Original Research Article

Antifungal activity of Antsbush (*Struchiumsparganophora*): a future remedy for candidiasis and cryptococcosis

Abstract

Background – The incidence of Candidiasis and Cryptococcosis has increased rapidly and the use of some current antifungal agents (AFAs) has become limited due to the emergence of acquired antifungal resistance.

Objectives-This study aims to evaluate the effectiveness of *S. sparganophora*leaf extracts against*C. albicans* and *C. neoformans* using different solvents and to also determine whether there was any difference between the antifungal effects of the *S. sparganophora*and conventional antifungal agents.

Method-Dried pulverised leaves were macerated using different solvents and concentrated using a rotary evaporator. Sterile filter paper discs were soaked in different concentrations of the various extracts. Antifungal discs were placed on SDA platesseeded with fungi. All plates were incubated and zone of inhibitions (ZOIs)were measured and expressed as mean ± SD.

Results-*S. sparganophora*leaf extracts showed large ZOIs especially at the 100mg/ml concentrations against all organisms tested. The largest ZOI was seen for the hexane extract against *C. neoformans* (35.5±5mm)at 100mg/ml.Large ZOIs against ATCC *C. albicans* was noted with the ethyl acetate extracts (11.3±1.5mm) at 100mg/ml. Large ZOI against*C. albicans*In-House B was noted for the hexane extract (18.5±3.5mm) at 100mg/ml. A minimum inhibitory concentration as low as 6.25mg/ml against *C. neoformans* suggest that small dosage of the antsbush extract may be effective in treating Cryptococcosis. No ZOI was observed against fluconazole against ATCC *C. albicans* and *C. albicans* In-House B, while that of the hexane extract was 10.0±0.0mm and 18.5±3.5mmat 100mg/ml concentration respectively and that of the ethyl acetate extracts was 11.3±1.5mm and 12.7±1.2mm at 100mg/ml concentration respectively. The ZOI for fluconazole against *C. neoformans* was 32.0±2.0mm, while that of the hexane extract was 35.5±5mmat 100mg/ml concentration.

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Conclusion-We conclude that *S. sparganophora*leaf extracts have antifungal activity and in some cases showed greater activity when compared to the conventional fluconazole.

Keywords: Antifungal agents, *S. sparganophora*, Candidiasis, Cryptococcosis, Solvents

Introduction

A wide variety of clinical infectious diseases are caused by fungi, some of which may be severe or fatal. The most common ones are*Cryptococcus neoformans, Candida albicans, Candida auris, Aspergillus fumigatus, Rhizopus oryzae* and *Aspergillus fumigatus*[1].The prevalence of co-infections with fungal pathogens has been increasing steadily for a number of years in association with patients who have HIV/AIDS, cancer, chronic respiratory diseases and those who undergo transplants [2]. Patients with invasive fungal infections have high risk of mortality if there is co-morbidity. A 90-day mortality rate for transplant patients with candidemia is reported to be 22-44% depending on the species involved [3]. The prevalence of hospital acquired fungal infections and community acquired fungal infections especially the COVID-19 has increased exponentially [4]. The rapid onset of the COVID-19 pandemic has posed great difficulty worldwide including the emergence or reappearance of fungal diseases which are resistant to conventional antifungal treatments [5].Such is the seriousness of the problem that the WHO has released a list of priority fungal pathogens and the critical priority group includes *C. albicans*, *Candida auris*, *Cryptococcus neoformans,* and *Aspergillus fumigatus* [6].

Antifungal agents (AFAs) such as amphotericin B, azole antifungals, echinocandins and flucytocyine are current antifungal medications [7].The use of AFAs has become limitedbecause of the emergence of acquired antifungal resistance tothe some of the currently available AFAs. For example, triazole drugs such as itraconazole, fluconazole, voriconazole is exponentially increasing and is thought to be associated with the over-expression of the ABC transporters that transport drugs extracellularly [8]. Antifungal therapy especially for invasive antifungal infections is now more worrying because of the recent emergence of fungi that are resistant to more than one class of AFA [9]. For example, the resistance to the azoles and echinocandins by *Candida sp.* has been recorded.

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Resistance to AFAs presents a monumental problem to hospitalisedpatients; especially those who are immunocompromised. Some fungi develop resistance to AFAs naturally even without being exposed to the AFA. The resistance to AFAs by fungal pathogens has been noted such as the resistance to echinocandins by *Cryptococcus sp*[10].

Due to the toxicity of currently prescribed antifungal medications along with the rising incidence of etiologic agent resistance, therapy for *Candida albicans* and *Cryptococcus neoformans* are becoming more complex [11] [12]. *Candida* sp. causes several types of infections but bloodstream infections among hospitalized and immunocompromised patients are the most fatal [13]. *Cryptococcus neoformans* causes meningoencephalitis in immunocompromised patients that can also be fatal [14]. Therefore, new methods of treating fungal diseases especially *Candida albicans* and *Cryptococcus neoformans*will remain a priority for the foreseeable future. Medicinal plants offer alternative and complementary therapy for dealing with the prevalence of resistant strains of fungal species.

*Struchiumsparganophora*is part of a family of plants called Asteraceae. This family is very large, and the species have many uses. These include oil production, food preparation, and most importantly as herbal remedies [15] [16]. *Struchiumsparganophora,* often known as 'water bitter leaf,' in Africa and 'antsbush' in Guyana, is used to treat a range of communicable and non-communicable diseases including dysentery, malaria, candidiasis, cancer and diabetes [16][17].

Obtaining the best products or compounds fromplants (otherwise known as phytochemicals or metabolites) often depends on the type of solvents used during the extraction process, and the type of extraction technique employed. Plants contain different types of metabolites (such as flavonoids, alkaloids, sterols, saponins, and tannins) in their stem, roots and leaves and these have been actively studied for their antibacterial, antifungal and even anti-tumour properties [18].Solvents frequently used in extraction compounds from medicinal plants include polar solvent, semi-polar solvents, and non-polar solvents [19].Methanolwhich is polar in nature, acetone which is semi-polar and hexane which is non-polar are some of the solvents commonly used. Non-polar solvents are more likely to extract non-polar compounds such as alkaloids, whilst polar solvents would be good for extracting polar compounds like saponins [20].

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The bioactive compounds obtained from medicinal plants are utilized for a number of reasons. However, the method involved in producing them is usually the same. Some current extraction techniques used are maceration, soxhlet extraction, superficial extraction, infusion, digestion, decoction, percolation, ultrasound-assisted, and microwave-assisted extractions[19].The most common methods used to determine antifungal activity including the most effective concentration of the antifungal agent are the disc diffusion and broth dilution methods [21]. Phytochemical components are thought to be associated with specific activity. For example flavonoid is associated with antifungal effects [22]. The phytochemical composition and concentration in medicinal plants tends to vary based species-specific biochemical interactions, geographical location, extraction techniques, and extraction solvent and thus influence antimicrobial activity [23].

A review of the literature shows that antsbush contain several phytochemicals. The ethanolic extract of the Antbush leaves contains alkaloids, tannin, saponins, phlobatannin, anthraquinone, and glycosides [24]. Luteolin, 3 methyl 2, 6, hexacosedienol and vernodalin have been identified in Antbush and are said to inhibit the growth of *A. niger* and *C. albicans* [25]. The presence of these compounds is proof of their anti-infective use in herbal medicine and suggests their potential as natural therapy for various illnesses.

When exposed to leaf extracts, microbial cells could be destroyed by the irregular breakdown of the intracellular matrix which results in the rupturing of cell walls and membranes. This rupturing mechanism allows the cell membrane to be easily penetrated, thus allowing the escape of the cell's important components [23] [26].Antbush leaves have demonstrated antifungal activity against *A. niger* and to some extent *C. albicans* [25],however it is unclear whether the subspecies of *S. sparganophora*found on the coast of Guyana, has antifungal activity.

There is a lack of scientific information about the antifungal activity of Antbush in the literature. Furthermore, there is a paucity of information and even awareness about ant’s bush leaves usage in Guyana and it is not well known except in a few rural communities. We present the findings of an evaluation of the effectiveness of extracts from the leaves of a variety of *S. sparganophora*which is indigenous to Guyana, against two fungi -namely *Candida albicans* and *Cryptococcus neoformans,* using different solvents. Furthermore, the investigators sought to

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determine whether there was any difference between the antifungal effects of the *S. sparganophora*and conventional AFA's.

Methods

Collection and preparation of Plant materials

Antbush was obtained from a site in Kimbia, Berbice River. The plantswere identified and verified by the Centre for Study of Biological Diversity, University of Guyana. Leaves that showed no sign of deterioration were washed three times with distilled water and left to air dry at room temperature for 3-4 weeks, carefully avoiding sunlight. A disinfected food processor was utilized to grind the leaves into a coarse powder. The powdered plant material was packed into sealed bags.

Fig. 1 *S. sparganophora* leaves

Extractionof compounds

Thirty grams (30g) of pulverisedAntbush were soaked in 300mls of four different solvents namely: hexane, ethyl acetate, methanol, and chloroform.Maceration of the ground leaves were carried out in tightly sealed and dark bottles which were placed in a dark cupboard for 24 hours under occasional shaking. The different extracts were filtered using sterile Whatman No. 1 filter papers and sterile funnels. The extraction was repeated three times with the same amount of solvents and each time filtration was performed. The filtered extracts were consolidated and concentrated to dryness by evaporating the solvents under reduced pressure

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using a rotary evaporator at 45 °C (Figure 2). All the crude extracts were stored at 4°C in the dark until needed [27].

Fig. 2- The rotary evaporator used to concentrate the extracts

Antimicrobial susceptibility testing

Concentrations of 5mls each of 100mg/ml (crude extract), 50 mg/ml, 25 mg/ml and 12.5 mg/ml were prepared via serial dilution using the stock solutions of Chloroform, methanol, ethyl acetate and hexane with their respective crude extracts for Antbush. Six millimeter (6mm) antimicrobial discs were prepared using sterile Whatman No. 3 filter papers and then soaking them in the varying concentrations of the different leaf extracts overnight. These discs were then used to perform antimicrobial susceptibility testing with known microorganisms [27].

The test organisms used were *Candida albicans* (three strains- *C. albicans* 24058; and two in-house strains: In-house A (IHA) and In-House B (IHB)) and an in-house strain of *Cryptococcus neoformans.*The in-house strains were obtained from Eureka Medical Laboratory and the Microbiology Department of Georgetown Public Hospital Corporation.The Kirby-Bauer disc diffusion method on Sabouraud’s Dextrose Agar (SDA) for the fungi was performed using Clinical Laboratory Standards Institutes (CLSI) guidelines with a 0.5 McFarland standard.

To compare the performance of the plant extract discs, discs soaked in pure solvent, served as the negative controls. The discs were placed in triplicate on each plate after seeded with the appropriate microorganism. Plates were incubated at 37^{o}C for 48-72 hours to facilitate the longer incubation time of fungi. Zones of inhibition were measured in millimeters and these

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were validated by two microbiologists. For comparing the performance of the plant extract, we also include positive controls such as Ketoconazole and Fluconazole (1% each) [27]. For the purpose of this study, a mean zone of inhibition (ZOI) of ≥ 10.0 mm was considered as an effective antifungal, while a ZOI of ≥ 20mm is considered a very effective antifungal. If the fungus grew up to the disc, then it was considered resistant, and the value was recorded as 6mm.

Fig. 3- Antifungal susceptibility testing (Placing the antifungal discs on the SDA plates)

Determining the Minimum Inhibitory Concentration of the plant leaf extracts

The MinimumInhibitory Concentration (MIC) is the lowest concentration of the antifungal agent that inhibits the growth of a fungus within a fixed period of time [28] [29].Itgives details about the susceptibility or resistance of a fungus to an antifungal agent. This in turn helps to determine the right treatment options especially in terms of dosage to patients. In this study, the MIC for the various leaf extracts was determined using a method adopted by Balouiri et al (2016) [28].

Statistical analysis

The independent variables were the various concentrations of the Antsbush extract for the four solvents and the organisms they were tested against. The dependent variables were the ZOIs which denote susceptibility.This study used both descriptive and statistical analysis. The data

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from the observations were expressed as mean ± Standard Deviation (SD) using tables and graph via Microsoft excel 2007. Data were also analysed statistically using SPSS version 27.

Hypothesis

Null hypothesis

1. There is no statistically significant difference between the various concentrations of the extracts and ZOIs.
2. There is no statistically significant difference between the various fungi and ZOIs.
3. There is no statistically significant difference between the various solvents/positive controls and ZOIs.

Alternate hypothesis

1. There is a statistically significant difference between the various concentrations of the extracts and ZOIs.
2. There is a statistically significant difference between the various fungi and ZOIs.
3. There is a statistically significant difference between the various solvents/positive controls and ZOIs.

The Kolmogorov-Smirnov and Shapiro-Wilk tests were used to determine whether the ZOIs were normally distributed forAntsbush extracts. Both p-values were less than the common alpha level of 0.05 (Table 1) suggesting that the distribution of the ZOIs significantly deviates from a normal distribution in the dataset. Therefore, the non-parametric Kruskal-Wallis H test was used to determine statistically significant differences between thedifferent concentrations of the extracts and ZOIs,the different fungi and ZOIs, and different solvents/positive controls and ZOIs.

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Table 1- Tests of Normality

	Kolmogorov-Smirnov^a			Shapiro-Wilk		
	Statistic	Df	Sig.	Statistic	df	Sig.
Inhibition zones	0.290	56	0.000	0.606	56	0.000

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a. Lilliefors Significance Correction

Results

Antifungal activity of *S.sparganophora*leaf extracts

The result from this study showed that *S.sparganophora*leaf extracts showed antifungal activity (Fig. 5). The most effective extract was the 100mg/ml hexane extract. Table 2 shows the ZOI at different concentrations for the methanolic extracts against the different fungi. A mean ZOI ≥ 10.0 mm was noted only for *C. neoformans* at up to 50mg/ml.The largest ZOI was seen for *C. neoformans* at 100% concentration(11.0±0.0mm).

Table 2– Activity of *S.sparganophora* methanolic extracts against specific fungi.

	Zone of inhibition (mean±SD) in mm at different concentrations (%)				**MIC (mg/ml)**	**NC (mm)**
Fungi	**100**	**50**	**25**	**12.5**		**Pure Met**
C. albicans ATCC	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	-	6.0±0.0
C. albicans IHA	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	-	6.0±0.0
C. albicans IHB	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	-	6.0±0.0
C. neoformans	**11.0±0**	**10.0±0**	9.6±0.5	6.5±0.5	12.5	6.0±0.0

NC=Negative control, 6.0 mm ZOI indicates that the fungus grew right up to the disc, - = No MIC

Table 3 shows the ZOI at different concentrations for the for hexane extracts against the different fungi. A mean ZOI ≥ 10.0 mm was noted for all the fungi at 100mg/ml concentrations and up to 50mg/ml for *C. neoformans*. The largest ZOI was seen for *C. neoformans* at 100% concentration (35.5.0±5.0mm).

Table 3 – Activity of *S.sparganophora* hexane extracts against specific fungi.

	Zone of inhibition (mean±SD) in mm at different concentrations (mg/ml)				**MIC (mg/ml)**	**NC (mm)**
Fungi	**100**	**50**	**25**	**12.5**		**Pure Et**

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C. albicans ATCC	**10.0±0**	9.0±0.0	6.0±0.0	6.0±0.0	25	6.0±0.0
C. albicans IHA	**10.0±2.0**	8.0±0.0	6.0±0.0	6.0±0.0	25	6.0±0.0
C. albicans IHB	**18.5±3.5**	9.0±0.0	6.0±0.0	6.0±0.0	25	6.0±0.0
C. neoformans	**35.5±5**	**10.0±0.0**	7.0±0.0	6.0±0.0	12.5	6.0±0.0

NC=Negative control, 6.0 mm ZOI indicates that the fungus grew right up to the disc.

Table 4 shows ZOI at different concentrations for ethyl acetate extracts against the different fungi. A mean ZOI ≥ 10.0mm was noted for the 100mg/ml concentration for *C. albicans* ATCC, *C. albicans* IHB and up to 12.5mg/ml for *C. neoformans*.The largest ZOI was seen for *C. neoformans* at 100mg/ml concentration (15.6±0.6mm). Large ZOI was noted up to the last concentration (12.5mg/ml) for *C. neoformans.*

Table 4 – Activity of *S.sparganophora* EA extracts against specific fungi.

	Zone of inhibition (mean±SD) in mm at different concentrations (mg/ml)				**MIC (mg/ml)**	**NC (mm)**
Fungi	**100**	**50**	**25**	**12.5**		**Pure Eth**
C. albicans ATCC	**11.3±1.5**	8.3±0.6	7.0±0.0	6.0±0.0	12.5	6.0±0.0
C. albicans A	7.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	50	6.0±0.0
C. albicans B	**12.7±1.2**	8.3±0.6	6.0±0.0	6.0±0.0	25	6.0±0.0
C. neoformans	**15.6±0.6**	**14.0±1.0**	**13.3±1.1**	**10.3±0.6**	6.25	7.3±1.6

NC=Negative control, 6.0 mm ZOI indicates that the fungus grew right up to the disc.

Figure 4- ZOI of*S.sparganophora* leaf extracts against the fungi.

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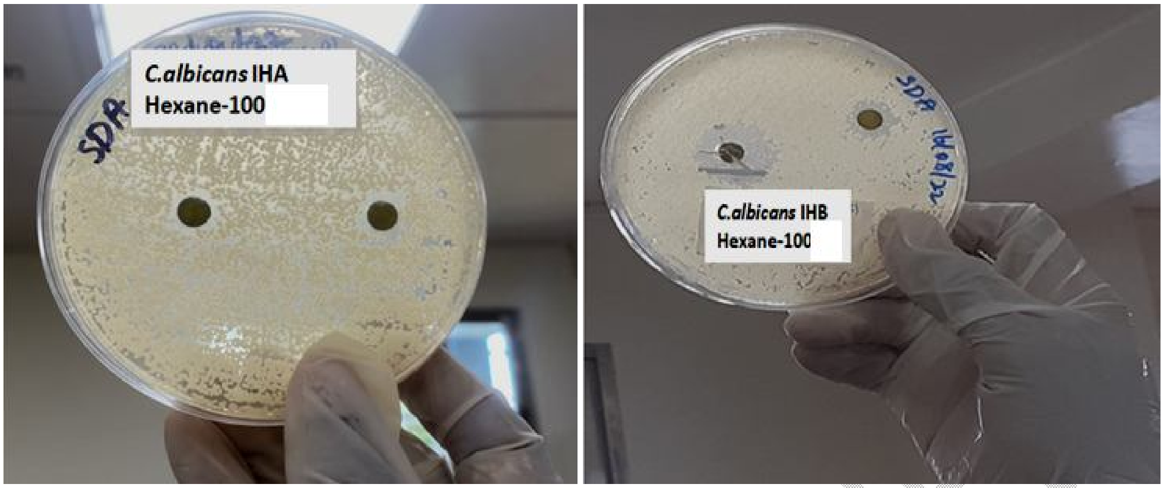
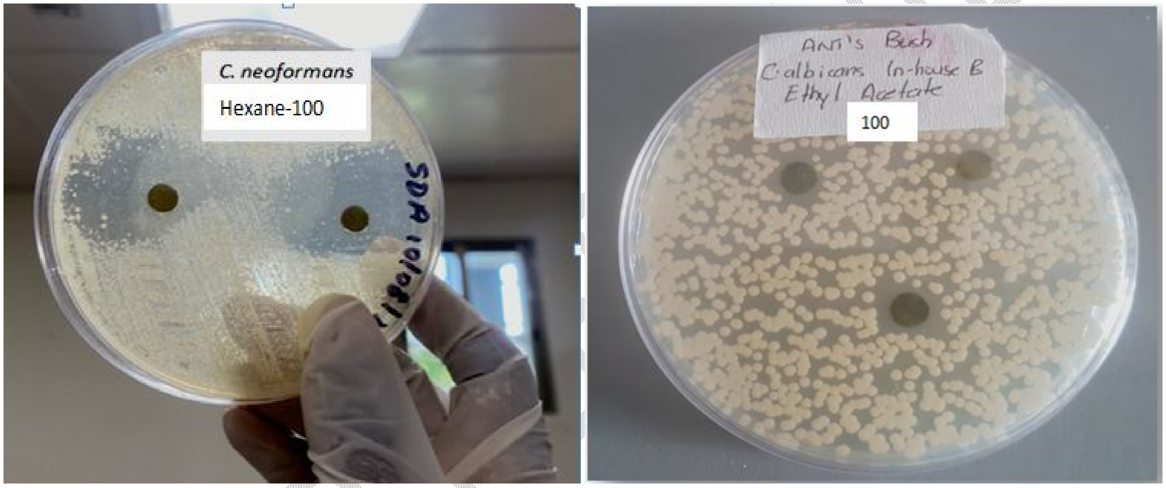


a. *C. albicans* IHAat 100mg/ml concentration (HE) b. *C. albicans* IHBat 100mg/ml concentration (HE)



c. *C. neoformans* at 100mg/ml concentration (HE) d. *C. albicans* IHBat 100mg/ml concentration (EAE)

e. C. neoformans at 100 mg/ml and 50mg/ml concentration (EAE)

HE-Hexane extract, EAE-Ethyl Acetate Extract

It is worthy to note that no ZOI was noted for the different concentrations for Antbush-chloroform extracts against the different fungi.

Antifungal activity of two current AFAs

Table 5 shows the antifungal susceptibility to the different fungi. It must be noted that there was no ZOI seen for ATCC *C. albicans* and *C. albicans* IHB to fluconazole, whilst ZOI was seen with the other strains of fungi. The largest ZOI was seen for *C. neoformans* followed by*C. albicans* IHA with both ketoconazole and fluconazole.

Table 5- Activity of Antifungals against the specific fungi.

Fungi	**Ketoconazole** **ZOI (mean±SD) in mm**	**R/S**	**Fluconazole** **ZOI (mean±SD) in mm**	**R/S**
C. albicans ATCC	11.0 ± 0.9	R	6.0 ± 0.0	R
C. albicans IHA	31. 0 ± 0.9	S	14.0 ± 4.0	S
C. albicans IHB	23.3 ± 1.3	S	6.0 ± 0.0	R
C. neoformans	39.5±0.5	S	32.0±2.0	S

R- Resistant, S-Sensitive

Comparing the Antifungal activity of the leaf extracts against two current AFAs.

The ZOI was smaller with fluconazole than those seen for some of the Antbush extracts. The ZOI for fluconazole against ATCC *C. albicans* and *C. albicans* IHB was 6mm, while that of the hexane extract was 10.0±0.0mm and 18.5±3.5mmat 100mg/ml concentration respectively and that of the EA extracts was 11.3±1.5mm and 12.7±1.2mm at 100mg/ml concentration respectively. The ZOI for fluconazole against *C. neoformans* was 32.0±2.0mm, while that of the hexane extract was 35.5±5mmat 100mg/ml concentration.

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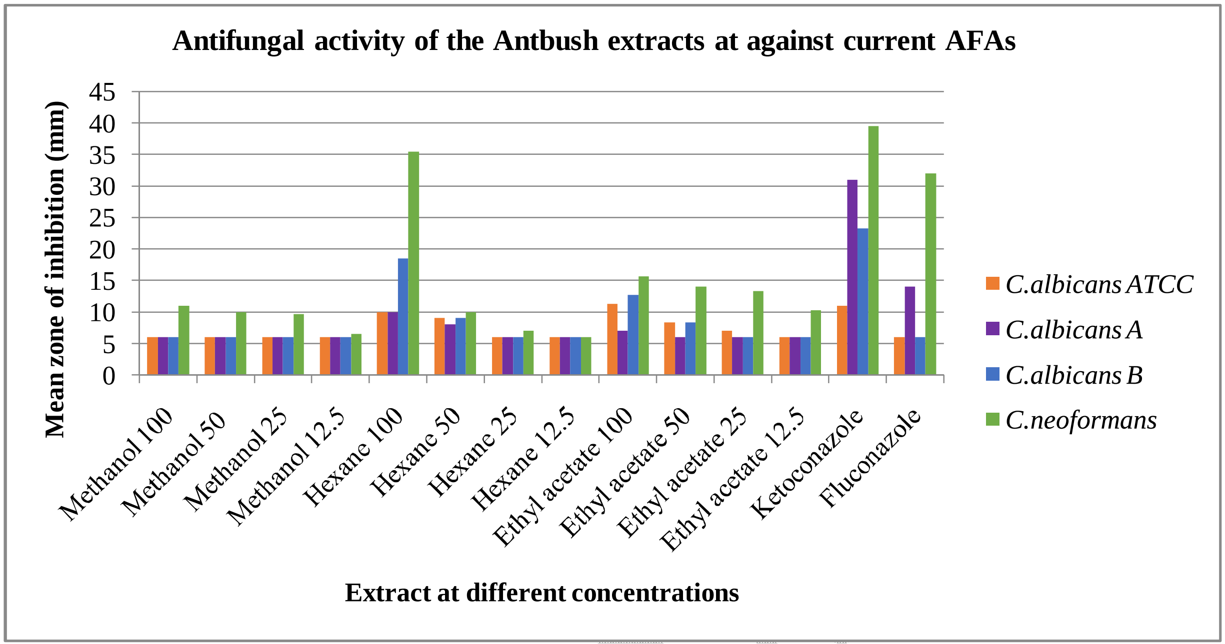
Fig 5 - The antifungal effects of the Antbush extracts at varying concentrations against current AFA (Ketoconazole and Fluconazole).



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The MIC of the S.sparganophora leaf extracts

There was no MIC exhibited by methanolic extracts against the threes stains of *C.albicans*, whilst the MIC exhibited against *C. neoformans* was 12.5mg/ml. The MIC exhibited by hexane extracts against *C.albicans*ATCC was 25mg/ml; against*C.albicans*IHA, it was 25mg/ml; against *C.albicans*IHB, it was 25mg/ml; and against *C. neoformans*, it was 12.5mg/ml. The MIC exhibited by EA extracts against *C.albicans*ATCC was 12.5mg/ml; against*C.albicans*IHA, it was 50mg/ml; against *C.albicans*IHB, it was 25mg/ml; and against *C. neoformans*, it was 6.25mg/ml.

Statistical findings

Concentrations of extracts and ZOIs

Table 6, 7 and 8 shows the descriptive statistics, ranks, and Kruskal-Wallis test results related to the concentration of the extracts and the ZOI respectively. The results shows that higher mean ranks indicate larger typical ZOIs, suggesting stronger inhibition at those concentration levels. Thus, 100 mg/ml has the largest inhibition effect, while 12.5 mg/ml has the smallest. The p-value of 0.004 is less than the 0.05 threshold.

Solventtype/ AFAs and ZOIs

Table 6, 7 and 8 shows the descriptive statistics, ranks, and Kruskal-Wallis test results related to the solvent/AFA and the ZOI respectively. This suggests that the type of solvent/AFA used has a significant impact on the effectiveness of inhibition. Specifically, ketoconazole seems to have the most substantial effect, as reflected by its high mean rank. However, of the three solvent extracts, the EA extract has the highest impact on ZOIs. The p-value of 0.006 is below the 0.05 threshold.

Type of fungi and ZOI

Table 6, 7 and 8 shows the descriptive statistics, ranks, and Kruskal-Wallis test results related to the type of fungi and the ZOI respectively. The higher ranks of *C. neoformans* compared to the three strains of *Candida albicans* suggest it has larger ZOIs, pointing to its greater susceptibility to the extracts/AFAs. The p-value of 0.003 is below the 0.05 threshold.

Table 6- Descriptive statistics for the different variables

Variable	N	Mean	Std. Deviation	Minimum	Maximum	Percentiles		
						Q1	Median	Q3
ZOI	56	10.26	7.698	6	40	6.00	6.75	10.83
Concentration	56	2.14	1.368	0	4	1.00	2.00	3.00
ZOI	56	10.26	7.698	6	40	6.00	6.75	10.83
Solvent/AFA	56	2.36	1.182	1	5	1.00	2.00	3.00
ZOI	56	10.26	7.698	6	40	6.00	6.75	10.83
Organism	56	2.50	1.128	1	4	1.25	2.50	3.75

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Table 7 – Ranks for the different variables

ZOI	Concentrations	N	Mean Rank
	100mg/ml	12	34.08
	50mg/ml	12	27.67
	25mg/ml	12	19.92
	12.5mg/ml	12	16.33
	Total	***48***	
	Solvents/ AFAs	**N**	**Mean Rank**
	Methanol	16	19.75
	Hexane	16	28.47
	Ethyl acetate	16	30.59
	Ketoconazole	4	51.13
	Fluconazole	4	32.63
	Total	***56***	
	Organisms	**N**	**Mean Rank**
	C. albicans ATCC	14	24.21
	C. albicans IHA	14	23.50
	C. albicans IHB	14	24.57
	C. neoformans	14	41.71
	Total	***56***	

Table 8- Kruskal-Wallis test

ZOI	Chi-Square	df	Asymp. Sig.
Concentrations	13.527	3	0.004
Solvents/AFAs	14.449	4	0.006
Organisms	13.844	3	0.003

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Discussion

Antifungal activity of *S.sparganophora* leaf extracts

Thehexane, methanol and ethyl acetate extracts showed antifungal activity, whilst the Chloroform extract did not. The most effective extract was the hexane, although statistical analysis revealed that it was the ethyl acetate extract because a higher mean rank was obtained.We postulate that the hexane extract was most effective because interestingly it worked better against all the fungi at 100mg/ml concentration and for *C. neoformans*, at 50mg/ml. The hexane extracts worked best against100mg/ml *C. neoformans* and *C. albicans* IHB. The ethyl acetate extract worked well against three out of the four fungi at 100mg/ml concentration and for *C. neoformans*up to 12.5 mg/ml.The EA extracts worked best against *C. neoformans* and *C. albicans* IHB at 100mg/ml. The methanolic extract was effective only against *C. neoformans* up to 50mg/ml concentration.

The hexane and ethyl acetate extracts worked better for the *C. albicans* than the methanolic extracts. The finding for *C. albicans*, where hexane extracts seemed to work better than methanolic extracts, were also found in a similar study done in Nigeria [16]. Further research, using different extraction techniques, led to the isolation of the metabolite vernodalinol, from chloroform-methanol extracts. Thismetabolite showed antifungal activities against *C. albicans* and *A.niger* [25]. Contrasting results were noted in a study done by Oboh (2006) where the ethanolic leaf extracts was effective against *Candida albicans* [24]. However, a similar zone of inhibition (18.0 mm) was noted in our study with the hexane extract for *C.albicans* IHB. He also showed that the extract was effective against *Penicillium sp.*(14.0 mm) and *Saccharomyces cerevisiae* (15.0 mm)but ineffective against *Aspergillus fumigatus*, *Fusarium solani*, and *Aspergillus flavus*. However we did not test the antifungal activity of our extractsagainst those fungi.

Our current study revealed that the hexane extracts worked best when compared to the ethyl acetate and methanolic extracts against *C. neoformans*and the extracts seemed to work better for *C. neoformans* than for *C. albicans.* A perusal of the literature shows that no study was done with *C. neoformans*and *S. sparganophora*;nevertheless, we are enthusiastic about our preliminary results for the activity of all three extracts against this fungus which show great promise as an alternative for treatment for cryptococcosis. Treatment of cryptococcosisoften entails the use of highly toxic medications that have difficultymoving through the blood-brain

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barrier. Some of the AFA used to treat cryptococcal meningitis are fluconazole, amphotericin B, and 5-flucytosine. Oguro (2013) indicated that newer strategies for HIV patients with cryptococcosis are immediately required [30].

MIC of *S.sparganophora*leaf extracts against the different fungi

Based on the MIC noted for the antsbush leaf extracts against the different fungi, the MIC was as low as 6.25mg/ml against *C. neoformans* and as high as 25mg/ml and 12.5mg/ml for the *C. albicans* strains. We postulate that high amount of phytochemicals with the respective antifungal activity would be needed to treat *Candida* infections and, a relatively small amount might be sufficient to treat *Cryptococcus* infections.

Resistance of AFAs

This study revealed that there is antifungal resistance to the current commercial antifungals ketoconazole and fluconazole by ATCC *C. albicans* was revealed.*C. albicans* IHB was resistant to fluconazole, whilst the other strains were susceptible. All the fungi were susceptible to ketoconazole. Both extracts worked well against ATCC *C. albicans*.

The *S. sparganophora*extracts were more effective than the antifungals in many cases. The hexane and ethyl acetate extract was effective when compared to fluconazole for the ATCC *C. albicans* and *C. albicans* IHB at 100mg/ml concentration. Although fluconazole was effective against *C. neoformans*, thehexane extract was more effective at 100mg/ml concentration. Our findings suggest that Antbush has great potential as antifungals agents when compared to current conventional antifungals like fluconazole and in a few cases ketoconazole.

Ketoconazole and fluconazole are designed to target and inhibit the growth of a wide range of fungal species. They have been extensively studied and optimized for this purpose. Antsbush extracts, on the other hand, are a natural remedy and may have greater activity against fungi.

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Statistical interpretations

Our statistical analysis indicates that the concentration level significantly impacts the inhibition size, with higher concentrations providing greater inhibition effects. It also highlights the importance of concentration on the effectiveness of inhibition, reinforcing the need for optimized dosages for desired biological effects. A statistically significant difference in inhibition zones among the solvent/AFA groups was also found suggesting that particular solvent/AFA worked better when compared to others. For example, the AFA ketoconazole continues to be effective and the ethyl acetate solvent extract showed more ZOIs. Statistical analysis also suggests that *C. neoformans*has notably different performance compared to *C.albicans* variants under the given experimental conditions and that this fungi is more sensitive to antsbush treatments. We therefore accept all three alternate hypotheses.

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Recommendations

Our findings indicate that further studies should be done to identify and eventually isolate the phytochemicals in *S. sparganophora*using different solvents and perhaps combinations of solvents. In addition, efforts should be made to test other fungal species (including *Candida auris, Aspergillus fumigatus* and perhaps some of the dermatophytes), as well as different strains of *C. albicans* and *C. neoformans.* Of note, most of the isolates we used came from patients and therefore, this means that *S. sparganophora*has promising antifungal potential for treatment. Studies involvingmore clinical isolates from patients should be performed to confirm these findings.We also recommend an in-depth phytochemical analysis be done including quantification of the various metabolites. Further studies on this plant would be very valuable, both from a therapeutic and an economic perspective.

Limitations

This aspect of our study was limited because of the unavailability of a substantial quantity of good quality Antbush leaves. Although numerous plants were harvested, a thorough inspection of the leaves, led to rejection of those that had perforations or discolourations. This resulted in a smaller than expected quantity of crushed leaves, and adjustments had to be made with the corresponding volume of solvent. Nonetheless, we were able to demonstrate that the variety of *S. sparganophora*found in Guyana does have considerable antifungal properties.

Conclusion

*S.sparganophora*leaf extractshave antifungal activity, and could therefore be good candidates in the search fornewer, natural antifungals. The antifungal effects of

*S.sparganophora*leaf extracts showed greater activity when compared to some conventional antifungal therapy. This study also reveals a scientific understanding to further establish antifungal values and investigate other pharmacological activity.

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