Antifungal Activities of Ocimum gratissimum L. Hydroethanolic Extract against Candida albicans ATCC 35659 and Toxicity Analysis on Oreochromis niloticus Larvae

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Abstract

Background

The use of plant extracts as a sustainable substitute for antimicrobials in aquaculture is constrained by a poor understanding of their potential toxicity to aquatic organisms. This study aimed to investigate the antifungal activity of the hydroethanolic extract of *Ocimum gratissimum* leaves against *Candida albicans* ATCC 35659 while assessing its toxicity on Nile tilapia larvae.

Methods

The study included control bacterial germs, *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922. *In vitro*, growth toxicity on the yeast was evaluated using concentrations (50–500 mg/mL) of the plant extract in standard culture media. Nystatin was used as a control at 250 mg/mL. Subsequently, the toxicity of the extract was analysed using four serial two geometrical fold dilutions (0, 250–2000 mg/L) in a randomized duplicated trial with 20 fish larvae per treatment. The survival of the fish was monitored for up to 96 hours.

Results

Our findings showed that the extract did not have a bactericidal effect, but it exhibited significant differences in the inhibitory zones against the targeted *Candida albicans*. The extract showed an exhibitory zone of 35.51 ± 6.12 mm (500 mg/mL) and 20.45 ± 3.89 mm (250 mg/mL), while Nystatin had 33.53 ± 2.23 mm ($Df_{2.9}$, F: 19.03, p: 0.001). However, subjecting the fish to immersion in the extract at a concentration above 500 mg/mL resulted in a high mortality rate of 100%, indicating the potential occurrence of detrimental effects on aquatic fauna.

Conclusion

These findings underline the need for a comprehensive understanding of the potential toxicity of plant extracts to aquatic organisms when considering their use as sustainable alternatives in aquaculture. Future research should focus on elucidating the mechanisms of toxicity and identifying optimal concentrations that balance antifungal efficacy with minimal damage to aquatic life.

Highlights

The concentrations of 250 mg/mL and 500 mg/mL of the ecotype (Togo) of *Ocimum gratissimum* leaves hydroethanolic extract suggest the activities of the plant extract against *Candida albicans*

A significant decrease of Nile tilapia larvae survival was observed after two hours of immersion, at a concentration of 500 mg/L *Ocimum gratissimum hydroethyl* extract.

Background

Aquaculture production has the potential to play a significant role providing sustainable fish protein, employment opportunities and economic activity. The sector has experienced continuous growth, with increasing number of fish farms contributing to the expansion of aquaculture production [1, 2]. In Togo, while many extensive fish farms still remain common, new farms typically are of the semi-intensive, involving mainly Nile tilapia (*Oreochromis niloticus*) farming [3, 4]. Nile tilapia is species of great nutritional and economic importance. In 2021, the average annual Nile tilapia production reached around 11,000 tonnes [5]. This has generated a substantial turnover of around EUR 38 billion in 2022. However, despite the growth in production, the overall aquaculture production capacity in the country remains relatively low. The current production level only met about 60% of the country's expressed demand [4]. These figures highlight both the potential and the existing gap in aquaculture production in Togo. Expanding the sector's capacity could contribute significantly to meeting the country's demand for fish, enhancing food security, and fostering economic development. Further efforts and investments are needed to support the sustainable growth of aquaculture in Togo and bridge the gap between supply and demand.

Similar to livestock sector, one of the challenges faced by fish farmers worldwide is the presence of zoonotic bacterial, viral, and yeast diseases, which can have detrimental effects on fish farms and cause significant losses [6, 7]. Among these diseases, pathogenic fungi have gained attention due to their occurrence in fish waters and their implication in lethal symptoms in fish species [8–11], including those that are zoonotic [8–11]. In addition to the economic consequences that fungi can have in aquaculture, there are also risks of infection for fish farmers, particularly women who are most susceptible when they come into contact with contaminated fish farming waters [12, 13]. Furthermore, there is a health risk associated with the consumption of aquaculture products contaminated with fungal toxins.

Irrespective of the scale of infection incidences and fish mortality distributions, the pathogenic germs control in aquaculture currently depends largely on the use of antibiotics, which are illegally dumped directly in fisheries waters [14–16]. This has already resulted in some environmental problems such as the destruction of aquatic life, inducting resistance in sensitive germs and boosting the bioaccumulation of antibiotic residues in the environment [17]. It can also have resulted in the spread of antimicrobial resistance (AMR) [15, 18], and thus negatively impact on fish production and the livelihoods of the rural populations that depend on them [19]. These factors highlight the complex nature of the challenges faced in sustainable fish production. The presence of zoonotic fungi and their potential impact on both livestock, fish health and human health necessitate proactive measures to mitigate the risks. This includes implementing effective disease management strategies, improving water quality, and ensuring proper hygiene practices in fish farming operations. Addressing these challenges is crucial for the sustainable development of aquaculture and the well-being of fish farmers and consumers alike.

The use of environmentally friendly and cost-effective alternative methods like the medicinal plants extract that are known to have antimicrobial proprieties is especially important in Togo and at the same time to reduce the consumption of antibiotics by fish farmers [20, 21]. Several plant organs extracts, including the leaves extract, have often been used for treating microbial-related-diseases including the

fungus infection in livestock [22], and in human [23, 24]. Studies have reported antimicrobial effectiveness of some medicinal plant extract, used either in the form of a decoction or in a purified form (obtained by alcohol extraction), against helminth and pathogenic germs including bacteria and yeast (*M. canis. M. gypseum. T. rubrum and T. mentagrophytes*) [25].

Ocimum gratissimum L. (*O. gratissimum*) also named basil belongs to the Lamiaceae plant family and is used in traditional Togolese medicine among other, for the treatment of ophthalmic, acne, ringworm, and some skin disease [23, 24, 26]. It has been reported that the plant extract, either in the form of decoction or in purified form (obtained by alcohol extraction), can be effective against pathogenic germs including yeast (*M. canis. M. gypseum. T. rubrum and T. mentagrophytes*) [25]. This medicinal plants belonging to the naturalist class of antifungal, consists of a mixture of Thymol, p-cymene, γ-terpinene and many others. that exert their antimicrobial action on fungi and bacteria [23, 27–29]. Considering the application of the *O. gratissimum* extract in animals, aquaculture in particular,as observed amongst Togolese fish farmers, there are chances for the plants extract to enter the environment [30].

In aquaculture, several studies have reported the antimicrobial activity of *O. gratissimum* against fish pathogens bacteria [31, 32], fungi, internal and external parasites [31, 33, 34]. The essential oil of this plant showed *in vitro* antimicrobial activity against four species of *Candida albicans* [35]. Kone et al. [33] reported 100% efficacy of crude *O. gratissimum* at 800 mg/L for 36 hours when tested against *Argilus spp*. However, it must be noted that the antimicrobial properties of a plant species may differ from others depending on the ecotype of the plant species [36, 37].

This study aimed at unfolding two issues: first, to assess the antifungal properties of hydroethanolic extract of *O. gratissimum* leaves on *Candida albicans* and secondly to assess the effects of the plant extracts decoction (us traditionally used by farmers) on Nile tilapia larvae survival in the laboratory conditions.

Ethical Disposal

The present study was approved by the Animal Health and Welfare of Togolese, Ministry in charge of Livestock Breeding and Fisheries Production. Post-test fish specimens were placed in boxes on ice to induce general chilling anaesthesia and then euthanized.

Material and methods

Yeast strain

We evaluated the growth of *Candida albicans* ATCC 35659 (*C. albicans*) in a 24-hour of pure cultured colonies. This reference strain was provided by the laboratory of the National Institute of Hygiene (INH-Togo) where the trial was performed.

Experimental Fish

Native larvae of *Oreochromis niloticus* strain ("TIL-AQUA" strain) from the Netherlands were used for the test. The larvae were used since this population range is more vulnerable to fungal infection.

Methods

Hydroethanolic extraction

Occimum gratissimum leaves were used to prepare the hydroethanolic extract. The leaves were rinsed with clean tap water and thereafter, they were air-dried in an air-conditioner for four days and then grounded using a grinder into a fine powder (Model 4. Thomas scientific with a 2 mm mesh size). For the extract preparation, 500 g of dry powder was soaked with 5 litres of ethanol-water (8:2. v/v) in the room temperature (28 ± 2°C) for 72 hours and then the supernatant was separated and filtered through Wattman No. 3 filter paper. Dissolved solvent was then removed from the filter medium using a rotary evaporator under high vacuum (Buchi Rotavapor R-100) at 40°C. The extract was stored in a refrigerator at 4°C for the experimentations.

The extract was assessed for sterility using the method of membrane filtration. Briefly, a 1% dilution of the extract stock solution was done and filtered. Subsequently, the filtration membrane was poured into a pre-cast Petri dish with Agar. The mixture was then incubated at 37°C for 72 hours under observation every 24 hours. An extract was considered sterile if there were no colonies visible on the agar plate after incubation.

The extract solutions of 50 mg/mL, 100 mg/mL, 150 mg/mL, 200 mg/mL, 250 mg/mL, and 500 mg/mL were prepared and sterilized using 95% alcohol. The sterilization process involved covering the extract with 95% alcohol and allowing it to evaporate at 40°C in an oven.

Preparation of microbial suspension

The colonies of the *Candida albicans* were isolated on Sabouraud Chloramphenicol Agar (SCA) after being incubated aerobically at 25°C for 24 hours. The microbial suspensions were prepared with a density of 0.5 McFarland and diluted to 10^{-1}. To obtain this suspension, approximately five colonies of *Candida albicans* were collected with a sterile loop and introduced into 10 mL sterile normal saline solution of 0.9% NaCl. The solution was homogenized by vortexing, and the 0.5 McFarland density was obtained by adjusting it with a densitometer. The suspensions were then diluted to 10^{-1}.

In vitro Screening

Respectively, the Presumptive Diffusion Test (PDM) onto agar and Broth Dilution Test (BDT) [38, 39] were performed to assess the Antifungal Minimum Inhibitory Concentration (MICs). The PDM was used to assess the inhibition activities of the six different concentrations, 50 mg/mL, 100 mg/mL, 150 mg/mL, 200 mg/mL, 250 mg/mL, and 500 mg/mL of *Ocimum gratissimum* hydroethanolic extract on *Candida albicans* [40].

The extract's antimicrobial potential was evaluated by measuring the diameter of the inhibition zone around the drilled wells that contained the extract. In order to conduct the experiment, a triplicate of 50 µL of the extract at a specific concentration, as well as water as a negative control and the reference drug Nystatin, were each added to the wells created in the agar using a 6 mm diameter sterile tube on the culture plate. Five wells were randomly assigned for each medium. The extract solutions, distilled water, and Nystatin at 250 µg/mL were each placed in 50 µL quantities within the wells. Based on the preliminary results, concentrations of 250 mg/mL and 500 mg/mL were selected for further testing, as the lower concentrations were ineffective against the microorganisms.

The BDT was performed to assess the MICs of the extract by preparing a serial dilution on Muller Hinstong (MH) culture media. The extract was diluted to create six solutions ranging from 3.90 mg/mL to 125 mg/mL. To each extract concentration, 0.9 mL of the extract was mixed with 0.1 mL of a standard 0.5 McFarland microbial suspension that was diluted to 10^{-1}. Controls of the same extract concentration were prepared in parallel. In contrast to conventional culture media containing the extract, a negative control containing 0.1 mL of sterile SCA and a growth control containing 0.9 mL of MH and 0.1 mL of microbial culture suspensions were also prepared. After incubation, each test tube was evaluated by comparing it with the opposite control tube of the same concentration, using a calibrated 2µl loop to spread the sample in 5 cm strips on the culture medium. The antifungal concentration was identified as the tube with less than 0.01% colonies in comparison to the control.

Submersion challenge

The decoction was prepared by mixing 1172 g of the fresh leaves crude extract with 2.5 litres of water to obtain a mixture of 3372.2g. Considering the dry matter of 17% obtained after analysis, a concentration of 79.696 g/L was prepared as a standard solution. The *in vivo* analysis of the aquatic toxicity was performed at a concentration of 250 mg/L, 500 mg/L, 1000 mg/L, 2000 mg/L, and 4000 mg/L in duplicate using Nile tilapia larvae model. The stocking density was 0.5 g/L of larvae and there were 20 fish per treatment. The fish survival in the experimental tank containing water with the concentrations above including the control was monitored for 2, 4, 24, 48, 72, and 96 hours. This toxicity screening test using the Nile tilapia larvae model was consistent with the validation standards as mortality was not recorded during the acclimatization period nor in the control batch [41]. Furthermore, for all the treatments, the experiment was carried out on 20 fish, which exceeds the minimum size of 7 animals per treatment required [41].

Statistical Analysis

The chi-square test (χ2) was applied to compare frequencies at 5% threshold. For the comparison of the mean quantitative values, the Tukey test was performed. The Cox model was used to establish the survival curves [42]. The Lethal Concentration 50 (LC50) was determined using the regression Probit model:

$$\text{Probit}(p) = \text{Intercept} + BX \text{ (the covariates } BX \text{ were transformed using the base 10 logarithm)}$$

Data were analysed using IBM Statistical Package for Social Science (SPSS) software version 20.

Results

In vitro antimicrobial activity

The experiment performed in this study showed an activity of *O. gratissimum* hydroethanolic extract against *C. albicans* (Fig. 1). However, neither the minimal concentration of 50 mg/mL nor the maximum concentration of 500 mg/ml of this extract did not inhibit the growth of laboratory-tested bacterial isolates (Fig. 1). The inhibition zones in *C. albicans* colonies ranged from 20.45 ± 3.89 mm (250 mg/mL) to 35.51 ± 6.12 mm (500 mg/mL) for the extract concentrations, and 33.53 ± 2.23 mm for Nystatin (Fig. 1, Table 1, $Df_{2.9}$, F: 19.03, *p*: 0.001). The results further revealed that the plant extract at a concentration of 500 mg/mL exhibited the most pronounced activity compared to the lowest concentrations, but relatively comparable to that of Nystatin ($Df_{1.6}$, F: 0.67, *p*: 0.42). The turbidity induced by the growth of bacteria decreased inversely with the concentration of extracts in the experimental tubes. An indication of an antifungal activity of the extract against *C. albicans* with a minimum inhibitory concentration of 0.97 mg/mL and a minimum fungicidal concentration of 3.9 mg/mL (Fig. 2).

Table 1
Growth inhibition zone shown by *Candida albicans* against different concentrations of *Ocimum gratissimum* hydroethanolic extract

Parameters	*Ocimum gratissimum* extract		Control
	500 mg/mL	250 mg/mL	Nystatin (250 mg/mL)
Inhibition diameter per replicate (mm)	31.76	23.51	35.45
	32.2	16.08	34.06
	42.58	21.77	31.09
	35.51	20.45	33.53
Average (mm)	35.51 ± 6.12	20.45 ± 3.89	33.53 ± 2.23
Growth inhibition zone was not observed for the concentrations lower than 250 mg/ml); distilled water was used as control for each step of the test performed			

Fish Survival

The physico-chemical characteristics of water in the observation tanks were as follows: 30.15 ± 0.15°C, pH: 7.25 ± 0.04 and dissolved oxygen (DO): 6.21 ± 0.19 mg/L. During the acclimatization phase, no mortality or behavioral disturbances were observed in the fish larvae across all treatments. The control group and the concentration of 250 mg/L of the extract also did not result in any mortality. Throughout the 96-hour observation period, the survival rate of the fish showed significant differences among the treatments (Fig. 3. *df*: 4 ; $\chi 2$: 81.7 ; $p < 0.001$).

The mortality rate for the treatment 500 mg/L was relatively moderate, 35%; also three cases (15%) of abnormal swims were observed at this concentration of extract. High concentrations, 1000 mg/L, 2000 mg/L and 4000 mg/L extracts resulted in 100% mortality, suggesting a low potential for using the crude leaf extract for water immersion treatments against the pathogen. No larvae survived after 2 minutes of immersion at the highest concentration (4000 mg/L). The CL50 determined by Probit transformation was 532.63 mg/L (532.63×10^{-3} mg/mL) (Table 2), significantly lower, when compared to 500 and 250 mg/mL found in the *in vitro* challenge against the yeast strain.

Table 2
Nile tilapia larvae susceptibility to *Ocimum gratissimum* crud extract (Lethal doses, Probit and confidence limits)

Probability		95% Confidence Limits for Dose (mg/L)			95% Confidence Limits for log(Dose)[a]		
		Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
PROBIT	0.010 (LC1)	229.597	126.223	300.669	2.361	2.101	2.478
	0.020	252.885	148.189	323.459	2.403	2.171	2.510
	0.030	268.871	163.965	339.020	2.430	2.215	2.530
	0.040	281.559	176.857	351.362	2.450	2.248	2.546
	0.050	292.320	188.030	361.843	2.466	2.274	2.559
	0.060	301.803	198.044	371.104	2.480	2.297	2.569
	0.070	310.370	207.218	379.500	2.492	2.316	2.579
	0.080	318.247	215.750	387.254	2.503	2.334	2.588
	0.090	325.584	223.776	394.513	2.513	2.350	2.596
	0.100	332.488	231.389	401.379	2.522	2.364	2.604
	0.150	362.663	265.242	431.958	2.560	2.424	2.635
	0.200	388.587	294.779	459.259	2.589	2.469	2.662
	0.250	412.299	321.834	485.396	2.615	2.508	2.686
	0.300	434.824	347.283	511.539	2.638	2.541	2.709
	0.350	456.793	371.624	538.514	2.660	2.570	2.731
	0.400	478.666	395.183	567.018	2.680	2.597	2.754
	0.450	500.824	418.213	597.721	2.700	2.621	2.776
	0.500 (LC50)	523.632	440.947	631.329	2.719	2.644	2.800
	0.550	547.479	463.642	668.661	2.738	2.666	2.825
	0.600	572.823	486.609	710.737	2.758	2.687	2.852
	0.650	600.251	510.248	758.931	2.778	2.708	2.880
	0.700	630.579	535.106	815.234	2.800	2.728	2.911
	0.750	665.029	561.977	882.750	2.823	2.750	2.946

Probability	95% Confidence Limits for Dose (mg/L)			95% Confidence Limits for log(Dose)[a]		
	Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
0.800	705.610	592.119	966.765	2.849	2.772	2.985
0.850	756.048	627.795	1077.415	2.879	2.798	3.032
0.900	824.664	673.918	1238.167	2.916	2.829	3.093
0.910	842.150	685.314	1280.916	2.925	2.836	3.108
0.920	861.566	697.821	1329.206	2.935	2.844	3.124
0.930	883.433	711.735	1384.605	2.946	2.852	3.141
0.940	908.511	727.485	1449.446	2.958	2.862	3.161
0.950	937.982	745.735	1527.397	2.972	2.873	3.184
0.960	973.832	767.589	1624.718	2.988	2.885	3.211
0.970	1019.787	795.103	1753.412	3.009	2.900	3.244
0.980	1084.251	832.861	1941.224	3.035	2.921	3.288
0.990 (LC99)	1194.228	895.341	2280.697	3.077	2.952	3.358

a. Logarithm base = 10; Parameters Estimates for the Probit Model generated (Intercept: -17.665 ± 3.729, Dose: 6.497 ± 1.377, *p*-value < 0.001); Estimate: doses expressed in ppm (mg/L). PROBIT model: PROBIT(p) = Intercept + BX (Covariates X are transformed using the base 10,000 logarithm.); LC: Lethal concentrations (1: 99% of survival, 50: 50% of survival, and 99: 1% of survival)

Discussions

Traditionally, medicinal plants have been used in human disease treatment and this has been reported as an eco-friendly alternative to antibiotics [20, 21, 43]. The synthesis of new antimicrobial drugs is restricted by the challenges involved in identifying new substances that are both effective against microorganisms and non-toxic to targeted animals [17, 44–46]. In the present study, leaves crude extract from *Ocimum gratissimum* shows a CL50 value of 523 mg/L for Nile tilapia larvae, which is lower than the minimum inhibitory concentration of 0.97 mg/mL (970 mg/L) and a minimum fungicidal concentration of 3.9 mg/mL (3900 mg/L) against *Candida albicans*. These results are in agreement with the previous reports concerning the toxicity of *Ocimum gratissimum* in fish [47] and in a freshwater live *Daphnia magna* [48], and thus raising environmental concern for its application in fish pound water [49, 50].

The results of this study indicate that, the lowest concentrations of *Ocimum gratissimum* extract (i.e. concentrations < 200 mg/mL) were ineffective in inhibiting the growth of *Candida albicans*, contrary to previous reports [51]. The minimum inhibitory concentration (MIC) value obtained in this study was lower

than the value reported (50 mg/mL) for hydro-distilled volatile oils from the leaves of the plant in Eastern Kenya [43]. Furthermore, the MIC value obtained in this study was much higher compared to the reference antifungal drug routinely used [52]. A number of factors could contribute to the observed differences in MIC values. These include the extraction method used, the presence of active principles in low concentrations specific to the ecotype of the plant used in this study, or the in vitro culture conditions. It is worth noting that the phenomenon of MIC, which refers to growth effects that hinder the clear determination of MICs, has been reported to affect the Broth Dilution Test in yeast [52]. Therefore, the previous work conducted on the specific ecotype of *Ocimum gratissimum* used in this study has likely contributed to the existing knowledge of its chemical profile and may serve as a foundation for further investigations into its potential benefits and applications [23, 53]. The previous findings could also be affected by other factors like the season of harvesting and farming practices that could influence the chemical composition of the plant [54–56]. This will be also the case in our study.

Generally, the concentrations of 250 mg/mL and 500 mg/mL *of Ocimum gratissimum* ethanolic leaves extract indicate the presence of antifungal activities of the plant. The 250 mg/mL concentration of *Ocimum gratissimum* ethanolic leaves extracts dilution showed a moderate activity against *Candida. albicans* isolate compared to that of Nystatin with the same concentration. However, the high concentration (500 mg/mL) of the plant extract showed high antifungal activity. Some heterogeneity of the inhibition zones of the germ growth was observed, which may be attributed to some of the above mentioned factors. Similar observation was made on the effectiveness of *Ocimum gratissimum* extract against yeast (*M. canis. M. gypseum, T. rubrum and T. mentagrophytes*) [25].

In this study, the high concentration of 500 mg/mL of extract of *O. gratissimum* was found to have no effect on both populations of the control bacteria germs exposed. Studies conducted by Nweze et Eze [43] have reported also no inhibition effect of *O. gratissimum* extract on *E. Coli*. There was a relatively low inhibiting effect in *S. aureus*, *E. coli* and *S. typhimurium* when using a typical extract obtained by steam distillation [57]. In contrast, Mounerou et al. [58] have observed sensitive phenotypes in *E. coli* and *S. aureus* population. Junaid et al. [59] also reported the antibacterial effectiveness of *O. gratissimum* extract against *Escherichia coli*, *Salmonella typhimurium, Aeromonas hydrophila and Bacillus cereus* (However, as mentioned above, it must be noted that the antimicrobial properties of a plant species may differ depending on the ecotype of the plant species [36, 37].

Considering the previous studies, the main focus was on the antimicrobial activity of *Ocimum gratissimum* extract in waterborne germs. In the current study, we examined in addition the possible toxicity effect of *Ocimum gratissimum* plant extract in Nile tilapia larvae in submersion. A study conducted on the effects of *Ocimum gratissimum* plant extract on mosquito, *Anopheles gambiae* revealed negative effects of the *Ocimum gratissimum* ethanolic extract on larvae; with 100% mortality at 0.4% concentration within 24 hours of treatment [60]. A studies by Omoigberale et al. [47] and Pastorino et al. [48] reported a toxic effect of this plant extract in fish and in a freshwater live *Daphnia magna*, with 100% effect at high concentration. Our results show a similar effect on the *Oreochromis niloticus*, as the survival of the experiment larvae decreased in the submersion test, though the concentrations of the test

waters were well below those found to be effective against fungi in the *in vitro* tests. Under the experimental conditions, after 2 hours of immersions, the concentrations of the crude extract above 500 mg/mL led to a significant decrease in larvae survival. It important to note that, while the moderate concentration (250 mg/mL) of *Ocimum gratissimum* extract might be better tolerated in immersion for systemic sanitary control, the extract type has toxic potentialities that should not be overlooked. Finally, the CL50 value of the plant extract found *in vivo* test was significantly lower than that found in the *in vitro* tests against yeast. Our results are in agreement with the previous reports concerning the toxicity of *Ocimum gratissimum* in aquatic live [47, 48].

Finally, we found that, the specific effects of *Ocimum gratissimum* hydroethanolic extract in Nile tilapia (*Oreochromis niloticus*) aquaculture vary, depending on the concentration and the exposure duration. This implead that when using this plant extract in aquaculture, it will be important to start with lower concentrations and gradually increase the dosage while closely careful monitoring and observation the response of the targeted aquatic live. In addition, the effects of the extract can vary depending others factors, including the species and life stage of the aquatic live, and the specific formulation or extraction method used. These findings suggest that while the hydroethanolic extract of *Ocimum gratissimum* leaves showed antifungal activity against *Candida albicans*, its immersion in high concentrations resulted in significant mortality in Nile tilapia larvae. This highlights the importance of evaluating the potential toxicity of plant extracts on aquatic organisms [48] before considering their use in aquaculture.

Conclusion

The aim of this study was to assess the in vitro antifungal activity of ethanolic extract obtained from *Ocimum gratissimum* leaves against *Candida albicans* laboratory isolate and the toxicity analysis on *Oreochromis niloticus* larvae. Although using medicinal plant decoction as a traditional prophylaxis is a common practice in local animal farms, few studies have investigated their potential negative effects on aquatic life. This preliminary study aimed to provide a better understanding of the impact of *Ocimum gratissimum* leaves crude extract on Nile tilapia larvae. Results showed that the extract inhibited *Candida albicans* growth in laboratory conditions, indicating an existing potential for use in the formulation of antifungal compounds. However, even at the lowest concentration, the extract negatively affected Nile tilapia larvae survival compared to the *in vitro* test concentrations. We conclude that, the use of medicinal plant extracts in aquatic environments should be carefully considered, and possible negative side effects taken into account.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

Not applicable

Competing Interests

The authors declare no competing interests

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Authors' contributions

"All authors of this study made significant contributions to the study design and scientific drafting of the manuscript. The initial protocol design, data analysis, and proposal of the first draft were primarily conducted by Boma Soudah. The final protocol was validated through the involvement and contributions of N'Feide Toï, Kombaté Bignoate, and Imorou Toko Ibrahim. Laboratory analyses were carried out by Bidéma Noumonzeme and Kombaté Bignoate. Their expertise and efforts in conducting the laboratory experiments were invaluable to the study. All authors carefully reviewed and critically assessed the final manuscript, providing valuable feedback and suggestions for improvement. Following the collective input and revisions, all authors have read and given their approval for the final version of the manuscript."

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Figures

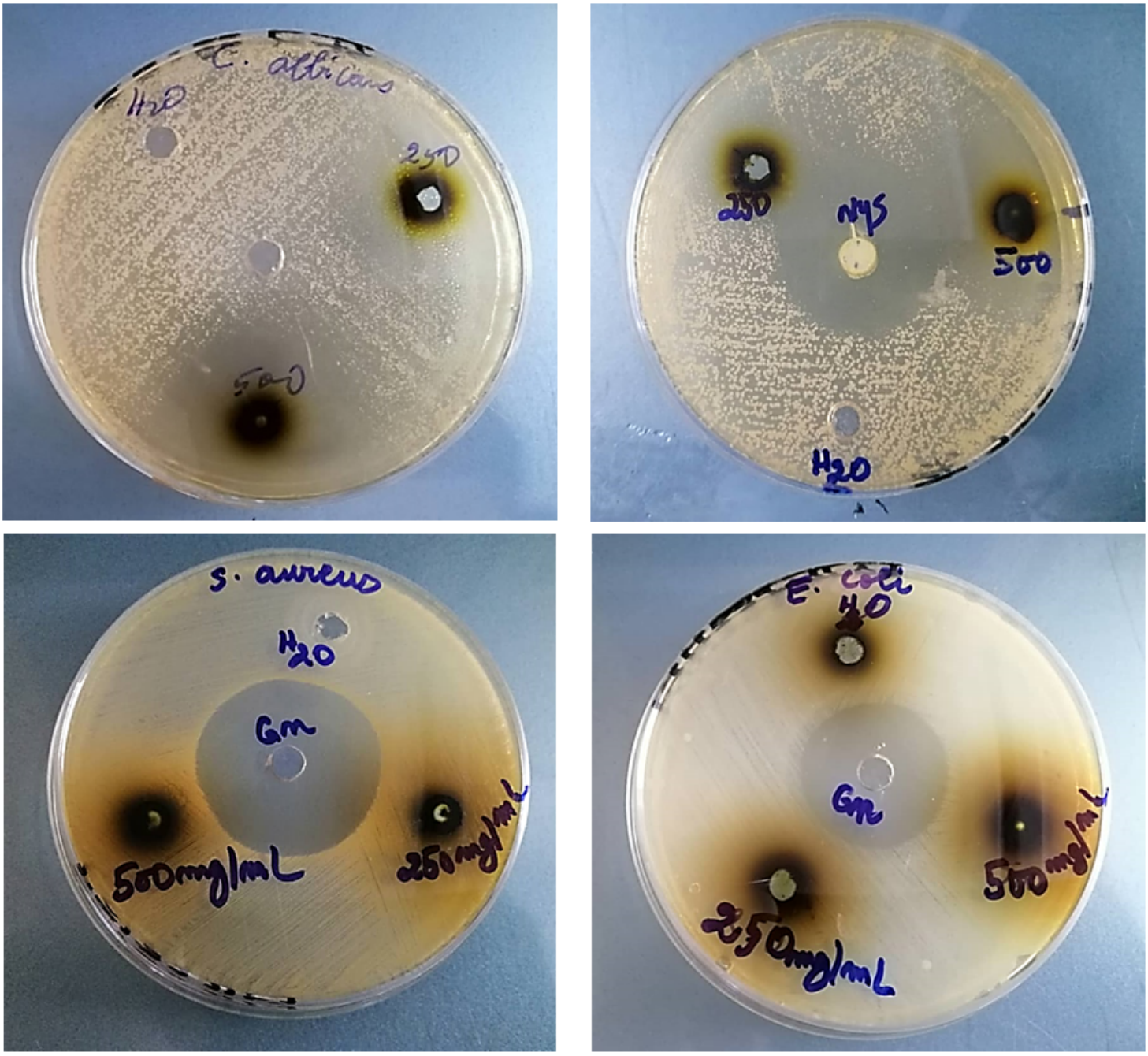

Figure 1

Illustration of the fungicidal effect of the hydroethanolic extract of *Ocimum gratissimum* on *Candida albicans* ATCC 35659. The drilled wells were filled with chemicals: water (H_20) as the neutral product, Nystatin as the control drug, and the decoction of the plant extract (250 and 500 mg/ mL). The controls chemical compounds used were: Gentamicin (Gn), Nyastitin (Nys) and water (H20). The test has included reference pathogen bacterial germ *Staphylococcus aureus* ATCC 29213 and the traditional sanitary indicator germ *E. coli* ATCC 25922. The two control products were used in separate Petri Discs to avoid eventual confusion factors related to Nystatin's broadest broadcast

Figure 2

Screenshot of the model tool home menu

Illustration of the dilution test procedure and the fungicidal effect of the hydroethanolic extract of *Ocimum gratissimum* on *Candida albicans*. MFC: Minimum Antifungal Concentration (A). +/-: positive /negative control without an extract of *Ocimum gratissimum*. The minimum inhibitory concentration of 0.97 mg/mL was identified as the tube with less than 0.01% colonies in comparison to the control (B)

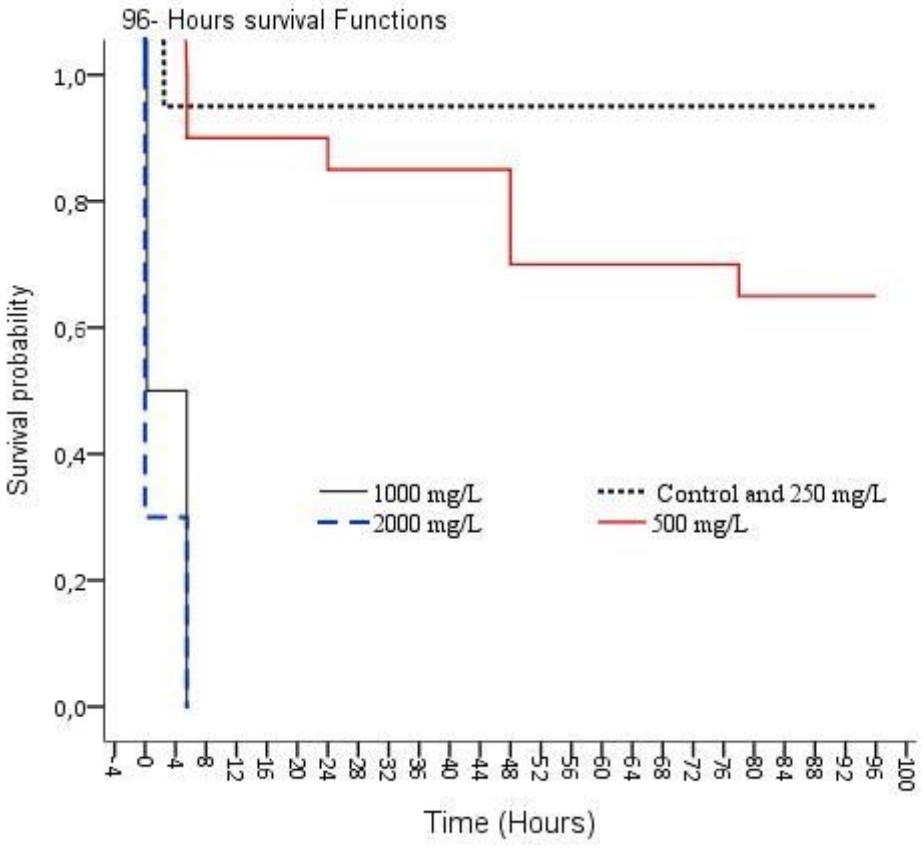

Figure 3

Survival curve of Nile tilapia larvae in immersion according to Ocimum gratissimum leaves crud extract concentration (control, 250 mg/L, 500 mg/L, 1000 mg/L, 2000 mg/L) and time after immersion. The 4000 mg/L led to 100% mortality after 2 min.

Supplementary Files

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