



Phytochemical Analysis and Antifungal Activity of Aqueous Leaf Extract of *Trema guineensis* (Ulmaceae), A Plant from the Ivoirian Pharmacopoeia

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Authors' contributions

This work was carried out in collaboration among all authors. Author LO wrote the protocol and wrote the draft of the manuscript. Author MEREN carried out the experiments (plant selection, phytochemical analysis and the antifungal tests). Author IB participated in the design of the study and performed the statistical analysis. Author AES carried out literature searches and revised the manuscript. Author KO conceived of the study and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Trema guineensis is a woody plant distributed in tropical forest of which leaf and bark extracts are traditionally used for the treatment of various diseases including fever, bronchitis, and gastrointestinal disorders. Previous studies have highlighted their antibacterial activity. So, present work was designed to examine the phytochemical and antifungal properties of aqueous crude extract of *T. guineensis* leaves. The bioactive components extracted from leaves were tested against pathogenic fungi using the agar tube dilution method. Antifungal activity of aqueous leaves extracts was carried out against selected pathogenic fungal strains as *Aspergillus fumigatus*, *Cryptococcus neoformans* and *Candida albicans*. The phytochemical analysis of the aqueous crude

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extract revealed the presence of secondary metabolites widely reported as antifungal such as flavonoids, saponins, quinones, alkaloids, polyphenols. The results showed that the aqueous crude leaves extract of *T. guineensis* was effective in inhibiting the fungal growth and were active against *A. fumigatus*, *C. neoformans* and *C. albicans* with MIC and MFC ranged from 20 to 200 mg/mL and 100 to 400 mg/mL, respectively. The efficient antifungal activity of *T. guineensis* from the present investigation revealed that aqueous leaf crude extract of the selected plant had a moderate potential to inhibit the growth of pathogenic fungal strains. This finding showed that the aqueous extract of *T. guineensis* exerted an antifungal effect on *C. albicans*, *A. fumigatus* and *C. neoformans* and supports its traditional use in herbal medicine.

Keywords: *Trema guineensis*; antifungal activity; aqueous extract; Minimum Inhibitory Concentration (MIC).

1. INTRODUCTION

Molecules from natural plants are considered a very important source of medicine. During the last decade, the use of traditional medicine has received renewed attention and interest in the world [1]. Thus, in many countries, a majority of population, mostly rural, uses traditional medicine and uses medicinal plants in primary health care [2,3]. According to WHO, more than 80% of the world's population use medicinal plants to treat several diseases [4]. Currently, about 25% of modern drugs are developed from plants [5]. The use of medicinal plants has gained a wide recognition as a result of its safety, low cost and effectiveness [6]. However, scientific work on medicinal plants is very low given the small number of studies carried out [7].

In Ivory Coast, traditional medicines are increasingly sought from traditional practitioners and herbalists for the treatment of various diseases. Among the plants used, *Trema guineensis* (Ulmaceae) is a woody plant distributed in the west central part of Ivory Coast. The leaves are locally used for the treatment of various diseases including cardiac failure and constipation [8]. The *in vivo* anti-inflammatory activity of this plant has been demonstrated by Kouakou et al. [9]. In West Africa, communities in southwestern Nigeria have used extracts from the leaves and bark of *T. guineensis* to treat fever, bronchitis, pneumonia and gastrointestinal disorders. Its phytochemical analysis indicated the presence of several secondary metabolites such as polyphenols, alkaloids, flavonoids, saponosids and tannins [10], who confer to the plant, several pharmacological activities including the analgesic activity. Previous studies conducted by Akinyemi et al., [10,11] revealed that aqueous extract of *T. guineensis* had antibacterial activity against strains of three food

borne pathogens that resisted conventional orthodox antimicrobials.

In recent times, no scientific report regarding the antifungal activity of *T. guineensis* extract has been published. After scrutiny of published literature showing its medicinal importance, the present protocol has been outlined regarding the antifungal activity on this plant aqueous extracts. The aims of this study were therefore to evaluate antifungal activity of aqueous extract of *T. guineensis* against some pathogenic fungi (*C. albicans*, *A. fumigatus* and *C. neoformans*) and to identify which components are deeply involved in this activity.

2. MATERIALS AND METHODS

2.1 Plant Collection and Authentication

Fresh leaves of *T. guineensis* (Ulmaceae) were collected from Korhogo and Abidjan (Ivory Coast) in February 2017. The botanical authentication of this plant was done by the herbarium of National Floristic Center of University FELIX HOUPHOUET BOIGNY (Abidjan, Ivory Coast), where a voucher specimen was conserved with reference number 8536; 10881 and 13968. The collected leaves were hand plucked and cleaned for debris using tap water and then rinsed with distilled water. The leaves were shade dried at room temperature. The dried leaves were powdered using an electric blender (IKAMAG RCT®) and powdered samples were stored in airtight glass containers protected from sunlight for subsequent extraction and further bioassay.

2.2 Aqueous Extract Preparation

The powder of *T. guineensis* leaves was used to prepare aqueous extract. Briefly, 100 g of the powder was soaked in 1 L of distilled water for 24 hours at room temperature, under shaking using

a mixer. The extracts obtained were filtered successively twice with absorbent cotton then once on Whatman filter paper number 1. After that, the resulting filtrate was concentrated under vacuum by using a Büchi rotary evaporator at 50°C [12]. The dark obtained powder constituted the aqueous extract (AqEx) used for the phytochemical screening and *in vitro* antifungal tests.

2.3 Fungi

Fungi strains, *C. albicans*, *A. fumigatus* and *C. neoformans* were obtained from the Laboratory of Mycology / Pasteur Institute, Côte d'Ivoire. These strains have been isolated from patients with deep mycoses. The fungi cultures were cultivated in Sabouraud agar (Bio-RAD/ref: 64494, lot: 7C2219) and incubated for 48 h at 30°C.

2.4 Phytochemical Screening

The extracts were subjected to preliminary phytochemical testing to detect for the presence of different chemical groups of compounds. Air-dried and powdered plant materials were screened for the presence of saponins, tannins, alkaloids, flavonoids, terpenoids, steroids, quinones by chemical test as described in literatures [13,14,15].

2.5 Assay for Antifungal Activity

The inhibitory effect of *T. guineensis* leaf aqueous extract against fungi's strains was measured by agar tube dilution method and minimum inhibitory concentration (MIC) [16]. Sabouraud agar (10 mL) was dispensed in screw capped tubes or cotton plugged test tubes. Then, Sabouraud agar was loaded with plant extracts using the method of the double dilution agar slopes. Tests tubes consisted of eight tubes containing plant extract and two controls tubes without plant extracts (one serving as control for the growth of germs; the other without germs serving as a witness sterility control of the culture medium). For the eight tests tubes, concentrations of plant extract ranged from 400 to 3.125 mg/mL binding by a geometrical reason of ½. After incorporation of extract in agar, all tubes were autoclaved at 121°C for 21 min. The tubes containing the media were then allowed to solidify in slanting position at room temperature. Standard drug Ketoconazole USP (20 mg) was used as a positive control. The tubes containing solidified media and plant extract were inoculated with a previously prepared inoculum (10⁵

cells/mL), obtained from culture cells of fungus on day 2. The tests tubes were then incubated at 30 °C for 48 hours. After this period, the colonies were counted and growth in experimental tubes was determined. Growth in the eight tubes of each experimental series was assessed as a percentage of survival compared to 100% survival in the control tube growth control [17]. The formula of this calculation has been mentioned below. Control experiments were carried out under similar condition by using Ketoconazole USP (20 mg) for antifungal activity as standard drugs. Treatment of experimental data was used to determine the antifungal parameters viz., MIC, MFC (minimum fungicidal concentration), and IC₅₀. Values of IC₅₀ (concentration producing 50% inhibition) were determined on the survival curves of microorganism's strains established with Graph Pad software, U.S.A. The MIC values were taken as the lowest concentration that inhibited the visual growth of the tested organisms. The MFC was determined by culturing 20 µL of the mixed broth culture from the tubes with no visible turbidity on Sabouraud at 30°C for 48 h on the MIC assay. The MFC was defined as the lowest concentration completely inhibiting the fungi's growth. [18,19]. The mechanism of antibiosis (fungicidal or fungistatic) of the extracts was calculated using the ratio of MFC/MIC [20] to elucidate whether the observed antifungal effect was fungicidal or fungistatic. When the ratio of MFC/MIC is ≤ 2.0, the extract is considered fungicidal or otherwise fungistatic. If the ratio is ≥ 16.0, the extract is considered ineffective. All assays were carried out in three replicates to ensure accuracy.

Formula to calculate the survival percentage:

$$S = \frac{n}{N} \times 100$$

S: The survival (%)

N: Number of colonies in one experimental tube and n: Number of colonies in the witness tube of growth control

2.6 Statistical Analysis

All experiments were repeated at least in triplicate and the results were presented as the average value with standard deviation.

3. RESULTS

The results of the preliminary phytochemical screening of aqueous plant leaf extracts of *T. guineensis* revealed the presence of various

bioactive agents. This is because the leaves aqueous extracts were found to contain compounds such as flavonoids, polyphenols, quinones, saponins and alkaloids while tannins and terpenoids are absent (Table 1).

The antifungal activity of the aqueous extracts of *T. guineensis* were studied in different concentrations (3.125; 6.25; 12.5; 25; 50; 100; 200 and 400 mg/mL) against three fungal strains (*A. fumigatus*, *C. neoformans*, *C. albicans*). Antifungal effect of *T. guineensis* extract of was quantitatively assessed by measuring minimum inhibitory concentration (MIC, mg/mL), minimum fungicidal concentration (MFC, mg/mL), IC₅₀ (mg/mL), and the efficacy ratio (MFC/MIC). The results of the antifungal activities are presented in Table 2. The assays showed that the fungi exhibited varied susceptibilities to the extract at different concentrations used. The fungi were sensitive to the aqueous extract tested in a dose-response relationship. The survival percentage of fungal germs decreased with increasing concentration of aqueous extracts (Fig. 1). The three fungal strains were sensitive to the aqueous extract tested. Thus, the sensitivity of aqueous extract decreases in the following order: *C. albicans* > *C. neoformans* > *A. fumigatus*. *A. fumigatus* is the most sensitive seed of the aqueous extract (MIC = 50 ± 0.00 mg/mL). *C. neoformans* has moderate sensitivity (MIC = 100 ± 0.00 mg/mL), whereas *C. albicans* has low sensitivity (MIC = 200 ± 0.00 mg/mL) (Table 2).

In addition, taking into account both MFC and IC₅₀, the aqueous extract presented the highest sensitivity with *A. fumigatus* (MFC = 100 ± 0.00 mg / mL, IC₅₀= 2.94±0.00 25 mg/mL) followed respectively by *C. neoformans* (MFC = 50

mg/mL, IC₅₀ = 8.82±0.003 mg/mL) and *C. albicans* (MFC = 400 mg/mL, IC₅₀ = 14.33±0.006 mg/mL).

Regarding the antifungal effect of *T. guineensis* aqueous extract and ketoconazole, the results recorded indicate that the MFC/MIC activity varies between 1 and 2. Ketoconazole showed fungicidal action on the strains tested excepted on *A. fumigatus*, while aqueous extract of *T. guineensis* showed fungistatic action against the three strains studied (*A. fumigatus*, *C. neoformans* and *C. albicans*). This study also showed that, ketoconazole standard antifungal molecules was more active than water crude extracts of *T. guineensis* against the three fungi strains as revealed by the MICs and MFCs values calculated (Table 2).

4. DISCUSSION

Antifungal activity of aqueous crude extract of *T. guineensis* leaves have been evaluated in the present research work. The *in vitro* antifungal activity of plant extract is a first step toward the development of new potential drugs. The results obtained from this investigation showed that the highest antifungal activity was exhibited by the standard drugs Ketoconazole USP and the lowest by the aqueous extracts. Thus, the aqueous crude extract of *T. guineensis* revealed antifungal activity against the three fungal strains (*A. fumigatus*, *C. neoformans* and *C. albicans*) in different degree. *A. fumigatus* was more sensitive to aqueous extract (MIC = 50 ± 0.00 mg/mL) than *C. neoformans* (MIC = 100 ± 0.00 mg/mL) and *C. albicans* (MIC = 200 ± 0.00 mg/mL). The basis of varying degree of sensitivity of test organisms of fungi may be

Table 1. Phytochemical analysis of *T. guineensis* leaves extracts

Sample	Tannin	Alkaloid	Polyphenol	Flavonoid	Terpenoid	Saponin	Quinone
<i>T. guineensis</i> leaves extract	-	+	+	+	-	+	+

+: Present, -: Absent

Table 2. Antifungal parameters crude aqueous extract of *T. guineensis* against clinical fungi isolates (*C. albicans*, *C. neoformans* and *A. fumigatus*)

Fungi strains	Extract	Antifungal parameters (mg/mL)			MFC/MIC	Antifungal effect
		MIC	MFC	IC ₅₀		
<i>A. fumigatus</i>	AqEx	50 ± 0.00	100 ± 0.00	2.94 ± 0.003	2	Fungistatic
	Ket	0.156 ± 0.00	0.313 ± 0.00	0.078 ± 0.0001	2	Fungistatic
<i>C. neoformans</i>	AqEx	100 ± 0.00	200 ± 0.00	8.82 ± 0.003	2	Fungistatic
	Ket	0.039 ± 0.00	0.039 ± 0.00	0.012 ± 0.00	1	Fongicidal
<i>C. albicans</i>	AqEx	200 ± 0.00	400 ± 0.00	14.70 ± 0.006	2	Fungistatic
	Ket	0.039 ± 0.00	0.039 ± 0.00	0.012 ± 0.00	1	Fongicidal

AqEx: Aqueous extract; Ket: ketoconazole

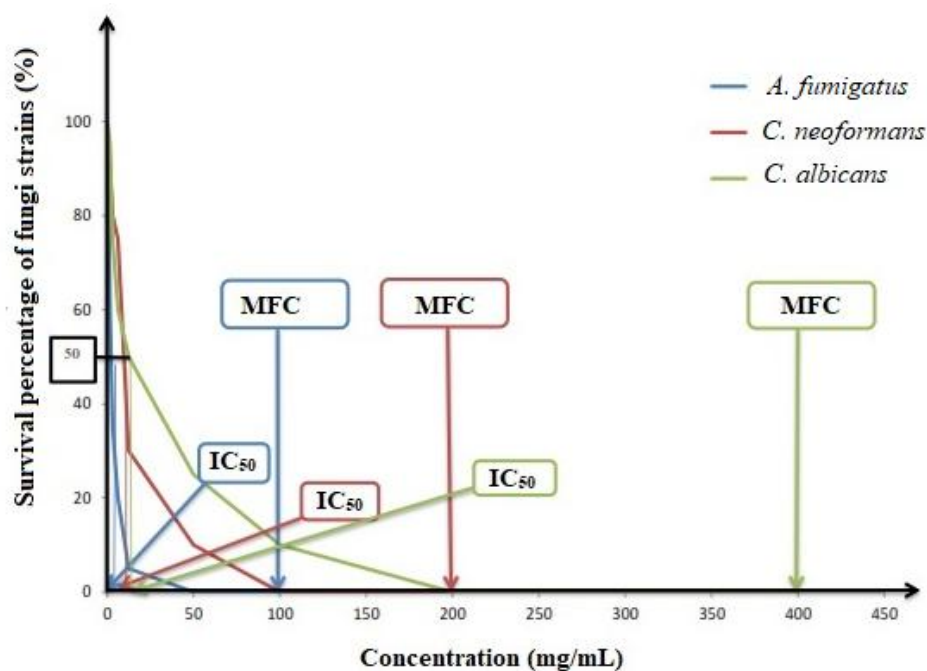


Fig. 1. Effect of aqueous extract of *T. guineensis* on in vitro growth of *A. fumigatus*, *C. neoformans* and *C. albicans*

due to the intrinsic tolerance of microorganisms and the nature and combinations of phytochemicals present in the crude extracts. This result also supported the reports of other researchers that plant extracts were very effective in the treatment of candidiasis [21,22]. However, this activity, compared to other should be considered moderate. In fact, the analysis revealed that water extract of *T. guineensis* (MFC = 400 mg/mL) is less active on *C. albicans* than aqueous extract of *Terminalia catappa* (MFC = 0.78 mg/mL) [23]. Similarly, previous studies of Ouattara et al. [24], have shown a highly effective inhibition by *Terminalia ivorensis* (MFC = 0.195 mg/mL) on pathogenic fungi *A. fumigatus*. The moderate values recorded for the plant extracts may be attributed to the fact that the extracts being in crude form, contain very small amounts of bioactive compounds. Probably, a more refined preparation would have antifungal activity at a lower concentration. It is known that the potency of the plant extracts depends on the solvent used, and this may be due to the degree of solubility of the bioactive constituents. Thus, it has been documented that different solvents have diverse solubility capacities for different phytochemicals [25]. The above results also showed that aqueous crude extracts of *T. guineensis* had fungistatic action against all fungi's strains. It is worthy of note that

MFC values obtained for the extracts against fungal strains are higher than MIC, indicating that the extract is fungistatic. The phytochemical screening revealed the presence of various bioactive agents such as flavonoids, polyphenols, saponins, alkaloids and quinones. In a previous finding, Akinyemi et al. [10], reported the presence of additional phytoconstituents, such as tannins in leaf extract of *T. guineensis*. There is therefore a difference between our results and those of these authors at the level of the major phytochemical components of *T. guineensis*. Such difference may be explained by several factors. Indeed, according to Sofowora [26], the composition of plant secondary metabolites varies according to the geographical location, the organ taken, the period of sampling, the time of sampling, the storage conditions, and the solvent for extraction. The degree of the antifungal activity of the extract may be accounted for by the presence of flavonoids as indicated in the phytochemical screening of *T. guineensis* [10]. The detected compounds in this extract may be responsible for antimicrobial observed activity of the plant and thus justifying its traditional use as medicinal plants for many diseases. This agrees with previous reports that associated the antifungal activity of the plant extracts to the presence of flavanoids, steroids, alkaloids and triterpenoids

and other natural polyphenolic compounds or free hydroxyl groups [27,28].

5. CONCLUSION

The present study focused on the effect of *T. guineensis* leaf crude extract on *C. albicans*, *A. fumigatus* and *C. neoformans*. The results showed antifungal activity. This study justified the claimed uses of leaves in the traditional system of medicine to treat various infectious disease caused by the microbes This study showed that this plant could be used as new antimicrobial agents instead of drugs. However, further studies are needed to better evaluate the potential effectiveness of the crude extracts as the antifungal agents. More research is required to toxicity testing, isolate active compounds, and elucidate the structures of this plant to develop new antifungal and antimicrobials therapeutic principles.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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