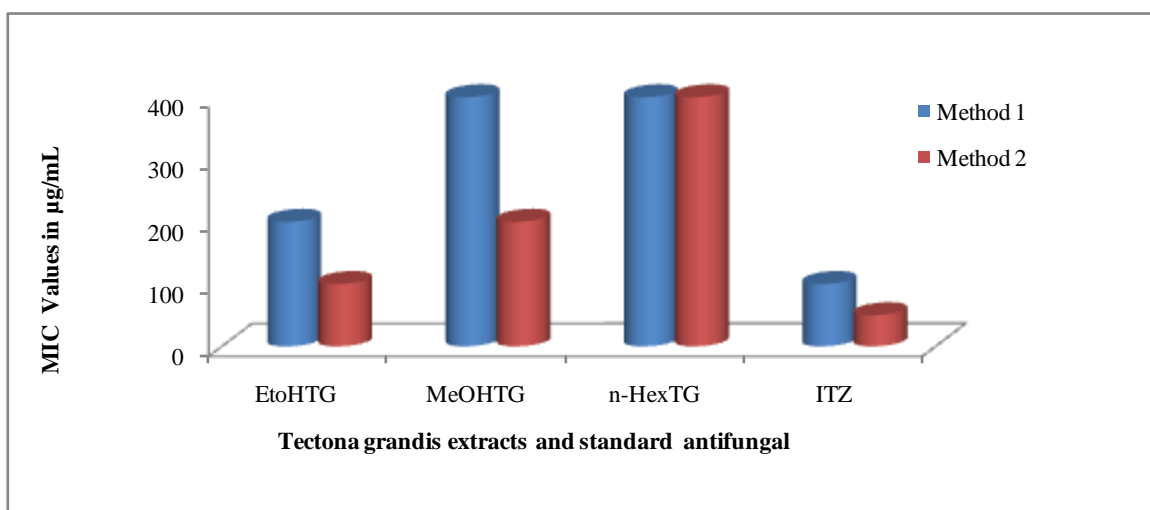


Figure 1: Histogram MIC values compared on *A. Flavis* 1006/OM

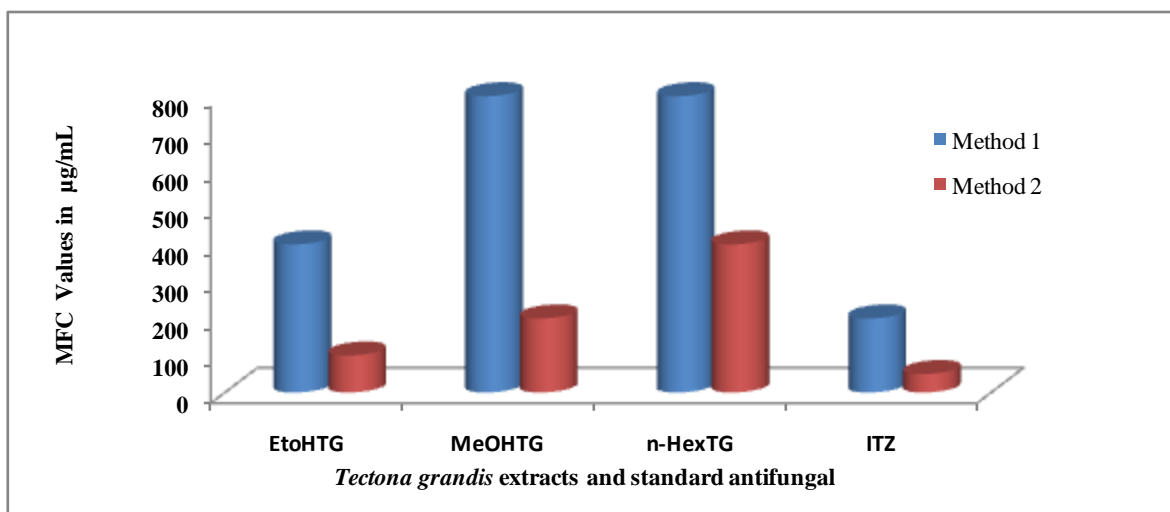


Figure 2: Histogram MFC values compared on *A. Flavis* 1006/OM

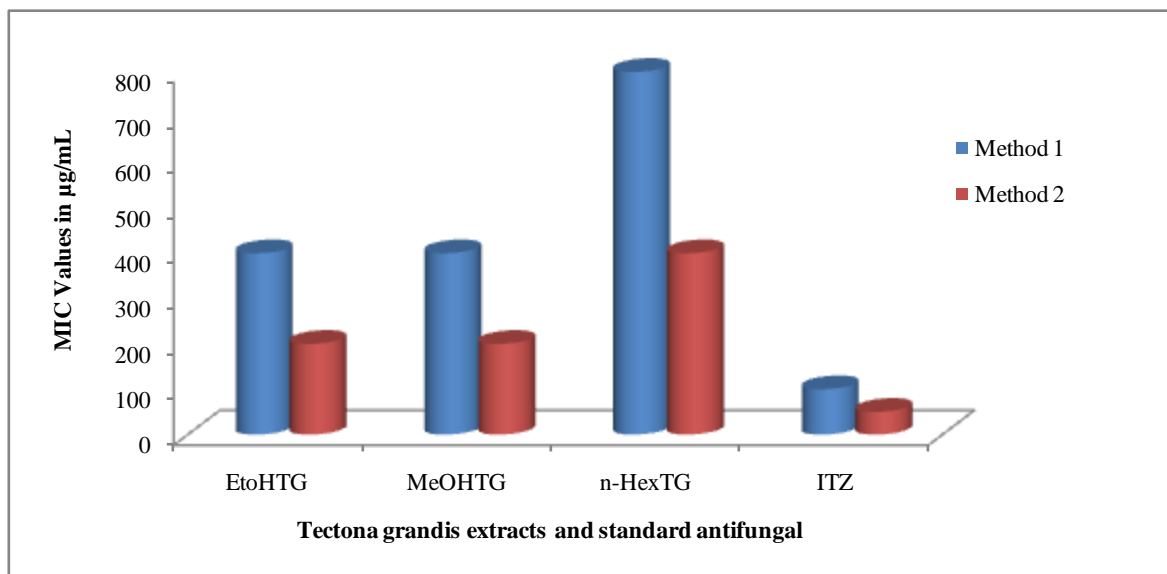


Figure 3: Histogram MIC values compared on *A. fumigatus* 896/AB

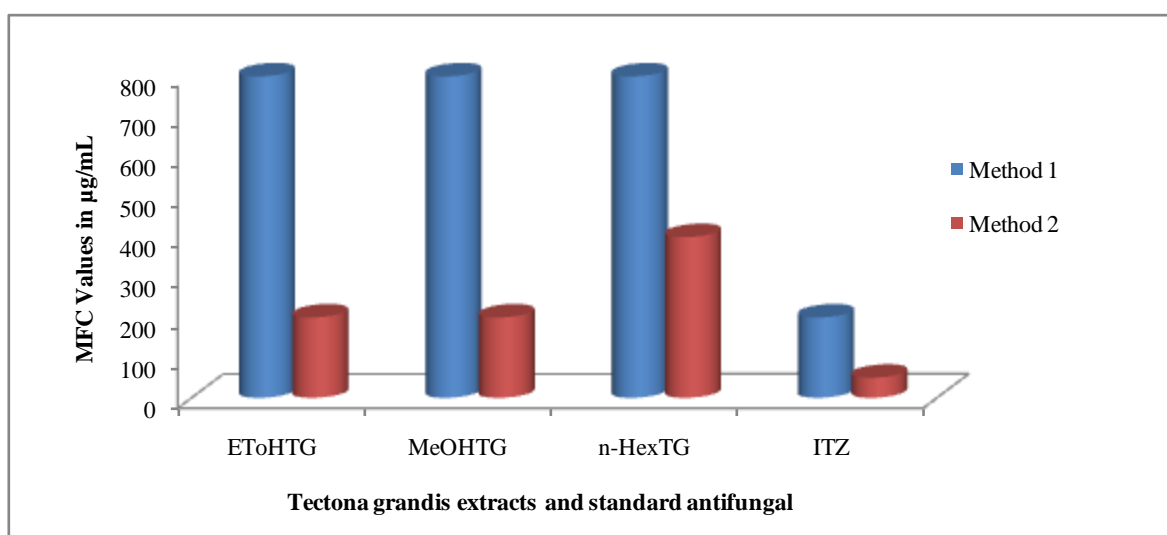


Figure 4: Histogram MFC values compared on *A. fumigatus* 896/AB

Table 2: Zones of inhibition leaves extracts against *A. flavus* and *A. fumigatus*

Fungi	Zones of inhibition of leaves extracts and Itraconazole against moulds (mm)				
	EtOH _{TG}	MeOH _{TG}	N-Hex _{TG}	ITZ	DMSO
<i>A. flavus</i>	29 ± 0.1	23 ± 0.1	20 ± 0.2	37 ± 0.1	5 ± 0.1
<i>A. fumigatus</i>	26 ± 0.3	23 ± 0.1	20 ± 0.2	35 ± 0.1	5 ± 0.1

Diameter (D): D < 9 mm (Resistant); 9 mm ≤ D < 20 mm (Sensible); D ≥ 20 mm (Très sensible)

Phytochemical screening

The results of preliminary phytochemical tests conducted on hydroalcoholic, methanol and n-hexane leaves extract of *T. grandis* are as shown in Table 3. Nine (9) groups of compounds were

screened. On the 9 groups, Hydroalcoholic extract (EtOH_{TG}) contained 8 groups without anthracenes derivatives, Methanol extract (MeOH_{TG}) included 6 groups without saponins and anthracenes derivatives, n-Hexane extract (n-Hex_{TG}) contained 3 groups (Flavonoids, Sterols/Terpenes and Tannins).

Table 3: Preliminary phytochemical screening from leaves extract from *T. grandis*

Tests	EtOH _{TG}	MeOH _{TG}	n- Hex _{TG}
Flavonoids			
Shinoda test	+	+	+
Ferric Chloride test	+	+	+
Sulphuric acid test	+	+	+
Sodium hydroxide test	+	+	+
Steroles/terpenes			
Liebermann-Buchard test	+	+	+
Salkowski's test	+	+	+
Saponins			
Frothing test	+	-	-
Tannins			
Ferric chloride tests	+	+	+
Alkaloids			
Mayer's reagent test	+	+	-
Dragendoff's reagent	+	+	-
Wagner's reagent	+	+	-
Coumarins			
Ammonia solution Test	+	+	-
Anthracenes derivatives			
Microscopic observation	-	-	-
Reducing compounds			
Fehling test	+	+	-

+: Present, -: Not detected

DISCUSSION

Percentages of yield of plant extracts grown according to the solvent. And yield of Hydroalcoholic extract (EtOH_{TG}) was highest because this solvent extracted both some polar, semi polar and non polar compounds and the removal in larger quantities of more hydrophilic molecules such as sugars polymers, proteins and tannins larger molecular weights. Using different solvent according their polarity allowed to separate compounds considering their solubility. This method of extraction to ambient temperature and in agitation allowed to extract maximum of bioactives compounds and prevent the inactivation of phenolics compounds Percentages of yield should not be considered as a criterion of efficiency but will estimate future returns to scale in production and to select the solvent depending on the antifungal activity [32].

According to phytochemical screening, the group of compounds identified possessed antimicrobial activity [33]. The mechanisms of actions of these compounds have been proven to be through cell membranes perturbations [34]. Flavonoids and tannins have been reported to possess antimicrobial activity, the antimicrobial activity of flavonoids was due to their ability to complex with extracellular, soluble protein and to complex with fungi cell wall while that of tannins may be related to their ability to inactivate microbial adhesions, enzymes and cell envelope proteins. The presence of alkaloids, Tannins and Flavonoids in plants extracts has also been shown to enhance antimicrobial properties as reported by Singh and Bhat (2003) [35]. By using hydroalcoholic extract which contained 8 tested groups of compounds, antimicrobial activity was better with each of antisusceptibility testing method. Therefore, it can be inferred that this extract would be able to serve as basis in the goal to improve activity of *Tectona grandis* leaves. However, CLSI method (M₂) brought best antifungals parameters on each fungi and with each

extract. The results obtained by CLSI method were better because in this method the extract didn't undergo effect of temperature. In contrary, in the slant method, extract incorporated to the medium were sterilized at 121°C during 15 mn before sowing fungi. Thus, by the slant method, temperature contributed to deteriorate some compounds so effect observed on the tubes by this method was due to residual activity of some compounds in the extracts. The n-hexane extract (n-Hex_{TG}) was the least efficiency extract. These was due to the presence of only three group of compounds on 8 groups tested. Based on findings from phytochemical screening, it can be deduced that the hydroalcohol extract contained the highest number of compounds. Findings from the antimicrobial screening showed that all extracts (crude hydroalcohol extract, Methanol extract, and n-hexane extract) had antimicrobial activities against *A. fumigatus* and *A. flavus*. These findings were partly in consonance with those of Ouattara and al. (2013) [36] who investigated hydroalcoholic extract on *Aspergillus fumigatus*, although some differences in antimicrobial activities existed, the variation might be attributable to differences not only in the polarity of solvent used but also the antifungal susceptibility testing. Zones of inhibition (ZI) were highest with Ethanol extracts. Therefore, this is an indication that Ethanol extract contained highest concentrations of the active compounds in the leaves of *T. grandis* responsible for the antifungal actions. The low MIC (100 µg/mL) with EtOH_{TG} as reflected by our results suggest that our plant extract has good antifungal activities against the susceptible fungi considering that compounds with MICs of less than 10 mg/mL are regarded as having strong antimicrobial potential [37,38]. The MFC showed that ethanol extract killed susceptible fungi at lowest concentration 100 µg/mL to 200 µg/mL with CLSI method, and highest concentrations at 400 µg/mL to 800 µg/mL with slant double dilution method. According method used for tests, other extracts had MFC 200 µg/mL to 800 µg/mL for MeOH_{TG} and 400 µg/mL to 800 µg/mL for N-Hex_{TG}. These extracts had moderate antimicrobial activities Considering antifungal test used, the lowest MFC were obtained with CLSI. With this Method, MIC=MFC so extracts were fungicidal but with slant double dilution, MIC≠MFC and it indicates that extracts were fungistatic. All tested organic extract's of leaves of *T. grandis* were found to contain some potent phytochemical. These compounds were responsible for its antifungal activities. In comparison to parameters obtained with Itraconazole (ITZ), all the extracts (EtOH_{TG}, MeOH_{TG}, n-Hex_{TG}) had MIC and MFC superior to this reference antifungal.

According to disc diffusion method (CLSI), extracts of *T. grandis* were more active on *A. flavus* than *A. fumigatus* because diameters evaluated were highest. Also, it was noticed that EtOH_{TG} had the best activity on *Aspergillus* tested. This result was in concordance with MIC/MFC determined by broth dilution (CLSI). By this last method, antifungals parameters were also best with EtOH_{TG}.

In fact, results obtained with hydroalcoholic extract of *T. grandis* (100 µg/mL to 200 µg/mL) according to CLSI method were close to results with Itraconazole a reference antifungal (50 µg/mL). This result suggest that an action in the goal to improve activity of EtOH_{TG} would allow to get more lower antifungal parameters than Itraconazole

CONCLUSION

Hydroalcoholic, Methanol and n-Hexane extracts of leaves of *Tectona grandis* proved to be very effective against both *Aspergillus flavus* and *Aspergillus fumigatus* by CLSI method. Antifungals parameters obtained by this method were between 100 µg/mL and 200 µg/mL for the two first extracts and to 400 µg/mL for n-hexane extract. The most active extract was Hydroalcoholic extract. This extract contained 8 groups of compounds. Thus, this study supports also ethnomedicinal use of leaves extracts of *T. grandis* in treating infections caused by these susceptible fungi. By this lowest parameters obtained with CLSI method, it's recommended to use this method in the goal to evaluate antifungal testing susceptibility of natural substances (plant's extract).

In perspectives to know nature of active compounds contained in *T. grandis*, there is a need to improve the activity of hydroalcoholic extract of *T. grandis*, to standardize and conduct more detailed phytochemical analyzes on this plant.

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