Original Research Article

In-vitro and *In-vivo* Anti-Candidal Effect of *Cnidoscolus aconitifolius* Leaves Extracts

Abstract

Candidiasis is an opportunistic infection caused by pathogens of the genus *Candida*. Due to the increase in antifungal resistance by *Candida* species, there is a need for alternatives in its treatment. *Cnidoscolus aconitifolius* is a medicinal plant with vast nutritional and antimicrobial properties. This study evaluated the in-vitro and in-vivo anti-candidal effect of *Cnidoscolus aconitifolius* (spinach tree) leaves extract. Clinical isolates of *Candida albicans*, *Candida krusei*, *Candida tropicalis*, *Candida dublinensis*, *Candida glabrata*, and *Candida parapsilosis* were used in this study and Cold maceration technique was employed in the plant extraction process, to obtain its ethanolic, methanolic, and aqueous extract. The agar well diffusion method and 2-fold dilution process -to obtain 250 mg/ml to 15.625mg/ml of the extracts -were used for the antimicrobial sensitivity test and three weeks old female Wistar rats infected with 20µl of standardized *C. albicans* were used for the *in-vivo* evaluation. Statistical analysis was done using Statistical package for Social Sciences (SPSS) version 22. *In-vitro* assay revealed ethanolic extract as the most potent extract with the highest inhibition zone diameter of 12.67 ± 1.15^{c}, 11.67 ± 1.15^{c}, and 12.33 ± 0.58^{c} at 250mg/ml against *C. tropicalis*, *C. krusei*[2], and *C. parapsilosis* respectively. *C. parapsilosis* was the most sensitive to all the extracts. *In-vivo*, the disappearance of disease symptoms and progressive decrease in rats *Candida* burden- from 6.35×10^{3} CFU/ml obtained after infection to 2.15×10^{3}CFU/ml after treatment revealed the ability of the plant to treat candidiasis. The study suggests that extract of *Cnidoscolus aconitifolius* leaves could act individually or synergistically as effective drug candidates in the treatment of candidiasis; thus if its individual antifungal constituents are purified, this plant could be the next major anti-candidal agent.

Keywords: Candidiasis, *Cnidoscolus aconitifolius,* female Wistar rats, *Candida* species, anti-candidal effect.

Introduction

Candida is an opportunistic normal flora of the human reproductive and gastrointestinal mucosa and may be isolated from the mouth, vagina, gut, and throat (Ezeadila *et al.*, 2020). The transition from a harmless commensal to a disease causing pathogen depends on the immune system of the host and virulence factors of the *Candida* species (Yang, 2003). Candidiasis mostly manifest as oral thrush or vagina candidiasis. More than 90% of immunocompromised persons, which are HIV patients, present with oral candidiasis (Raesi *et al.*, 2019). Seventy-five percent of women experience a *Candida* infection at least once in their lifetime (Ezeadila *et al.*, 2020) and

this could result in systemic infections that are fatal to pregnant women and critically unwell patients with a fatality rate of approximately 30% (Sexton *et al*., 2007).

Candida infections are majorly treated with antifungal medications from the polyene and azole families (Fang *et al*., 2020) but resistant strains keep springing up due to the frequent usage of these drugs. Pfaller *et al*. (2019) reported the statistics of the drug resistance towards Miconazole, Ketoconazole, Clotrimazole, and Fluconazole as shown by various *Candida* species as *C. krusei* (20%), *C. albicans* (89.3%), *C. glabrata* (14.2%), and *C. glabrata* (42.9%) respectively. Resistance to polyene has been discovered mostly in clinical isolates of non-albicans *Candida* such as *Candida glabrata, Candida parapsilosis, Candida tropicalis*, and *Candida lusitaniae* (Ezeadila *et al*., 2020). Thus, there is a need for alternative treatment, which is where medicinal plants come in.

"A medicinal plant is one that contains substances that can be used for therapeutic purposes and/or can serve as active ingredients for the synthesis of new drugs" (WHO, 2005). *Cnidoscolus aconitifolius*, generally called spinach tree, chaya, catholic vegetable or "Hospital too far" is a medicinal and perennial herb from the Euphorbiaceae family and originates from South-East Mexico (Fagbohun *et al.*, 2012). The root, stem, leaves, fruit, seed, bark, and latex of the plant are largely used for the treatment of many diseases in different parts of the world (Rajore and Batra, 2003). Traditionally, extracts of the leaves are used in treating diabetes, anemia, cardiovascular diseases etc. (Iwalewa *et al.*, 2005) and the leaf decoction is utilized in treating venereal disease in Colombia (Morton, 1981; Viswanathan *et al.*, 2018). Bautista-Robles *et al.* (2020) reported the ability of *Cnidoscolus aconitifolius* to treat insect bites, snakebites, and skin irritation. Its antimicrobial abilities have also been confirmed by various researchers.

In a study by Fagbohun *et al.* (2012) on the analysis of antimicrobial activity of the methanol extract of *Cnidoscolus aconitifolius*, *Aspergillus niger* and *Aspergillus tamarii* showed susceptibility with increasing concentrations of the extract. Jayashree and Gopukumar (2018) recorded considerable sensitivity of *Aspergillus niger*, *Aspergillus flavus* and *Rhizopus spp* to the latex of *Cnidoscolus aconitifolius*; the research analyzed the efficacy of petroleum ether, ethyl acetate, methanol, aqueous and ethanol extracts of the plant and the fungi showed clear zones of inhibition that revealed their susceptibility. Using n-Hexane, ethyl acetate, and methanol extracts of the plant in Northern Nigeria, Hamid *et al.* (2016) showed positive antifungal activity of *Cnidoscolus aconitifolius* against *Candida albicans, Aspergillus niger, Penicillium notatum,* and *Rhizopus stolonifer*. But Senjobi *et al*. (2011) and (Oyagbemi and Odetola, 2010) recorded no

antifungal activity of the plant against *Candida albicans* using hot ethanol extract. Limited scientific research has been done on the anticandidal activity of *Cnidoscolus aconitifolius* using different *Candida* species hence this research aims at analyzing the efficacy of *Cnidoscolus aconitifolius* leaves extract in the treatment of candidiasis caused by various *Candida* species *In-vitro* and *In-vivo*.

Materials and Methods

Collection of Plant Material

Cnidoscolus aconitifolius leaves were obtained from a garden in Ifite, Awka, Anambra state and Authentication of the herbal leave was done by a Taxonomist in the Herbarium section of the Department of Botany, Nnamdi Azikiwe University, Awka, Nigeria.

Preparation of plant extracts

The plant leaves were air-dried at room temperature, pulverized, and kept in sterile containers. The crude ethanol, methanol, and aqueous extracts were prepared using cold maceration technique. Three hundred and fifty grams (350g) each of the leaf powder was soaked in 2L of ethanol, methanol, and sterile water separately for three (3) days. These were later sieved and the filtrates, except that of the aqueous extract, concentrated at 40°C using a rotary evaporator. The extracts were kept refrigerated afterwards at 4°C in the refrigerator until needed (Buss and Butler, 2010).

In-vitro Anticandidal Assay of *Cnidoscolus aconitifolius* Leaf Extract

Preparation of stock extracts solution

The agar well diffusion method as described by Perez *et al.* (1990) was used for the *In-vitro* antifungal susceptibility testing using the various extracts of *Cnidoscolus aconitifolius* leaf.

Stock solution of each of the plant extract was prepared by dissolving 1000mg of the extract in 2ml of Dimethyl sulfoxide (DMSO) to obtain a final stock concentration of 500mg/ml.

Dilutions of 250 mg/ml, 125 mg/ml, 62.5 mg/ml, 31.25 mg/ml and 15.625 mg/ml in DMSO (100% v/v) was prepared from the 500mg/ml stock solution of each of the plant extract. A 2-fold dilution process was used for this. First, 250mg/ml was obtained by transferring 1ml of the stock solution (500mg) into an equal volume (1ml) of DMSO in another test tube and so it continued until the last concentration was obtained.

Test Microorganisms

Clinical isolates of *Candida* species were obtained from the oral cavity and high vagina swabs of patients with oral thrush and vaginal candidiasis attending Chukwuemeka Odumegwu Ojukwu University Teaching Hospital (COOUTH) Awka, Anambra state, Nigeria. The species were *Candida albicans, Candida krusei, Candida tropicalis, Candida dublinensis, Candida glabrata* and *Candida parapsilosis.*

Standardization of Inoculum for *In-vitro* Susceptibility Testing

About 25µl of the 0.5 McFarland standardized suspension of the yeast isolates equivalent to 1.5 x 10^8 CFU/ml was used as described by Cheesbrough, (2010). McFarland standard was prepared by mixing 0.6ml of 1% Barium chloride with 99.4ml of 1% sulphuric acid (H_2SO_4). Then a colony of the *Candida* species (24 hours pure culture) was picked and diluted in 5ml of 0.85% normal saline. The resulting turbid solution was placed beside the standard and compared visually. The too turbid solution was lightened by adding normal saline while a little more inoculum was added to the less turbid solution until it matches with that of the standard.

Susceptibility Testing Using Plant Extracts

First, Petri dishes containing Muller Hinton medium were prepared and using spread plate method, 0.1ml of the standardized concentrations (0.5 McFarland) of test isolates were seeded on the plates evenly. Then with a sterile cork borer, wells of 6mm were dug in each of the plates and 25µl of the various dilutions of the plant extracts were put in each of the wells under aseptic condition. DMSO served as the negative control while Fluconazole was the positive control. This was allowed to stand for an hour for the agents to diffuse into the agar medium before incubation. The plates were incubated at 25-27°C for 24 hrs.

The inhibition zones diameters (IZDs) obtained afterwards were measured and test samples with no inhibition were assigned the value of 0 mm. This experiment was conducted in duplicate, and the mean IZDs calculated afterwards.

***In-vivo* Anticandidal Evaluation of *Cnidoscolus aconitifolius* Leaf Extract**

Experimental Animals

Twenty (20) female Wistar rats (*Rattus norvegicus*) weighing between 27g and 56g were used. The animals were placed under standard laboratory animal house condition in accordance with

established guidelines on the care of laboratory animals following approval by Nnamdi Azikiwe University, Awka Animal Ethical Committee.

Standardization of *Candida* species Concentration for Infection

Candida albicans previously stored in Bijou bottle was reactivated by subcuturing in Sabouraud Dextrose Agar (SDA) at 25°C for 24-48h. Then the *Candida* inoculum was prepared in 0.05M phosphate buffer saline (pH 6.8) in concentrations of 5×10^5-5×10^{10} yeasts/ml according to the method of Marcia *et al.,* (2010); this was counted using a Neubauer chamber and used to standardize the inoculum to induce vaginal candidiasis in the rats.

The *Candida* inoculum was reactivated again from the Bijou bottle each time vaginal infection of the rats was to be carried out afresh. This was done 48 hours prior to the experiment.

Experimental Procedure

After an adaptive period of one week, the rats were divided into four groups of 5 animals (Rat A, B, C, D and E) each namely:

Group 1: Healthy rats not infected (Positive control).

Group 2: Rats infected without treatment (Negative control).

Group 3: Rats infected and treated with Nystatin (100unit/ml)

Group 4: Rats infected and treated with ethanol extract, the most effective plant extract invitro.

Groups 2, 3 and 4 were infected with 20µl of the standardized *Candida* yeast suspension of *C. albicans*, intra-vaginally and left for a period of four days to give room for disease establishment (Ikele *et al.,* 2019). This process was done without prior administration of estradiol hexa-hydrobenzoate, which helps sustain and maintains the estrus state of the rats to facilitate the infection process (Esther and Michael, 2018). This was because we wanted to mimic real life clinical situation; the rats that showed no clinical symptoms of the disease by day 3 were re-infected with twenty microlitre of the standardized *Candida* suspension for the disease state to be initiated and sustained.

By Day 5, the mean colony count and CFU/ml of the vaginal lavage for Group 2, 3 and 4 was carried out to ascertain disease establishment and recorded. Apparently healthy animals in group 1 showed no signs of vaginal candidiasis hence no record of them was taken.

Microbial Enumeration and Treatment

To calculate the CFU/ml, vagina lavage was done. Twenty microlitres of phosphate buffer saline was injected in the vagina and immediately removed. The content was seeded in Petri dishes containing ChromAgar Candida and incubated for 24-48 hours at 25°C. Colony forming units from lavage cultures after 24 hours was used to determine the vaginal *Candida* burden as described by Ikele *et al.* (2019).

Treatment Regimen

Treatment administration started on Day 5. Daily treatment was administered orally and intravaginally. Colony count determination via vaginal washing was done within the interval of three days, on Day 8 and 11 to determine the fungal burden.

Statistical Analysis

The data obtained was analyzed using Statistical Package for Social Sciences (SPSS) version 22 and the results expressed as mean ± standard deviation. Significant differences of the result were established by one-way ANOVA and the acceptance level of significance was $p < 0.05$ for all the results.

Results

Analysis of the inhibition zone diameters of *Cnidoscolus aconitifolius* leaves extracts against *Candida* isolates

Ethanol Extracts

Candida tropicalis was the most susceptible *Candida* species to this extract followed by *C. parapsilosis* and *C. krusei* as shown in Table 1. Statistically, the differences in their IZDs are not significant. The inhibition zones diameters (IZDs) were concentration dependent: as concentration of the plant extract decreased, there was a corresponding decrease in the IZDs. However, *C. krusei*[1] showed an increase in IZD at lower concentrations while *C. glabrata* demonstrated a constant result irrespective of the concentration.

Methanol Extracts

Table 2 shows *C.krusei*[2] and *C. tropicalis* had the highest IZD at 250mg/ml. Most organisms were concentration independent but *C. parapsilosis* had an almost constant IZD.

Aqueous Extracts

The lowest inhibition zone diameter amongst the three extracts was recorded by the aqueous plant extract (Table 3): most isolates showed no inhibition zone diameter even at 250mg/ml.

TABLE 1: Inhibition zone diameters of *Candida* isolates to ethanol extracts of *Cnidoscolus aconitifolius*

	Inhibition zone diameter (mm)				
	250mg/ml	**125mg/ml**	**62.5 mg/ml**	**31.25mg/ml**	**15.625mg/ml**
Candida spp					
C.tropicalis	12.67±1.15^{c}	9.00±0.00^{abc}	8.00±0.00^{a}	8.00±0.00^{ab}	8.33±0.58^{ab}
C.albicans	11.00±1.00^{bc}	10.33±0.58^{cd}	9.33±0.58^{a}	9.33±0.58^{b}	9.33±0.58^{b}
C. dublinensis	10.67±0.58^{bc}	10.00±0.00^{bc}	8.33±0.00^{a}	8.33±0.58^{ab}	8.00±0.00^{ab}
C.krusei[1]	9.33±0.58^{ab}	9.33±0.58^{abc}	9.33±0.58^{a}	8.33±0.58^{ab}	11.33±0.58^{c}
C.krusei[2]	11.67±1.15^{c}	8.67±0.58^{ab}	9.67±1.15^{a}	9.00±0.00^{b}	9.33±0.58^{b}
C.glabrata	8.33±0.58^{a}	8.33±0.58^{a}	8.33±0.58^{a}	8.33±0.58^{ab}	8.33±0.58^{ab}
C.parapsilosis	12.33±0.58^{c}	11.67±0.58^{d}	12.33±0.58^{b}	11.33±0.58^{c}	8.00±0.00^{ab}
C.krusei[3]	8.33±0.58^{a}	8.33±0.58^{a}	8.00±0.00^{a}	7.33±0.58^{a}	7.67±0.58^{a}

Mean values along same column with different affixes (a, b, c, ab, bc) are significantly different (P>0.05)

TABLE 2: Inhibition zone diameters of *Candida* isolates to methanol extracts of *Cnidoscolus aconitifolius*

	Inhibition zone diameter (mm)				
	250mg/ml	**125mg/ml**	**62.5 mg/ml**	**31.25mg/ml**	**15.625mg/ml**
Candida spp					
C.tropicalis	11.00±1.73^{b}	8.67±0.58^{a}	8.00±0.00^{a}	8.00±0.00^{ab}	8.33±0.58^{a}
C.albicans	10.67±1.15^{ab}	10.33±0.58^{bcd}	9.33±0.58^{a}	9.33±0.58^{bc}	9.33±0.58^{ab}
C. dublinensis	9.33±0.58^{ab}	9.67±0.58^{abc}	8.33±0.58^{a}	8.33±0.58^{abc}	8.00±0.00^{a}
C.krusei[1]	10.67±0.58^{ab}	11.00±0.00^{cd}	8.33±0.58^{a}	8.33±0.58^{abc}	11.00±1.73^{b}
C.krusei[2]	11.67±0.58^{b}	11.67±0.58^{d}	11.67±0.58^{b}	9.67±0.58^{c}	8.33±0.58^{a}

C.glabrata	9.33±0.58^{ab}	8.33±0.58^{a}	8.33±0.58^{a}	8.33±0.58^{abc}	8.33±0.58^{a}
C.parapsilosis	10.33±0.58^{ab}	11.33±0.58^{d}	11.33±0.58^{b}	11.33±0.58^{d}	8.00±0.00^{a}
***C.krusei*^{3}**	8.33±0.58^{a}	9.33±0.58^{ab}	9.00±0.00^{a}	7.33±0.58^{a}	8.00±0.00^{a}

Mean values along same column with different affixes (a, b, c, ab, bc) are significantly different (P>0.05)

TABLE 3: Inhibition zone diameters of *Candida* isolates to aqueous extracts of *Cnidoscolus aconitifolius*

	Inhibition zone diameter (mm)				
	250mg/ml	**125mg/ml**	**62.5 mg/ml**	**31.25mg/ml**	**15.625mg/ml**
Candida spp					
C.tropicalis	8.67±0.58^{b}	8.00±0.00^{b}	0.00±0.00	7.33±0.58^{b}	0.00±0.00
C.albicans	11.00±1.00^{c}	0.00±0.00^{c}	0.00±0.00	0.00±0.00	7.00±0.00^{b}
C. dublinensis	8.67±0.58^{b}	8.33±0.58^{bc}	8.33±0.58^{b}	8.33±0.58^{cd}	8.00±0.00^{cd}
***C.krusei*^{1}**	9.00±0.00^{b}	9.33±0.58^{cd}	8.33±0.58^{b}	9.00±0.00^{d}	9.00±0.00^{e}
***C.krusei*^{2}**	0.00±0.00	9.67±0.58^{d}	8.67±0.00^{b}	0.00±0.00	8.33±0.58^{de}
C.glabrata	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	7.33±0.58^{bc}
C.parapsilosis	11.33±0.58^{c}	8.33±0.58^{bc}	8.33±0.58^{b}	7.67±0.58^{bc}	7.67±0.58^{bcd}
***C.krusei*^{3}**	9.33±0.58^{b}	8.33±0.58^{bc}	0.00±0.00	0.00±0.00	0.00±0.00

Mean values along same column with different affixes (a, b, c, ab, bc) are significantly different (P>0.05)

Anti-candidal Effect of Ethanol Extract of *Cnidoscolus aconitifolius* Leaf Extract *In-vivo*

By Day 4, key symptoms of vaginal candidiasis, such as pruritus, erythema and tenderness of the vagina region, reduced activity, weakness, and ulcer in the nose region of some rats were seen. Mortality rate of 15% was also recorded. Five animals per group were used in the work but after infection, there was a decrease in their number (Fig. 1) due to "Too few to count" colonies (Rats A and B in Group 2) and death of some animals (Rat B in Group 3 and Rats A and D in Group 4).

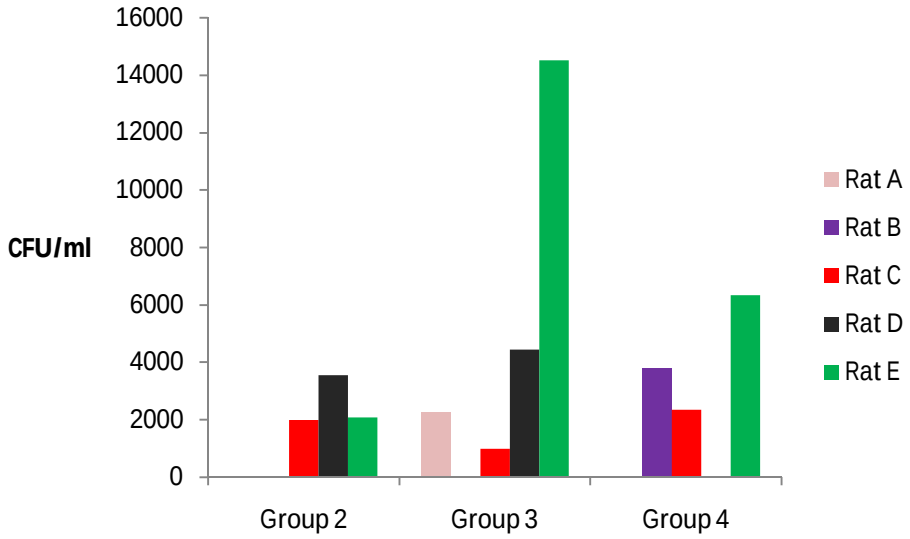

Figure 1: Colony forming unit, five days after infection.

Treatment regimen 1

Group two, being the negative control, had an increased CFU/ml for Rats A and B (Fig. 2) compared to the previous graph while Rats C and D died. After three days of treatment, the colony-forming unit of rats in Group 3 reduced drastically to "Too Few to Count" colonies, only Rat E had a CFU/ml of 4.1×10^3 from 1.45×10^4 CFU/ml obtained after infection (Fig. 3). The highest colony-forming unit in Group 4 (Rat E) after infection was 6.35×10^3 but after the first treatment regimen, this reduced to 4.35×10^3 (Fig. 4). The decrease in fungal burden led to a corresponding decrease in the number of animals presented in the graph: the too few to count (TFTC) colonies were not recorded.

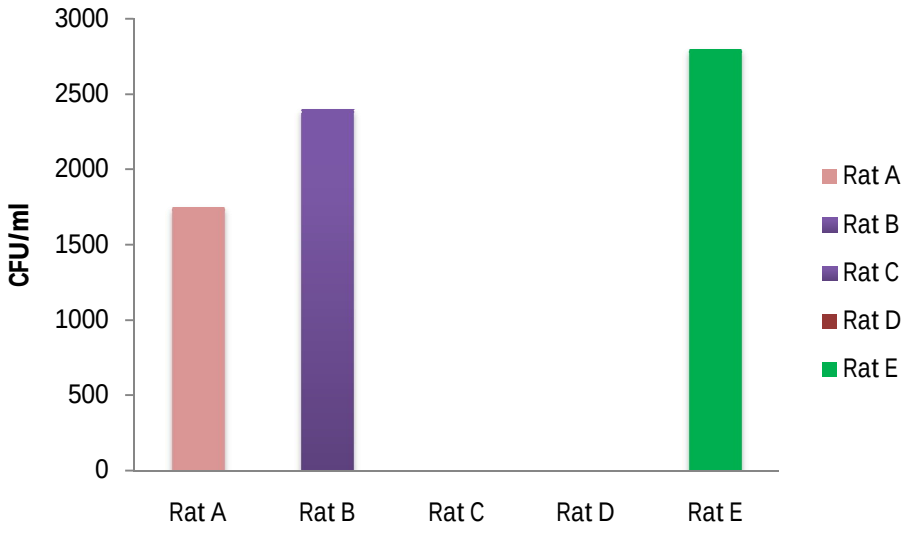

Figure 2: Colony forming unit for Group 2 after 3 days

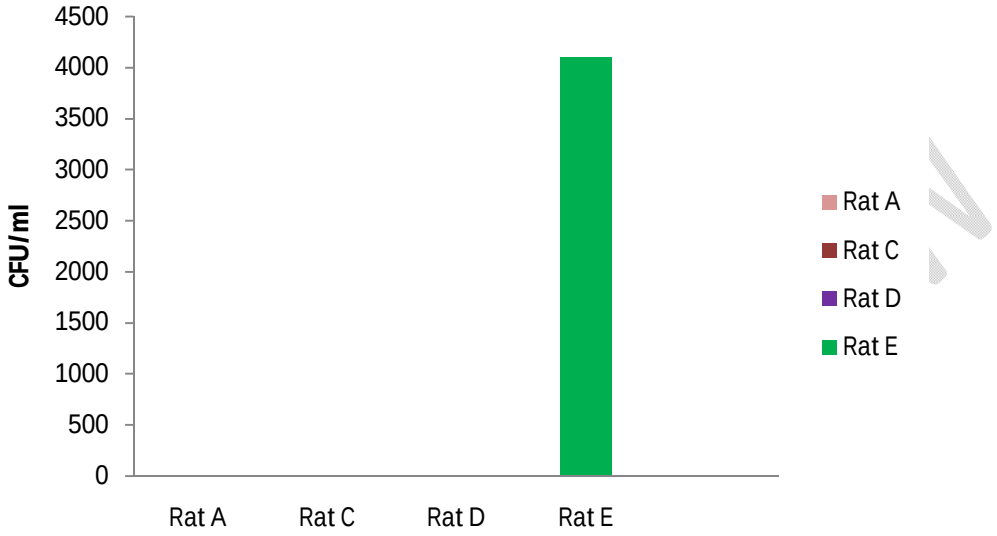

Figure 3: Colony forming unit for Group 3, after 3 days of treatment

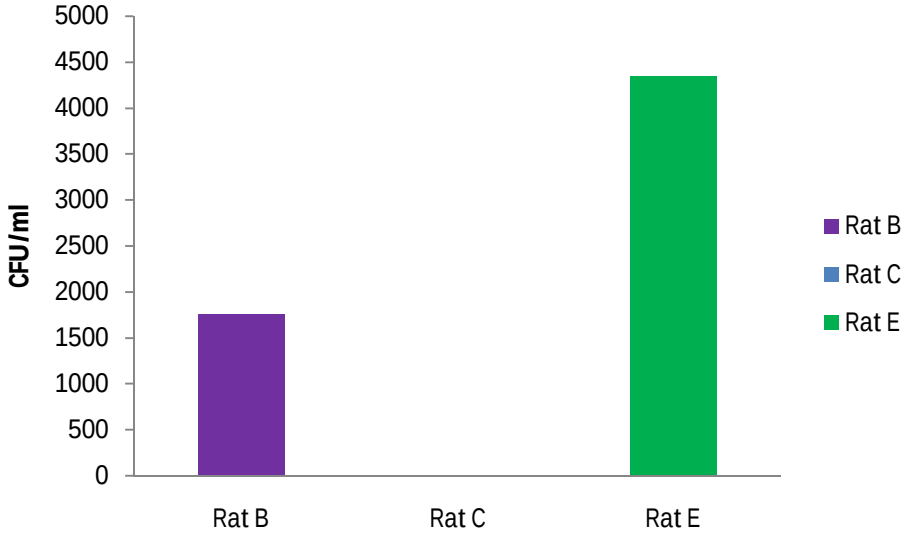

Figure 4: Colony forming unit for Group 4, after 3 days of treatment

Treatment regimen 2

After six days, the animals in Group 2 reduced to two (Fig. 5) and for Group 3, no animal was within the acceptable colony-forming unit range. For Group 4, one animal was left after six days of treatment, Rat E, and its *Candida* burden decreased further to 2.15×10^3 cfu/ml. A remarkable increase in body size was observed in Group 4 than those in other groups.

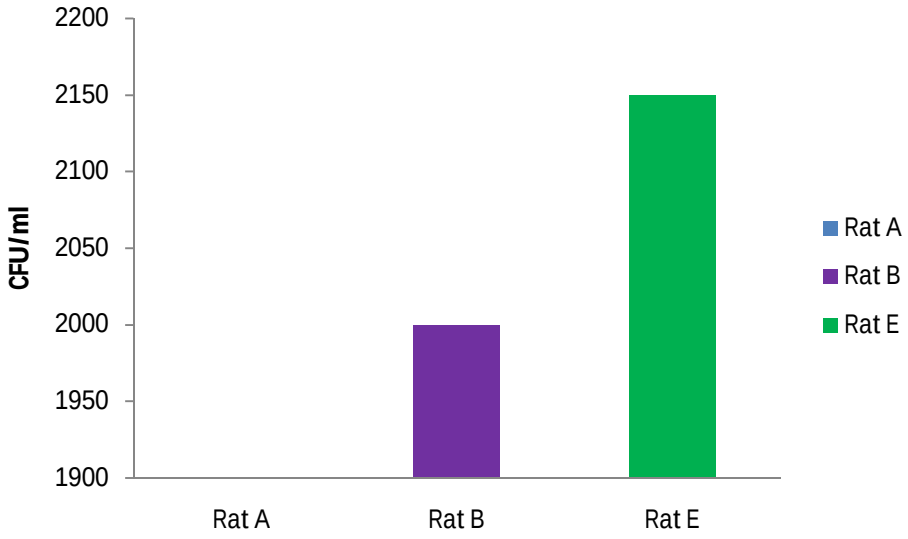

Figure 5: Colony forming unit for Group 2, after 6 days.

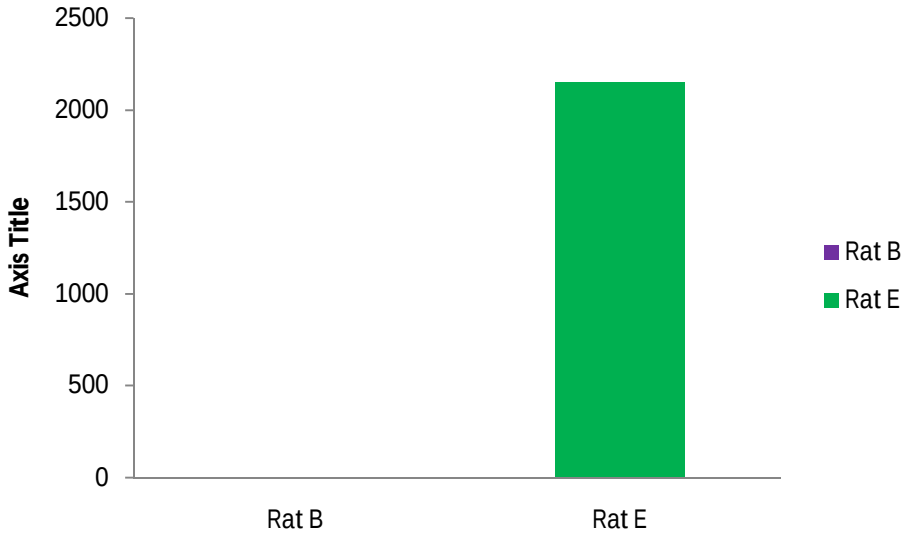

Figure 6: Colony forming unit for Group 4, after 6 days of treatment.

Discussion

This study shows that *Cnidoscolus aconitifolius* leaves extract has positive anti-candidal activity against various clinical isolates of *Candida* species and the plant anticandidal activity varied with the extract used. The ethanol extract was more potent than the methanol and aqueous extract. At the highest concentration of 250mg/ml, *Candida tropicalis*, *C. krusei* and *C. parapsilosis* showed the highest inhibition zone diameter against the ethanol plant extract followed by the methanol and aqueous extract. Most of the isolates were concentration dependent and sensitive to the extract.

Candida albicans was sensitive to both ethanol and methanol extracts at all concentrations but little or no IZDs to the aqueous extract were recorded. This correlated with the findings of Hamid *et al.,* (2016) who worked on the antifungal activities of *Cnidoscolus aconitifolius* using n-Hexane, ethyl acetate, and methanol extracts. The methanol extract of Hamid *et al.,* (2016) exhibited IZDs of 16mm, 14mm, 12mm, and 10mm at concentrations 200mg/ml, 100mg/ml, 50mg/ml, and 25mg/ml respectively against *C. albicans*, thereby confirming the plant's potency to clear the infection caused by *C. albicans*. This antifungal property may be due to the presence of phytochemicals such as phytate, tannins and sapogenin in *Cnidoscolus aconitifolius* leaves extract (Adaramoye *et al.,* 2011).

However, the study carried out by Senjobi *et al.,* (2011) and Oyagbemi *et al*., (2011) using ethanol extract of *Cnidoscolus aconitifolius,* recorded no zone of inhibition against *C. albicans.* Disparities in results seen in the various works could be due to preparation techniques of extracts of *C. aconitifolius* leaf, variation in the soil nutrient that contributes to the bioactive constituents of the plant, choice of solvent and different strains of *Candida* species worked on by the researchers (Ezeadila *et al.,* 2020).

Against *C. dublinensis* and *C. glabrata*, the extracts exhibited limited anti-candidal activity based on the IZDs shown. *C. glabrata* was sensitive to all concentrations of the ethanol extract but the IZDs gotten were not concentration dependent: 8.33mm was recorded at all concentrations. This trend was also seen with methanol extract but with aqueous extract, no zone of inhibition was seen except at 15.625mg/ml where 7.33mm was recorded.

The highest inhibition zone diameter produced by ethanol extracts of the plant against *C. krusei*[1] was at the lowest concentration (15.625mg/ml) while the least IZD was at the highest concentration (250mg/ml). According to Trease and Evans (1978), this may be because "at that high concentration the diffusion of the extracts through the medium was very slow and the growth of the microorganism was faster than the extract could diffuse". *C.krusei*[1], *C.krusei*[2] and

C.krusei[3] were sensitive to all the extracts at all concentrations but ethanol extract were better inhibitors than methanol and aqueous extracts.

The inhibition zones diameters produced by the ethanol, methanol, and aqueous extract against *C. parapsilosis* were not concentration dependent but the organism was sensitive to the extracts at all concentrations. High IZDs were recorded for the ethanol and methanol extract against *C. parapsilosis* than for other *Candida* species. "The zone of inhibition exhibited by the plant extracts gives evidence to the fact that *Cnidoscolus aconitifolius* is an antifungal plant and hence can be used for the treatment of illness caused by *Candida* species" (Hamid *et al.,* 2016). According to Nweze and Eze (2009), "In cases where there was no activity by any of the plant extracts, it may be due to the absence of some secondary metabolites, or the presence of some in low concentration. The type of strains used or changes in any of the factors that affect rate of microbial growth or rate of diffusion of the test agent might also be a factor".

In addition, since crude extracts of the plants were used, the efficacy of the unpurified secondary metabolites in the extracts might not be optimum (Chipinga *et al.*, 2018). Otitolaiye and Asokan (2016), Rahman and Anwar (2008) and Oyegbemi and Odetola, (2010), who worked on extracting phytochemicals from *Cnidoscolus aconitifolius* leaves using GC-MS technique, confirmed the presence of various phytochemicals with antifungal properties. Phytochemicals such as Caryophllene oxide, IH-cycloprop (e) azulene, Farnesol, acetate, 1-0-Tolylprop - 2 - en-1-1one, Longiverbenone and Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl. These bioactive substances further support the antifungal efficacy of this plant, thereby confirming its use in ethnopharmacology. Ethanol extract was the most potent extract in this study followed by methanol and aqueous extract. The presence of considerable amounts of phytate, sapogenin, tannins and flavonoids that have antifungal properties (Thangjam *et al*., 2020; Alexander-lindo *et al*., 2020), in the ethanol extract of *Cnidoscolus aconitifolius* leaves could be the reason for the extract's better antifungal effect than others and gives credence to the claims of the plant's antimicrobial ability.

In-vivo study of the ethanol extract of *Cnidoscolus aconitifolius* leaves using female Wistar rats infected with *Candida albicans* revealed its antifungal activity and ability to increase body weight. During administration of the plant extract, the symptoms of vaginal candidiasis waned and a decrease in *Candida* burden from 6.35×10^3 CFU/ml gotten after infection to 2.15×10^3 CFU/ml after 6 days of treatment was observed. The negative control showed a decrease in *Candida* burden with time because as the disease progresses, the immune system becomes

enabled to clear the pathogens though at a slower rate. The findings of this study also showed that Nystatin had a better anti-candidal effect than the ethanol extract of the plant. This could be due to the crude nature of the extract compared to the purified standard antifungal agent. The results however revealed the anti-candidal potential of the plant extract.

Limited scientific research has been done on the anti-candidal activity of *Cnidoscolus aconitifolius* leaf using different *Candida* species, most are on *C. albicans.* However, our results demonstrate that apart from *C. albicans, Cnidoscolus aconitifolius* also has a potent activity against other *Candida* species; thus, the plant can apparently be used in producing antifungal drugs for the eradication of candidiasis.

We suggest further research on the antifungal properties of each phytochemical possessed by *Cnidoscolus aconitifolius* for their individual and synergistic anti-candidal abilities to be properly maximized for drug development.

Conclusion

The research provided information on the anticandidal activity of *Cnidoscolus aconitifolius* leaves against clinical isolates of different *Candida* species *In-vitro* and *In-vivo*, providing data that will serve as a baseline on the ability of ethanol extract of *Cnidoscolus aconitifolius* leaves to treat candidiasis.

Ethical Approval and Consent

Informed consent was obtained from patients who tested positive with oral thrush and vaginal candidiasis attending Chukwuemeka Odumegwu Ojukwu University. Ethical approval for the use of animal Wistar Rat) was obtained from Ethics Committee for Animal Experimentation of Nnamdi Azikiwe University, Awka. protocol number NAU/AREC/2022/0014.

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