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Phytochemical Composition, Antioxidant, Anti-*Candida* and Antifungal Resistance Modulatory Activities of Extracts of *Irvingia gabonensis* Against Multidrug-Resistant *Candida albicans* and *Candida glabrata*

Benjamin Kingsley Harley[1] | Theophilus Duku Asamoah[1] | Cedric Dzidzor K. Amengor[2] | Annabel Asantewaa Yeboah[1] | Nii Korley Kortei[3] | Priscilla Eghan[4]

[1]Department of Pharmacognosy and Herbal Medicine, School of Pharmacy, University of Health and Allied Sciences, Ho, Ghana | [2]Department of Pharmaceutical Chemistry, School of Pharmacy, University of Health and Allied Sciences, Ho, Ghana | [3]Department of Sports Nutrition, School of Sports and Exercise Medicine, University of Health and Allied Sciences, Ho, Ghana | [4]Department of Midwifery, School of Nursing and Midwifery, University of Health and Allied Sciences, Ho, Ghana

Correspondence: Benjamin Kingsley Harley (bkharley@uhas.edu.gh)

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ABSTRACT

The present study investigated the antifungal activity of the 70% ethanol extracts of the leaves (IGL), stem bark (IGSB) and roots (IGR) of *Irvingia gabonensis* against multidrug-resistant *Candida albicans* and *Candida glabrata* strains and clinical isolates. The inhibitory activities of extracts against biofilm formation and mature biofilms were also investigated. Furthermore, the effects of the combinations of *I. gabonensis* extracts with amphotericin B, ketoconazole, voriconazole, nystatin and caspofungin, against the resistant *Candida* strains and isolates were evaluated using the checkerboard assay. Qualitative and quantitative assessments of the phytoconstituents in the plant extracts, as well as their antioxidant activities, were then analysed. IGSB displayed considerable anti-*Candida* activity with minimum inhibition concentrations (MICs) of 15.63 μg/mL–500 μg/mL, while IGR showed MICs of 125 μg/mL–1000 μg/mL. IGL demonstrated limited activity with MICs of 500 μg/mL–> 2000 μg/mL. IGSB also exerted significant antibiofilm activity against the drug-resistant *C. albicans* and *C. glabrata* strains and isolates with $SMIC_{90}$ of 31.25 μg/mL–1000 μg/mL and 125 μg/mL–2000 μg/mL against the formation of biofilms and mature biofilms, respectively. In the checkerboard assay, IGSB recorded the most synergistic interactions with the conventional antifungal drugs producing median FICI values that ranged between 0.35 to 0.61 and 0.30 to 0.68, and synergistic action that ranged between 50% to 66.67% and 55.56% to 100%, against the MDR *C. albicans* and *C. glabrata* isolates, respectively. The extracts displayed strong antioxidant activities, which correlated with their total phenol, flavonoid and tannin contents. The presence of phytochemicals such as glycosides, coumarins and triterpenoids was also detected in *I. gabonensis* extracts. The study findings have demonstrated that *I. gabonensis*, particularly its stem bark, is a potential source of bioactive compounds that could be developed into novel antifungal agents.

1 | Introduction

Fungal infections pose a significant global health challenge, affecting millions of individuals across various demographics [1]. Opportunistic fungal infections have become a critical concern, especially among patients with compromised immune systems, individuals undergoing prolonged antimicrobial therapy and those with metabolic disorders such as diabetes [2, 3]. The rise in fungal infections has also been accompanied by an increasing

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prevalence of antifungal resistance, further complicating treatment efforts [4, 5]. Among fungal pathogens, *Candida* species stand out as the leading cause of invasive fungal infections, with significant morbidity and mortality rates [6, 7].

Vulvovaginal candidiasis (VVC) is one of the most common fungal infections, affecting approximately 75% of women at least once throughout their lives, with a high probability of recurrence [8]. *Candida albicans* is the primary causative pathogen, although non-albicans *Candida* (NAC) species such as *Candida glabrata* have been increasingly implicated in VVC cases [9]. Several factors on the part of the host including behavioural factors, such as hormonal fluctuations, antibiotic use, immunosuppression, metabolic disorders and stress, contribute to VVC [10]. Clinical manifestations include vaginal discharge, dysuria, dyspareunia, vulvar hyperaemia and intense vaginal itching or soreness, which significantly impact the quality of life of affected individuals [11, 12].

Current antifungal therapies for VVC include azoles (e.g., fluconazole, voriconazole and itraconazole), polyenes (e.g., nystatin) and echinocandins (e.g., caspofungin) [13]. Despite their effectiveness, the widespread use of antifungal in treating various fungal infections has led to the emergence of antifungal resistance [5, 14]. Additionally, these drugs are associated with adverse effects such as gastrointestinal discomfort, hepatotoxicity, nephrotoxicity and hypersensitivity reactions, making long-term treatment challenging [15, 16]. This underscores the urgent need for the discovery and development of novel antifungal agents with improved efficacy and safety profiles.

Traditional medicine plays a crucial role in primary healthcare, particularly in developing countries where resources are limited [17]. Medicinal plants are used extensively for treating various ailments, including microbial infections. However, the lack of scientific validation of the therapeutic potential and safety of the majority of plants employed in traditional medicine remains a major limitation to their integration into mainstream healthcare in many countries, including Ghana [18, 19]. Our recent research efforts have focused on identifying bioactive compounds from medicinal plants that can serve as alternatives or adjuvants to conventional antifungal therapies [20, 21].

Irvingia gabonensis (Aubry Lecomte ex O'Rorke) Baill. is an approximately 20- to 40-m multipurpose tree native to Central and West Africa [22]. Popularly referred to as the bush mango, *I. gabonensis* produces a sweet edible fruit which forms part of the diet of several rural African homes [23]. Various parts of the plant are utilized as therapeutic agents in folk medicine. The leaves are used in the management of diabetes and to stop haemorrhage in pregnant women [24]. The stem bark of *I. gabonensis* is prescribed for the treatment of skin diseases, dysentery, hepatic disorders and as an antidote to poisons, whereas the root bark is employed as a wound healing and antidiarrhoeal agent [25]. The presence of alkaloids, glycosides, terpenoids and phenolic compounds, which contribute to the pharmacological activities of the plant such as antidiabetic, antimicrobial, analgesic, anticancer and hepatoprotective effects, has been identified in the plant [26, 27]. Extensive chemical studies on the plant by various research groups have led to the isolation of pyrrole alkaloids, methyl 2-[2-formyl-5-(hydroxymethyl)-1*H*-pyrrol1yl]-propanoate, 4-[formyl-5-(methoxymethyl)-1*H*-pyrrol-1-yl]butanoic acid, 5-(methoxymethyl)-1*H*-pyrrole-2-carbaldehyde, 5-(hydroxymethyl)-1*H*-pyrrole-2-carbaldehyde and flavonoid glycosides, kaempferol-3-*O*-*β*-D-glucopyranoside, chrysoeriol-7-*O*-*β*-D-glucopyranoside, 3,3′-di-*O*-methyl ellagic acid-4′-*O*-*β*-D-glucopyranoside and 3,3′-di-*O*-methyl-4′-*O*-*α*-L-rhamnopyranosylellagic acid 4-sulphate ester [28, 29]. From the stem bark, oleanolic acid, betulinic acid, 3-friedelanone, 3,3′,4′-tri-*O*-methylellagic acid, hardwickiic acid and 3,4-di-*O*-methylellagic acid were isolated [30], while the triterpene hederagenin has been isolated from the root bark [31].

While previous studies have reported the antibacterial and antifungal properties of *I. gabonensis* [30], limited research has been conducted on its antifungal activity against drug-resistant *Candida species*. Furthermore, little attention has been given to the potential synergistic effect of extracts prepared from the plant with conventional antifungal drugs. Given the rising cases of antifungal resistance and the challenges of current treatments, the present study aims to evaluate the leaves, stem bark and roots of *I. gabonensis* against multidrug-resistant (MDR) strains and isolates of *C. albicans* and *C. glabrata*, exploring their potential use as alternative or adjunct therapy for the treatment of VVC.

2 | Materials and Methods

2.1 | Reagents and Chemicals

Nystatin, caspofungin, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Folin–Ciocalteu's phenol reagent came from Sigma-Aldrich (Sigma Chemical Co., St. Louis, MO, United States), while fluconazole was bought from Pfizer (Pfizer Inc., New York, NY, United States). Sabouraud dextrose agar (SDA), XTT assay reagent, RPMI 1640 medium, chloramphenicol, miconazole, amphotericin B, voriconazole, clotrimazole, ketoconazole and Mueller–Hinton (MH) agar were sourced from Thermo Fisher (Oxoid Limited, Hampshire, UK). 2,2′-Azino-bis-(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS) came from AG Scientific (AG Scientific Inc., San Diego, CA, United States). All organic solvents used in the work were of analytical grade and were supplied by BDH laboratory supplies (Merck Ltd., Lutterworth, UK).

2.2 | Plant Material Collection, Processing and Extraction

The leaves, stem bark and roots of *I. gabonensis* were collected from Kwahu-Asakraka in the Eastern Region of Ghana (coordinates: 06°37.356′N/000°41.393′W) between January and March 2024, with the aid of a local herbalist. The collected plant parts were identified and authenticated at the Institute of Traditional and Alternative Medicine, University of Health and Allied Sciences, Ho, by Mr. Michael Baidoo, a Medical Herbalist. Afterwards, voucher specimens were deposited at the herbarium of the Department of Pharmacognosy and Herbal Medicine, University of Health and Allied Sciences, Ho (Table 1).

The collected materials were washed thoroughly under running water, chopped into pieces, air-dried for seven days and crushed into coarse powder using a mixer-grinder. 500 g of the powdered plant materials was extracted by cold maceration using 70% ethanol for 3×3 days. The extracts obtained were then evaporated to dryness on a rotary evaporator at 40°C [32]. The weight of the extracts obtained is also depicted in Table 1.

TABLE 1 | Information on plant materials under study.

Plant part/extract acronym	Voucher specimen number	Weight of extract (g)	Yield (% *w*/*w*)
Leaves/IGL	UHAS/PCOG/2024/L003	11.15	2.23
Stem bark/IGSB	UHAS/PCOG/2024/SB005	28.60	5.72
Roots/IGR	UHAS/PCOG/2024/R003	34.25	6.90

2.3 | Phytochemical Investigation of *I. gabonensis*

2.3.1 | Qualitative Phytochemical Screening

Phytochemical investigations of the 70% ethanol extracts of the leaves, stem bark and roots of *I. gabonensis* were carried out using standard pharmacognostic methods described by Harley et al. [33]. The extracts were screened for the presence of various secondary metabolites, including tannins, flavonoids, alkaloids, terpenes, plant sterols, coumarins, saponins, anthraquinones and reducing sugars.

2.3.2 | Quantification of Total Phenolic Content (TPC)

The TPC present in the 70% ethanol extracts of *I. gabonensis* was measured by the Folin–Ciocalteu's colorimetric technique utilizing gallic acid as the standard phenolic compound [34] with slight modifications. 1 mg/mL (100 μL) concentrations of the extracts prepared in methanol were added to 0.9 mL distilled water and 0.1 mL of Folin–Ciocalteu's reagent with intermittent shaking. Five minutes later, sodium carbonate (7%, 1 mL) was added and the volume adjusted to 2.5 mL with distilled water. The mixtures were incubated for 2 h at 25°C, and the absorbance measured at 750 nm using a UV/vis spectrophotometer (Jenway, Bibby Scientific Ltd., Stone, Staff., UK). A standard calibration curve of seven points from 7.81 μg/mL to 1000 μg/mL of gallic acid in methanol was prepared and used to determine the total content of phenolic compounds in the extracts in gallic acid equivalents (GAEs) according to the following formula:

$$\mathrm{TPC} = \frac{v}{m} \times c, \tag{1}$$

where TPC is the total content of phenolic compounds in mg/g dry weight of extract, in GAE; v is the volume of extract in mL; c is the concentration of gallic acid established from the calibration curve in mg/mL; and m is the mass of plant material extracted with methanol. The experiment was carried out in duplicate with three technical replicates per treatment and on different days.

2.3.3 | Quantification of Total Flavonoid Content (TFC)

The aluminium chloride colorimetric method was utilized in determining the TFC of the 70% ethanol extracts of *I. gabonensis* [35]. Briefly, 2 mg/mL of the extracts (100 μL) was thoroughly mixed with 50 μL of aluminium chloride (1.2%) and 50 μL of potassium acetate (120 mM). The resulting mixtures were incubated for 30 min at 25°C, and the absorbance measured at 415 nm using a UV/vis spectrophotometer at 415 nm. Distilled water was employed as a control.

A standard calibration curve of seven points from 7.81 μg/mL to 1000 μg/mL of quercetin in distilled water was prepared and used to determine the TFC in the extracts in quercetin equivalents (QEs) according to the following formula:

$$\mathrm{TFC} = \frac{v}{m} \times c, \tag{2}$$

where TFC is the total content of flavonoids in mg/g dry weight of extract, in QE; v is the volume of extract in mL; c is the concentration of quercetin established from the calibration curve in mg/mL; and m is the mass of plant material extracted. The experiment was carried out in duplicate with three technical replicates per treatment and on different days.

2.3.4 | Determination of Total Tannin Content (TTC)

Folin–Ciocalteu's colorimetric technique previously described with slight modifications was used to estimate the TTC of the *I. gabonensis* extracts with tannic acid employed as the standard tannin compound [34]. Mixtures consisting of 100 μL of 2 mg/mL of the extracts in methanol, 500 μL of the Folin–Ciocalteu reagent (10 times diluted in distilled water), 1 mL of sodium carbonate (35%) solution and 8.4 mL of distilled water were stirred and incubated for 30 min at 25°C. Afterwards, the absorbances of the mixtures were determined at 700 nm using a UV/vis spectrophotometer. Methanol was employed as control.

A calibration curve was plotted with tannic acid prepared in methanol and used to determine the TTC in the extracts, measured in tannic acid equivalents (TAEs) according to the following formula:

$$\mathrm{TTC} = \frac{v}{m} \times c, \tag{3}$$

where TTC is the total content of phenolic compounds in mg/g dry weight of extract, in TAE; v is the volume of extract in mL; c is the concentration of tannic acid established from the calibration curve in mg/mL; and m is the mass of plant material extracted with methanol. The experiment was carried out in duplicate with three technical replicates per treatment and on different days.

2.4 | Antioxidant Assays

2.4.1 | ABTS Radical Scavenging Assay

The ABTS scavenging activity of the 70% ethanol extracts of *I. gabonensis* was determined according to the method described by Li et al. [36] with slight modifications. A stock solution of ABTS free radicals ($ABTS^{\bullet+}$) was prepared by reacting an aqueous solution of ABTS (7 mM, 5 mL) with 2.45 mM aqueous solution of potassium persulphate (5 mL) at room temperature overnight in the dark. Then, 1 mL of the $ABTS^{\bullet+}$ radical solution was mixed with 0.50 mL of the aqueous extract or standard (ascorbic acid) at different concentrations (1000 μg/mL–3.91 μg/mL). The mixtures were incubated at 30°C for 10 min in the dark

before measuring their absorbances at 734 nm on a UV/vis spectrophotometer. The negative control was prepared by mixing 1 mL of $ABTS^{\bullet+}$ solution with 0.50 mL of distilled water. All experiments were carried out in duplicate with three technical replicates per treatment and on different days.

The percentage scavenging activities were calculated as % inhibition using the following equation:

$$\%\,\text{Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100. \tag{4}$$

2.4.2 | DPPH Scavenging Assay

The DPPH free radical scavenging activity of the extracts was assessed using the method described by Harley et al. [33] with some modifications. Briefly, 0.50 mL of the extracts or standard (ascorbic acid) with varying concentrations (1000 μg/mL–1.95 μg/mL) was added to 1.0 mL of DPPH• methanolic solution (0.1 mM). The mixtures were vigorously shaken and incubated in the dark for 20 min at room temperature. The absorbances of the mixture were measured at 517 nm against a blank of methanol without DPPH• using a UV/Vis spectrophotometer. The experiment was carried out in duplicate with three technical replicates per treatment and on different days.

The percentage inhibition of DPPH• was calculated using the above equation.

2.4.3 | Nitric Oxide (NO) Scavenging Activity

The NO scavenging activity of the extracts was determined using the Griess reagent [33]. A 2 mL of 10 mmol/L sodium nitroprusside in phosphate buffer was incubated with 150 μL of a methanol solution containing 50 μL of extracts at 25°C for 2 h. Afterwards, 5 mL of Griess reagent was added and absorbances measured at 540 nm using a UV/vis spectrophotometer. Methanol was used as a control, while α-tocopherol was used as positive control. The experiment was carried out in duplicate with three technical replicates per treatment and on different days.

2.5 | Antifungal Assay

2.5.1 | Fungal Strains and Growth Conditions

Twenty-three *Candida* strains were used for the study. They included two reference strains (*C. albicans* ATCC 10231 and *C. glabrata* ATCC 2001), twelve MDR *C. albicans* clinical isolates (CA-01, CA-02, CA-05, CA-07, CA-09, CA-10, CA-12, CA-13, CA-16, CA-19, CA-23 and CA-25) and nine MDR *C. glabrata* clinical isolates (CG-03, CG-06, CG-08, CG-14, CG-15, CG-17, CG-20, CG-21 and CG-24) (Table S1). The clinical isolates were collected from the Microbiology Laboratory unit of the Ho Teaching Hospital, Ghana; were maintained on SDA at 4°C; and subcultured on a fresh SDA for 48 h prior to any antifungal assay.

2.5.2 | Assessment of Anti-*Candida* Activity

The anti-*Candida* activity of the 70% ethanol extracts of *I. gabonensis* was investigated using our previously described method, adopted from document M27-A3 by the Clinical and Laboratory Standards Institute (CLSI) (2008), with modifications [37]. Caspofungin and ketoconazole were used as positive controls, whereas the culture media was employed as a negative control. The activity, measured in terms of the minimum inhibitory concentration (MIC), was measured visually and spectrophotometrically at 490 nm using a microplate reader. The experiment was performed in triplicate with three technical replicates per treatment and on different days.

2.5.3 | Determination of Minimum Fungicidal Concentrations of Compounds

Aliquots from the wells corresponding to the MIC of the extracts and three subsequent wells (higher) were inoculated onto SDA plates and incubated for 24 h at 37°C. The plates were then examined for the presence or absence of growth.

2.6 | Assessment of the Combinations of *I. gabonensis* Extracts and Antifungal Drugs Using the Checkerboard Assay

Combinations of the leaves, stem bark and root extracts of *I. gabonensis* with amphotericin B, ketoconazole, voriconazole, nystatin and caspofungin were evaluated using the checkerboard method from EUCAST AFST guidelines [38] with slight modifications. Briefly, twofold serial dilutions of the extracts and antifungal drugs were made in RPMI 1640 medium in 96-well microtitre plates. 50 μL from each extract dilution was transferred into new microtitre plates in the vertical direction. The same amounts of antifungal drugs were added in the horizontal direction to obtain various combinations of the compound and antifungal drugs. Afterwards, 100 μL of 0.5 McFarland inoculum suspension of each *Candida* clinical isolate was added to the wells. The plates were then incubated at 37°C for 72 h, and the MIC values determined at 490 nm using a microplate reader. Each experiment was carried out in triplicate with three technical replicates per treatment and on different days.

Analysis of the results was carried out by the determination of the fraction inhibitory concentration index (FICI), which was calculated as follows:

$$\text{FICI} = \text{FIC}_\text{A} + \text{FIC}_\text{B}, \tag{5}$$

where $\text{FIC}_\text{A} = (\text{MIC}_\text{CA}/\text{MIC}_\text{A})$ and $\text{FIC}_\text{B} = (\text{MIC}_\text{CB}/\text{MIC}_\text{B})$.

MIC_A and MIC_B are the MICs of drugs A and B alone; and MIC_CA and MIC_CB are the concentrations of drugs A and B at the iso-effective combinations.

The FICIs were interpreted as follows: $\text{FICI} \leq 0.5$ synergism; 0.5–4.0 indifference and > 4.0 antagonism [39].

2.7 | Antibiofilm Activity of *I. gabonensis* Extracts

2.7.1 | Inhibition of Biofilm Formation

The antibiofilm formation activities of the 70% ethanol leaves, stem bark and root extracts of *I. gabonensis* and caspofungin, used as a positive control, against the *Candida* strains/isolates were determined using a microplate-based method [40]. Briefly, 50 μL each of extract in RPMI 1640 medium was placed in wells of Column 1 of a flat-bottom 96-well microplate. Twofold serial dilutions were carried out to obtain concentrations from 4000 μg/mL–3.91 μg/mL from Column 1 to Column 10. The wells of the plates were then inoculated with 50 μL of 2×10^6 cells/mL

Candida suspensions, and the plates were incubated for 24 h at 37°C. After incubation, the media in each well were aspirated carefully and the plates washed with 100 μL PBS three times to remove planktonic cells. Afterwards, 100 μL of XTT/menadione solution was added to each well and the plates incubated in the dark at 37°C for 2 h. 80 μL of the supernatant obtained from the wells was then transferred into new plates before measuring the absorbances at 490 nm using a microplate reader.

2.7.2 | Inhibition of Mature Biofilm

The inhibitory activities of the 70% ethanol leaves, stem bark and root extracts of *I. gabonensis* and caspofungin, used as a positive control, against mature biofilms of the *Candida* strains/isolates were determined using a 96-well plate method [41]. Briefly, 100 μL of *Candida* suspensions of 1×10^6 cells/mL in RPMI 1640 of each reference *Candida* strain was added to wells of flat-bottom 96-well plates and incubated at 37°C for 48 h to enable the formation of biofilms. The media in the wells were aspirated carefully in order not to disturb the biofilms formed. The wells were washed with 100 μL PBS to remove planktonic cells. Serial dilutions of the compounds were made from 4000 μg/mL to 3.91 μg/mL in new plates before transferring to the plates containing the preformed biofilms. The plates were then incubated at 37°C for 24 h. The media in the wells were removed carefully, and the plates were washed twice with 100 μL PBS.

100 μL of XTT/menadione solution was added to each well, and the plates incubated at 37°C for 2 h in the dark. 80 μL of the supernatant from the wells was then transferred into new plates before measuring the absorbances at 490 nm using a microplate reader.

The absorbances were analysed with GraphPad for Windows Version 8 (GraphPad Prism Software, San Diego, USA).

The experiment was also replicated thrice in both assays with three technical replicates per treatment and on different days.

2.8 | Statistical Analysis

Data are displayed as mean ± SD. Analyses were carried out using one-way analysis of variance (ANOVA) with GraphPad Prism for Windows Version 8 (GraphPad Prism Software, San Diego, USA). Multiple comparisons between groups were done using Tukey's post hoc test. For the checkerboard assay data, median FICI values with interquartile ranges (IQR, Q1–Q3) were calculated to represent data dispersion for the nonparametric datasets. Pearson correlation analyses were performed to evaluate the relationship between phytochemical contents (TPC, TFC and TTC) and activities (antioxidant IC_{50} values and antifungal MIC values) using Python (Version 3.11.0) with the SciPy library (scipy.stats.pearsonr function). Spearman's rank correlation was also performed as a nonparametric alternative using the scipy.stats.spearmanr function. For all analyses, $p < 0.05$ was considered statistically significant.

3 | Results

3.1 | Qualitative Phytochemical Evaluation of *I. gabonensis* Extracts

Phytochemical analysis of the leaves, stem bark and root extracts of *I. gabonensis* led to the detection of several major groups of secondary metabolites in the leaves, stem bark and roots of the plants (Table 2). Whereas alkaloids and anthraquinones were detected in the leaves and stem bark extracts, they were found absent in the root extract. Saponins, however, were absent in all parts of the plant.

3.2 | Quantitative Phytochemical Analysis of the Extracts

The total amounts of phenols, flavonoids and tannins present in the 70% ethanol leaves, stem bark and root extracts of *I. gabonensis* were quantified. The leaf extract recorded the highest amount ($p < 0.001$) of phenol, flavonoid and tannin contents with TPC, TFC and TTC of 72.96 ± 0.78 mg·GAE/g, 10.15 ± 0.13 mg·QE/g and 38.03 ± 0.75 mg·TAE/g, respectively. This was followed by the stem bark and root extracts in that order (Table 3).

3.3 | In Vitro Antioxidant Activity

3.3.1 | ABTS Radical Scavenging Activity

The ABTS radical scavenging activities of the 70% ethanol leaves, stem bark and root extracts of *I. gabonensis* measured in terms of half maximal inhibitory concentration (IC_{50}) are displayed in Table 4. The stem bark extract showed the greatest inhibition of the radical with IC_{50} of 7.54 ± 0.18 μg/mL (Table 4). The scavenging activity of the stem bark extract was however significantly lower ($p < 0.001$) compared to ascorbic acid (3.45 ± 1.66 μg/mL).

3.3.2 | DPPH Radical Scavenging Activity

The 70% ethanol leaves, stem bark and root of extracts of *I. gabonensis* demonstrated a concentration-dependent scavenging of the DPPH free radicals with IC_{50}s of 25.06 ± 1.17 μg/mL, 15.25 ± 0.16 μg/mL and 68.41 ± 0.22 μg/mL, respectively (Table 4). The scavenging activity of the stem bark extract was significantly ($p < 0.001$) higher than the others; however, it was lower ($p < 0.001$) than ascorbic acid ($IC_{50} = 2.28 \pm 0.29$ μg/mL).

3.3.3 | NO Radical Scavenging Activity

The IC_{50} values of the 70% ethanol leaves, stem bark and root of extracts of *I. gabonensis* were found to be 87.11 ± 1.08 μg/mL, 40.35 ± 0.64 μg/mL and 120.28 ± 0.26 μg/mL, respectively, in the NO scavenging assay. The NO scavenging activity of the stem bark extract was significantly ($p < 0.001$) higher than the others; however, it was lower ($p < 0.001$) than α-tocopherol (8.68 ± 0.81 μg/mL) (Table 4).

3.4 | Anti-*Candida* Activities of the Leaves, Stem Bark and Root Extracts of *I. gabonensis*

The inhibition of growth activities of the 70% ethanol leaves, stem bark and root extracts of *I. gabonensis* on the *Candida* strains and clinical isolates are displayed in Table 5. The ethanol stem bark extract demonstrated significant anti-*Candida* activity with MICs of 15.63 μg/mL–500 μg/mL against *C. albicans* and *C. glabrata* reference strains and MDR clinical isolates. This was followed by root extract with MICs that ranged from 125 μg/mL–1000 μg/mL against the tested strains and isolates, while the leaf extract showed low inhibitory activity with MICs of 500 μg/mL–> 2000 μg/mL.

TABLE 2 | Major phytochemical groups in the leaves, stem bark and root extracts of *I. gabonensis*.

Phytochemical groups	IGL	IGSB	IGR
Tannins	+	+	+
Hydrolysable tannins	−	+	+
Condensed tannins	+	+	+
Flavonoids	+	+	+
Triterpenoids	+	+	+
Coumarins	+	+	+
Phytosterols	+	+	+
Reducing sugars	+	+	+
Saponins	−	−	−
Anthraquinones	−	+	+
Alkaloids	−	+	+

Note: IGL: 70% ethanol *I. gabonensis* leaf extracts; IGSB: 70% ethanol *I. gabonensis* stem bark extracts; IGR: 70% ethanol *I. gabonensis* root extracts. +: detected; −: not detected.

TABLE 3 | Quantitative phytochemical analysis of the leaves, stem bark and root extracts of *I. gabonensis*.

Plant extract	TPC (mg·GAE/g)	TFC (mg·QE/g)	TTC (mg·TAE/g)
IGL	72.96 ± 0.78	10.15 ± 0.13	38.03 ± 0.75
IGSB	68.55 ± 0.22^{a}	8.94 ± 0.18^{a}	36.84 ± 0.40^{c}
IGR	$47.20 \pm 0.05^{a,b}$	$6.77 \pm 0.09^{a,b}$	$14.98 \pm 0.28^{a,b}$

Note: Values are expressed as mean ± SD. IGL: 70% ethanol *I. gabonensis* leaf extracts; IGSB: 70% ethanol *I. gabonensis* stem bark extracts; IGR: 70% ethanol *I. gabonensis* root extracts.
Abbreviations: GAE = gallic acid equivalent, QE = quercetin equivalent, TAE = tannic acid equivalent, TFC = total flavonoid content, TPC = total phenolic content, TTC = total tannin content.
[a] $p < 0.001$ compared to IGL.
[b] $p < 0.001$ compared to IGSB.
[c] $p < 0.01$ compared to IGL.

TABLE 4 | Radical scavenging activity of the leaves, stem bark and root extracts of *I. gabonensis*.

Plant extract	IC_{50} (μg/mL)		
	ABTS radical	DPPH radical	NO radical
IGL	18.42 ± 0.80^{a}	25.06 ± 1.17^{a}	87.11 ± 1.08^{a}
IGSB	7.54 ± 0.18	15.25 ± 0.16	40.35 ± 0.64
IGR	$40.27 \pm 0.56^{a,b}$	$68.41 \pm 0.22^{a,b}$	$120.28 \pm 0.26^{a,b}$
Ascorbic acid	3.45 ± 1.66^{a}	2.28 ± 0.29^{a}	—
With α-tocopherol	—	—	8.68 ± 0.81^{a}

Note: Values are expressed as mean ± SD. IGL: 70% ethanol *I. gabonensis* leaf extracts; IGSB: 70% ethanol *I. gabonensis* stem bark extracts; IGR: 70% ethanol *I. gabonensis* root extracts.
[a] $p < 0.001$ compared to IGSB.
[b] $p < 0.001$ compared to IGL.

Caspofungin and ketoconazole used as positive controls also demonstrated strong anti-*Candida* activities with MICs of 0.50 μg/mL–2.00 μg/mL and 4.00 μg/mL–16.00 μg/mL, respectively.

3.5 | Checkerboard Assay

The interactions produced because of the combinations of *I. gabonensis* extracts with conventional antifungal drugs against the MDR *C. albicans* and *C. glabrata* isolates are outlined in Tables 6 and 7, respectively. In general, the FICIs for all the combinations of the extracts and antifungal drugs indicated either synergism or indifference, and most importantly, none of the combinations showed an antagonistic action (FICI > 4.0). The combinations of the antifungal drugs with *I. gabonensis* stem bark extract (IGSB) produced median FICI values that ranged from 0.35 to 0.61 (IQR: 0.28–0.88) and 0.30 to 0.68 (IQR: 0.25–0.88), and synergistic action that ranged from 41.67% to 66.67% and 44.44% to 100%, against the MDR *C. albicans* and C. *glabrata* isolates, respectively. The narrow interquartile ranges observed for most IGSB combinations demonstrate consistent synergistic interactions across the tested isolates. Combinations with the root extract (IGR) induced little to no synergistic action against the *C. albicans* and *C. glabrata* isolates, with median FICI values of 0.86 to 1.13 (IQR: 0.75–1.31) and 0.56 to 1.05 (IQR: 0.44–1.16), respectively. Median FICI values of the leaf extract (IGL) in combination with the antifungal drugs were in the range

TABLE 5 | Anti-*candida* activity of the leaves, stem bark and roots of *I. gabonensis* ethanol extracts.

Candida species	Minimum inhibitory and fungicidal concentration (μg/mL)							
	IGL		IGSB		IGR		KTC	CAS
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MIC
C. albicans ATCC 10231	1000	> 2000	31.25	125	500	> 2000	16.00	2.00
C. glabrata ATCC 2001	500	1000	15.63	125	250	2000	4.00	0.50
C. albicans CA-01	1000	> 2000	125	500	125	2000	16.00	2.00
C. albicans CA-02	500	2000	62.50	250	250	2000	8.00	1.00
C. albicans CA-05	2000	ND	62.50	500	250	2000	16.00	2.00
C. albicans CA-07	2000	ND	31.25	> 250	125	1000	8.00	1.00
C. albicans CA-09	2000	ND	62.50	> 500	250	2000	16.00	2.00
C. albicans CA-10	500	1000	15.63	62.50	250	1000	4.00	0.50
C. albicans CA-12	500	> 2000	31.25	250	500	> 2000	8.00	0.50
C. albicans CA-13	2000	ND	125	> 1000	500	> 2000	16.00	1.00
C. albicans CA-16	> 2000	ND	500	2000	1000	> 2000	16.00	2.00
C. albicans CA-19	500	2000	15.63	> 250	500	> 2000	4.00	0.50
C. albicans CA-23	500	> 2000	31.25	250	250	2000	4.00	0.50
C. albicans CA-25	1000	> 2000	125	1000	1000	> 2000	8.00	0.50
C. glabrata CG-03	> 2000	ND	250	1000	1000	> 2000	16.00	1.00
C. glabrata CG-06	> 2000	ND	250	> 2000	500	> 2000	4.00	0.50
C. glabrata CG-08	500	2000	62.50	500	125	1000	16.00	1.00
C. glabrata CG-14	> 2000	ND	250	1000	500	> 2000	8.00	0.50
C. glabrata CG-15	1000	> 2000	62.50	500	125	500	8.00	0.50
C. glabrata CG-17	> 2000	ND	500	2000	500	> 2000	16.00	1.00
C. glabrata CG-20	1000	> 2000	62.50	500	250	2000	8.00	0.50
C. glabrata CG-21	500	2000	15.63	125	125	500	4.00	0.50
C. glabrata CG-24	2000	ND	250	2000	500	2000	8.00	0.50

Note: IGL: 70% ethanol *I. gabonensis* leaf extracts; IGSB: 70% ethanol *I. gabonensis* stem bark extracts; IGR: 70% ethanol *I. gabonensis* root extracts. KTC: ketoconazole; CAS: caspofungin. > 2000: fungicidal activity not achieved at the highest concentration tested (2000 μg/mL). ND: not determined—MFC assay could not be performed as the MIC was at or above the upper limit of the tested concentration range (2000 μg/mL), leaving no higher concentration wells available for subculture.
Abbreviations: MFC = minimum fungicidal concentration, MIC = minimum inhibition concentration.

of 0.38 to 1.39 (IQR: 0.28–1.69) and 0.76 to 0.92 (IQR: 0.56–1.00), and synergistic actions ranged from 33.33% to 75% and 11.11% to 33.33%, against the MDR *C. albicans* and *C. glabrata* isolates, respectively, with the VRC–IGL combination being the most prevalent at 75% against *C. albicans* isolates (Table 6).

3.6 | Antibiofilm Activities of the Leaves, Stem Bark and Root Extracts of *I. gabonensis*

The inhibitory action of the leaves, stem bark and root extracts of *I. gabonensis* against biofilm formation and mature biofilms of the *C. albicans* and *C. glabrata* strains and isolates measured in terms of the lowest treatment concentration with > 90% sessile inhibition ($SMIC_{90}$) is displayed in Table 8. As shown in the table, the mature biofilms of the *Candida* species were resistant to the *I. gabonensis* extracts as compared to planktonic *Candida* cells, requiring higher concentrations with $SMIC_{90}$ ranging from 31.25 μg/mL–> 4000 μg/mL. The stem bark extract (IGSB) exhibited considerable antibiofilm activity with $SMIC_{90}$ of 31.25 μg/mL–1000 μg/mL and 125 μg/mL–2000 μg/mL against the formation of biofilm and mature biofilms, respectively, whereas the leaf extract (IGL) and root extract (IGR) demonstrated little to no antibiofilm activity with $SMIC_{90}$ of ≥ 1000 μg/mL and ≥ 2000 μg/mL against the formation of biofilm and mature biofilms, respectively.

3.7 | Correlation Between Phytochemical Content and Bioactivities

To evaluate the relationship between phytochemical content and antioxidant and antifungal activities, Pearson correlation analyses were performed among TPC, TFC, TTC and both antioxidant (IC_{50} values) and antifungal (MIC values) activities.

3.7.1 | Correlation Between Phytochemical Content and Antioxidant Activity

Strong negative correlations were observed between the quantitative phytochemical content and antioxidant IC_{50} values. For the TPC, the correlations with the three radical scavenging assays were as follows: ABTS ($r = -0.908$, $p = 0.276$), DPPH ($r = -0.944$, $p = 0.213$) and NO ($r = -0.709$, $p = 0.498$). Similarly, the TFC showed strong negative correlations with ABTS ($r = -0.806$, $p = 0.404$), DPPH ($r = -0.860$, $p = 0.341$) and NO ($r = -0.555$, $p = 0.626$). The strongest correlations were observed for TTC, particularly with DPPH radical scavenging activity ($r = -0.976$, $p = 0.140$), followed by ABTS ($r = -0.950$, $p = 0.203$) and NO ($r = -0.785$, $p = 0.425$).

TABLE 6 | Combinations of the leaves, stem bark and roots of *I. gabonensis* ethanol extracts with conventional antifungal drugs against 12 clinical isolates of *C. albicans*.

Combinations	FICI			% clinical isolates showing the following interactions:		
	FICI range	Median FICI	IQR (Q1–Q3)	Synergism (number/total)	Indifference (number/total)	Antagonism (number/total)
Combinations including IGL						
AMB–IGL	0.28–0.80	0.45	0.38–0.63	58.33 (7/12)	41.67 (5/12)	0 (0/12)
KTC–IGL	0.19–1.15	0.65	0.50–0.96	33.33 (4/12)	66.67 (8/12)	0 (0/12)
VRC–IGL	0.25–0.75	0.38	0.28–0.50	75 (9/12)	25 (3/12)	0 (0/12)
NYS–IGL	0.09–1.00	0.76	0.50–0.94	33.33 (4/12)	66.67 (8/12)	0 (0/12)
CAS–IGL	0.73–2.15	1.39	1.13–1.69	0 (0/12)	100 (12/12)	0 (0/12)
Combinations including IGSB						
AMB–IGSB	0.38–1.50	0.56	0.44–0.94	50 (6/12)	50 (6/12)	0 (0/12)
KTC–IGSB	0.25–1.25	0.50	0.38–0.75	66.67 (8/12)	33.33 (4/12)	0 (0/12)
VRC–IGSB	0.28–0.95	0.35	0.28–0.56	66.67 (8/12)	33.33 (4/12)	0 (0/12)
NYS–IGSB	0.20–1.00	0.61	0.45–0.88	41.67 (5/12)	58.33 (7/12)	0 (0/12)
CAS–IGSB	0.31–1.15	0.40	0.34–0.81	66.67 (8/12)	33.33 (4/12)	0 (0/12)
Combinations including IGR						
AMB–IGR	0.55–1.50	1.13	0.94–1.31	0 (0/12)	100 (12/12)	0 (0/12)
KTC–IGR	0.57–1.25	1.04	0.88–1.16	0 (0/12)	100 (12/12)	0 (0/12)
VRC–IGR	0.50–1.03	0.90	0.75–0.97	25 (3/12)	75 (9/12)	0 (0/12)
NYS–IGR	0.50–1.25	1.00	0.81–1.13	16.67 (2/12)	83.33 (10/12)	0 (0/12)
CAS–IGR	0.64–1.13	0.86	0.75–1.00	0 (0/12)	100 (12/12)	0 (0/12)

Note: IGL: 70% ethanol *I. gabonensis* leaf extracts; IGSB: 70% ethanol *I. gabonensis* stem bark extracts; IGR: 70% ethanol *I. gabonensis* root extracts. AMB: amphotericin B; KTC: ketoconazole; VRC: voriconazole; NYS: nystatin; CAS: caspofungin; IQR: interquartile ranges (Q1–Q3).
Abbreviation: FICI = fraction inhibitory concentration index.

These negative correlations indicate that extracts with higher phenolic, flavonoid and tannin content exhibited superior antioxidant activities (lower IC_{50} values). As shown in the blue-dominated left panel of the correlation heatmap (Figure 1), the antioxidant columns show consistently deep blue colouration across all three phytochemical rows, reflecting the uniformly strong negative associations.

3.7.2 | Correlation Between Phytochemical Content and Antifungal Activity

The correlation analysis between phytochemical content and antifungal activity revealed weak to moderate positive correlations between phytochemical content and average MIC values. The correlations were as follows: TPC vs. MIC ($r = 0.429$, $p = 0.718$), TFC vs. MIC ($r = 0.600$, $p = 0.590$) and TTC vs. MIC ($r = 0.322$, $p = 0.791$). As observed in the correlation heatmap (Figure 1), the mean MIC column displayed a warm red-pink gradient across all the phytochemical categories, which is opposite to the blue-toned antioxidant columns. The positive correlations suggest that antifungal activity may not be solely dependent on phenolic secondary metabolites, but rather on the synergistic action of multiple phytochemical classes present in the extracts.

4 | Discussion

The present study explored the antifungal potential of the leaves, stem bark and roots of *I. gabonensis* against MDR *C. albicans* and *C. glabrata* strains and clinical isolates. Qualitative and quantitative assessment of the secondary metabolites present in the plant was also carried out.

The anti-*Candida* testing revealed that the 70% ethanol stem bark extract (IGSB) exhibited the most significant antifungal activity against the panel of MDR *Candida* strains and isolates with MIC values ranging from 15.63 μg/mL to 500 μg/mL. This was followed by the root extract (IGR) (MIC = 125 μg/mL to 1000 μg/mL), with the leaves extract (IGL) demonstrating lowest antifungal activity. Resistance to antifungal drugs has become a serious concern worldwide, affecting clinical outcomes in *Candida* infection management in recent years, especially those caused by *C. albicans* and *C. glabrata* [42, 43]. Pressure exerted by the prolonged use and overexposure of azoles and echinocandins has resulted in the development of resistant mechanisms in both species [44, 45]. This has also led to the selection for infection with intrinsically resistant strains, particularly *C. glabrata* [14]. Thus, there is an urgent need for the discovery and development of new antifungal drugs, particularly agents active against drug-resistant species and strains. The considerable anti-*Candida* activity exerted by IGSB against the *C. albicans* and *C. glabrata* isolates, which was completely indifferent to their resistance patterns to conventional antifungals (Table S1), indicates that the stem bark of *I. gabonensis* may be an effective adjunct in the treatment of resistant and recurrent candidiasis caused by *C. albicans* and *C. glabrata* and a source of novel potent antifungal agents against drug-resistant *C. albicans* and *C. glabrata*. *C. albicans* and *C. glabrata* are the most frequently

TABLE 7 | Combinations of the leaves, stem bark and roots of *I. gabonensis* ethanol extracts with conventional antifungal drugs against 9 clinical isolates of *C. glabrata*.

Combinations	FICI			% clinical isolates showing the following interactions:		
	FICI range	Median FICI	IQR (Q1–Q3)	Synergism (number/total)	Indifference (number/total)	Antagonism (number/total)
Combinations including IGL						
AMB–IGL	0.20–1.05	0.76	0.56–0.94	33.33 (3/9)	66.67 (6/9)	0 (0/9)
KTC–IGL	0.19–1.00	0.81	0.63–0.94	22.22 (2/9)	77.78 (7/9)	0 (0/9)
VRC–IGL	0.31–1.15	0.87	0.63–1.00	33.33 (3/9)	66.67 (6/9)	0 (0/9)
NYS–IGL	0.10–1.05	0.78	0.56–0.94	33.33 (3/9)	66.67 (6/9)	0 (0/9)
CAS–IGL	0.42–1.02	0.92	0.75–1.00	11.11 (1/9)	44.44 (8/9)	0 (0/9)
Combinations including IGSB						
AMB–IGSB	0.42–2.05	0.48	0.42–0.75	77.78 (7/9)	22.22 (2/9)	0 (0/9)
KTC–IGSB	0.38–0.90	0.42	0.38–0.56	66.67 (6/9)	33.33 (3/9)	0 (0/9)
VRC–IGSB	0.09–0.48	0.33	0.25–0.38	100 (9/9)	0 (0/9)	0 (0/9)
NYS–IGSB	0.25–0.50	0.30	0.28–0.38	100 (9/9)	0 (0/9)	0 (0/9)
CAS–IGSB	0.50–1.13	0.68	0.56–0.88	44.44 (4/9)	55.56 (5/9)	0 (0/9)
Combinations including IGR						
AMB–IGR	0.55–1.50	0.94	0.75–1.13	0 (0/9)	100 (9/9)	0 (0/9)
KTC–IGR	0.57–1.25	1.05	0.88–1.16	0 (0/9)	100 (9/9)	0 (0/9)
VRC–IGR	0.25–1.03	0.56	0.44–0.75	33.33 (3/9)	66.67 (6/9)	0 (0/9)
NYS–IGR	0.50–1.25	0.88	0.69–1.06	11.11 (1/9)	88.89 (8/9)	0 (0/9)
CAS–IGR	0.64–1.13	0.90	0.81–1.00	0 (0/9)	100 (9/9)	0 (0/9)

Note: IGL: 70% ethanol *I. gabonensis* leaf extracts; IGSB: 70% ethanol *I. gabonensis* stem bark extracts; IGR: 70% ethanol *I. gabonensis* root extracts. AMB: amphotericin B; KTC: ketoconazole; VRC: voriconazole; NYS: nystatin; CAS: caspofungin; IQR: interquartile ranges (Q1–Q3).
Abbreviation: FICI = fraction inhibitory concentration index.

identified *Candida* pathogens responsible for vulvovaginal cases in Ghana. An investigation carried out in two tertiary healthcare facilities in two different regions of the country revealed that *C. albicans* is the most implicated causative agent, with the prevalence of 58% [46] and 22.9% [47] in Komfo Anokye Teaching Hospital, Kumasi, Ashanti Region, and Ho Teaching Hospital, Ho, Volta Region, respectively. However, another study in a secondary hospital recorded *C. glabrata* as the most predominant pathogen for VVC in pregnant women with a prevalence of 57.4% [48]. High resistance to antifungal drugs, particularly azoles, was also observed in the isolates. Thus, the anti-*Candida* activity of the stem bark extract of *I. gabonensis* against the drug-resistant *Candida species* in this study warrants further investigation of IGSB as a therapeutic agent for use against *Candida* infections including VVC in Ghana. Previous antimicrobial studies on the plant showed that the methanol extract of IGSB exhibited significant anti-*Candida* activity against three *Candida* clinical isolates (*C. albicans* LMP0204U, *C. glabrata* LMP0413U and *C. krusei* LMP0311U) from Cameroon [30], further augmenting *I. gabonensis* as a potent antifungal agent.

Antifungal drug combinations as a therapeutic strategy in the management of fungal infections are receiving increasing attention. The declining rate of the development of new antifungal drugs and the constant buildup of resistance mechanisms in *Candida* species to clinically used antifungal drugs suggest that combination drug therapy may be an effective way to overcome resistant fungi [5, 49, 50]. Combining new antifungal agents with known antifungal drugs, particularly those with different mechanisms of action, would be a novel therapeutic approach, which may circumvent the drug resistance problem [51]. Combinations of IGSB extract with the conventional antifungal drugs, especially the azoles (ketoconazole and voriconazole) and polyenes (nystatin and amphotericin B), exerted potent activities against the MDR *C. albicans* and *C. glabrata* isolates, with 50% to 66.67% and 55.56% to 100% synergistic action, respectively, by the checkerboard assay. This result is noteworthy as it suggests the presence of phytochemicals in the IGSB that could reverse the resistance of the *Candida* isolates to azoles and polyenes. The strong synergistic action observed also indicates that IGSB may possess a different mechanism of antifungal action than the azoles, which act by the inhibition of lanosterol 14α-demethylase and subsequent prevention of ergosterol synthesis, a fungal cell-membrane component [52], and polyenes, which act by distributing fungal cell membrane through pore formation after binding to ergosterol [53]. The combinations with IGL showed majorly indifferent against the isolates apart from AMB–IGL and VRC–IGL combinations, where there was more than 50% synergistic activity against *C. albicans* isolates. The combinations of IGR with the antifungal drugs on the other hand showed indifferent effects against all the *Candida* isolates.

The pathogenicity of *Candida* species is influenced by certain virulence mechanisms including the capacity of adhesion and invasion into host cells, secretion of tissue-damaging hydrolytic

TABLE 8 | Antibiofilm activity of the leaves, stem bark and roots of *I. gabonensis* ethanol extracts.

Candida species	$SMIC_{90}$ (μg/mL)							
	IGL		IGSB		IGR		CAS	
	BFI	MBI	BFI	MBI	BFI	MBI	BFI	MBI
C. albicans ATCC 10231	4000	> 4000	125	125	1000	> 4000	2.00	8.00
C. glabrata ATCC 2001	2000	4000	62.50	125	1000	4000	1.00	4.00
C. albicans CA-01	4000	> 4000	1000	2000	1000	2000	2.00	4.00
C. albicans CA-02	1000	4000	500	2000	2000	2000	1.00	4.00
C. albicans CA-05	4000	> 4000	250	1000	2000	4000	1.00	4.00
C. albicans CA-07	4000	> 4000	125	1000	1000	4000	2.00	8.00
C. albicans CA-09	> 4000	> 4000	250	1000	1000	2000	1.00	4.00
C. albicans CA-10	4000	> 4000	125	500	1000	4000	1.00	2.00
C. albicans CA-12	2000	> 2000	500	2000	2000	> 4000	0.50	4.00
C. albicans CA-13	2000	> 4000	250	2000	2000	> 4000	0.50	2.00
C. albicans CA-16	> 4000	> 4000	500	2000	2000	> 4000	0.50	1.00
C. albicans CA-19	4000	> 4000	31.25	500	1000	> 4000	2.00	4.00
C. albicans CA-23	2000	> 4000	125	1000	1000	> 4000	0.50	4.00
C. albicans CA-25	> 4000	> 4000	250	1000	2000	4000	0.50	2.00
C. glabrata CG-03	> 4000	> 4000	500	1000	2000	> 4000	0.50	4.00
C. glabrata CG-06	> 4000	> 4000	500	2000	1000	4000	0.50	2.00
C. glabrata CG-08	2000	> 4000	125	500	1000	2000	0.50	2.00
C. glabrata CG-14	> 4000	> 4000	250	1000	1000	> 4000	0.50	2.00
C. glabrata CG-15	> 4000	> 4000	62.50	1000	1000	4000	2.00	8.00
C. glabrata CG-17	> 4000	> 4000	1000	2000	2000	4000	0.50	4.00
C. glabrata CG-20	4000	> 4000	125	500	2000	4000	0.50	2.00
C. glabrata CG-21	4000	> 4000	31.25	250	2000	4000	0.50	4.00
C. glabrata CG-24	> 4000	> 4000	500	2000	2000	4000	0.50	2.00

Note: IGL: 70% ethanol *I. gabonensis* leaf extracts; IGSB: 70% ethanol *I. gabonensis* stem bark extracts; IGR: 70% ethanol *I. gabonensis* root extracts, CAS: Caspofungin. $SMIC_{90}$: lowest treatment concentration with > 90% sessile inhibition.
Abbreviations: BFI = biofilm formation inhibition, MBI = mature biofilm inhibition.

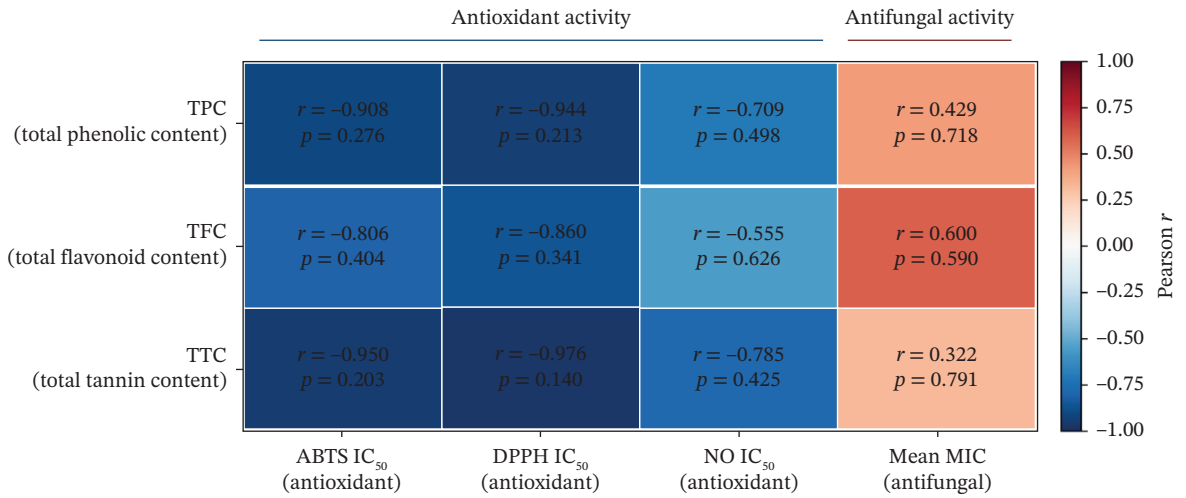


FIGURE 1 | Pearson correlation heatmap of phytochemical content vs biological activities of *I. gabonensis* extracts. Blue: negative correlations; red: positive correlations.

enzymes such as proteases and phospholipases and the ability to form biofilms [54]. The formation of biofilms serves as a protective mechanism against external attacks, contributing significantly to their drug resistance and the occurrence of recurrent *Candida* infections [55, 56]. Thus, new antifungal agents should not only inhibit the growth of free-floating *Candida* cells

but should also be able to interfere with the formation of biofilms and/or disrupt already-formed ones. IGSB demonstrated significant antibiofilm action by preventing the formation of biofilms in the *Candida* isolates with $SMIC_{90}$ that ranged from 31.25 μg/mL to 1000 μg/mL and disrupted already-formed biofilms of the isolates at 125 μg/mL–2000 μg/mL. The tendency of IGSB to demonstrate in vitro inhibition of planktonic *Candida* cells as well as *Candida* biofilms at low concentrations confirms the stem bark of *I. gabonensis* as a source of much needed novel antifungal agents against resistant fungi as majority of the available antifungal drugs can control the growth of planktonic cells alone, resulting in their inability in controlling biofilms in vivo, eventually leading to failure in clinical outcomes [57, 58]. The leaves and root extracts of the plant proved ineffective against the biofilms of the *Candida* strains and isolates.

The antioxidant activity of the extracts, assessed via ABTS, DPPH and NO radical scavenging assays, demonstrated strong free radical scavenging potential, particularly in IGL and IGR, which had very high TPC, TFC and TTC. Antioxidant properties are known to contribute to antifungal efficacy by mitigating oxidative stress in infected tissues and enhancing immune responses against fungal infections [59, 60]. The strong antioxidant activities of the leaves and IGSB extracts recorded in this study were consistent with previous investigations done on the plant [24, 25, 61, 62]. The antioxidant activities of the extracts could contribute to their overall anti-*Candida* activities. However, in vivo antioxidant investigations of the extracts must be carried out in animal models of *Candida* infection to confirm the findings of the study.

The variations in activity among the different plant parts could be attributed to the differences in their chemical compositions. Phytochemical screening revealed tannins, flavonoids, coumarins, sterols and triterpenoids as classes of secondary metabolites present in all parts of *I. gabonensis*. Some phytoconstituents belonging to these classes including the alkaloids and anthraquinones present in the stem bark and roots of *I. gabonensis* are known for their antimicrobial and antioxidant properties and are consistent with previous studies on medicinal plants that possess antifungal activity [63, 64]. The correlation analysis revealed strong negative associations between phenolic content and antioxidant activities, with correlation coefficients ranging from −0.71 to −0.98. The strongest correlation was observed between TTC and DPPH radical scavenging activity ($r = -0.976$), suggesting that tannins are particularly important contributors to the antioxidant capacity of *I. gabonensis* extracts. These findings align with the well-established antioxidant properties of phenolic compounds, which act through multiple mechanisms including hydrogen atom donation, electron transfer and metal ion chelation [65, 66].

In contrast, the relationship between phenolic content and antifungal activity showed significantly weaker correlations (TPC vs. MIC: $r = 0.429$; TFC vs. MIC: $r = 0.600$; TTC vs. MIC: $r = 0.322$), with positive rather than negative correlation coefficients. This clear divergence between strongly negative phenolic–antioxidant correlations and weakly positive phenolic–antifungal correlations indicates that antioxidant and antifungal activities are driven by distinct sets of constituents, with the antifungal activity of IGSB attributable to nonphenolic compounds. The presence of alkaloids, triterpenoids and coumarins in the stem bark extract, as revealed by qualitative phytochemical screening, likely contributes significantly to the observed anti-*Candida* activity alongside phenolic compounds [13, 67]. Some isolated compounds from IGSB such as betulinic acid, oleanolic acid, hardwickiic acid, 3,4-di-O-methylellagic acid and 3,3,4-tri-O-methylellagic acid have been shown to exert anti-*Candida* activity [30, 37]. These findings provide a strong rationale for future bioassay-guided fractionation focused on nonphenolic fractions enriched in alkaloids, triterpenoids and coumarins for antifungal agents, which can be used for further drug development, while phenolic-rich fractions may be appropriate for antioxidant-directed studies. Furthermore, mechanistic studies should be carried out to elucidate how the stem bark extract exerts its activity against the resistant *Candida* pathogens. While the current evidence is phenotypic, the potent antibiofilm activity of IGSB suggests interference with the *Candida* yeast-to-hypha morphological transition, a critical step in *Candida* biofilm formation. Key regulatory genes including EAP1 (encoding an enhanced adherence to polystyrene 1 GPI-anchored cell wall protein essential for biofilm initiation and epithelial adhesion) and HWP1 (hyphal wall protein 1, required for hyphal elongation and stable biofilm maturation) represent possible targets by which IGSB may have exerted its biofilm retardation [68, 69]. Upstream regulators of the hyphal programme, such as BCR1 (a transcription factor coordinating biofilm gene networks) and WOR1 (the main regulator of white-opaque switching and virulence), are additional molecular targets that could be involved in IGSB-mediated biofilm suppression [70–72]. Future transcriptomic and quantitative RT-PCR studies comparing IGSB-treated and untreated *Candida* cells would allow direct assessment of whether inhibition of these genes accompanies the observed inhibition of biofilm formation.

The concentrations of IGSB required to inhibit *Candida* growth (MIC: 15.63 μg/mL–125 μg/mL) and biofilms ($SMIC_{90}$: 31.25 μg/mL–1000 μg/mL) are within pharmacologically achievable ranges for topical and vaginal administration. These concentrations are readily achievable through conventional formulation strategies. Clotrimazole vaginal creams contain 10 mg/g (10,000 μg/g) of active ingredient, representing concentrations approximately 80–640 times higher than our observed MICs [73]. Similarly, miconazole vaginal suppositories deliver 100–200 mg doses that yield local concentrations of 133,000–400,000 μg/mL [52]. Nystatin vaginal tablets deliver local concentrations of 4000–6000 μg/mL, which is 30–250 times higher than our MIC values [74]. These comparisons demonstrate that formulations containing 1%–5% IGSB (10,000–50,000 μg/g) would deliver concentrations 80–3200 times higher than the MICs, providing substantial therapeutic margins even accounting for potential bioavailability differences between crude extracts and purified drugs.

The safety profile of *I. gabonensis* has been well-characterized in several studies. Acute toxicity studies demonstrated an LD_{50} exceeding 5000 mg/kg body weight in rats [24]. More importantly, subchronic toxicity evaluation showed no significant adverse effects on haematological parameters, liver function (ALT, AST, ALP), kidney function (creatinine, urea) or lipid profiles when rats received *I. gabonensis* extracts at doses up to 400 mg/kg/day for 90 consecutive days [75]. Histopathological examination revealed no treatment-related pathological changes, establishing a no-adverse-effect level (NOAEL) at ≥ 400 mg/kg/

day. For topical and/or vaginal application, systemic absorption would be minimal (< 5%–10%), further enhancing the safety profiles. Additionally, *I. gabonensis* has been used extensively in traditional medicine and as food across West and Central Africa for centuries without documented serious adverse effects [23, 27], providing ethnopharmacological evidence of safety. However, before clinical translation can be considered, vaginal irritation studies using validated mouse models such as the rabbit vaginal irritation model and demonstration of efficacy in an in vivo mouse model of VVC must be carried out. These studies are essential to confirm both mucosal safety and antifungal efficacy under conditions that more closely mimic the human vaginal environment. Genotoxicity studies, reproductive toxicity assessment, stability and formulation optimization studies and pharmacokinetic assessment of systemic absorption following vaginal administration must also be carried out.

The therapeutic profile of IGSB offers potential advantages over conventional antifungals. Azoles, while effective, are associated with hepatotoxicity, headache and gastrointestinal disturbances [76]. The synergistic interactions observed between IGSB and the conventional antifungals (50%−100% synergism rates) suggest potential for combination therapy approaches that could reduce required doses of synthetic drugs minimizing adverse effects while maintaining efficacy. This may be particularly valuable for managing drug-resistant *Candida* infections. Furthermore, the antibiofilm activity of IGSB represents an advantage over many conventional antifungals that demonstrate poor activity against sessile cells [58, 59], which is particularly relevant for treating recurrent VVC, where biofilm formation contributes to therapeutic failure. Thus, the concentration–efficacy relationship, substantial safety margins in reported preclinical studies, extensive traditional use history and synergistic potential collectively support the translational potential of IGSB extract for topical antifungal therapy. However, rigorous clinical investigation remains essential to confirm safety and efficacy in human populations.

Overall, the findings of this study highlight the potential of IGSB as a source of bioactive compounds with significant antifungal and antifungal resistance modulatory properties. The observed synergism with conventional antifungals, coupled with its strong biofilm inhibition and antioxidant activity, supports further investigation into its pharmacological applications.

5 | Conclusion

This study demonstrates that IGSB extract possesses significant antifungal potential against MDR *C. albicans* and *C. glabrata*, with considerable anti-*Candida* activity, antibiofilm inhibition and synergistic interactions with conventional antifungal drugs. Correlation analyses revealed that while phenolic compounds strongly contribute to its antioxidant activity, the antifungal efficacy depends on synergistic actions of multiple phytochemical classes, including alkaloids and triterpenoids. These findings are particularly relevant for Ghana and West Africa, where VVC prevalence is high and antifungal resistance is increasing. Future research should focus on isolation and characterization of bioactive compounds IGSB through bioassay-guided fractionation, targeting nonphenolic fractions enriched in alkaloids, triterpenoids and coumarins. Mechanistic studies should be conducted to evaluate whether IGSB suppresses key biofilm-regulatory genes, including EAP1, HWP1, BCR1 and WOR1, using transcriptomic and qRT-PCR approaches. Furthermore, in vivo efficacy studies should be assessed using a murine model of VVC, along with vaginal irritation studies to confirm mucosal compatibility and toxicological evaluations, and ultimately progressing toward clinical trials. The study contributes to the global effort to combat antimicrobial resistance through natural product-derived alternatives.

Author Contributions

Conceptualization: Benjamin Kingsley Harley.

Data curation: Benjamin Kingsley Harley, Theophilus Duku Asamoah, Annabel Asantewaa Yeboah, Priscilla Eghan.

Formal analysis: Benjamin Kingsley Harley, Theophilus Duku Asamoah, Annabel Asantewaa Yeboah, Priscilla Eghan.

Investigation: Benjamin Kingsley Harley, Annabel Asantewaa Yeboah, Theophilus Duku Asamoah.

Methodology: Benjamin Kingsley Harley, Annabel Asantewaa Yeboah, Theophilus Duku Asamoah, Priscilla Eghan.

Project administration: Benjamin Kingsley Harley, Cedric Dzidzor K. Amengor, Nii Korley Kortei.

Supervision: Benjamin Kingsley Harley, Nii Korley Kortei.

Validation: Benjamin Kingsley Harley, Theophilus Duku Asamoah.

Writing–original draft: Benjamin Kingsley Harley, Annabel Asantewaa Yeboah, Nii Korley Kortei, Priscilla Eghan.

Writing–review and editing: Benjamin Kingsley Harley, Cedric Dzidzor K. Amengor, Nii Korley Kortei.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available in the supporting information of this article.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. *(Supporting Information)*

The antifungal resistance profile of the *Candida* strains used in this study is included in the supporting information. The fraction inhibitory concentration indices of the extracts against the individual *Candida* strains and isolates are also included in the study.