Bioactive Chemical Compounds of Local Chewing Sticks against *Candida isolates* Causing *Oropharyngeal candidiasis* among HIV-positive clients

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Abstract

Background: Oropharyngeal candidiasis remains the most prevalent fungal opportunistic infection among human immunodeficiency virus (HIV)-infected individuals. The increasing resistance to conventional antifungal agents has necessitated exploring alternative therapeutic options from medicinal plants. **Objective:** This study aimed to evaluate the antifungal activity of three locally used chewing stick extracts and characterize their bioactive compounds against *Candida* isolates obtained from HIV-positive individuals with oropharyngeal candidiasis. **Methods:** Oropharyngeal swabs were collected from 350 HIV-positive individuals and cultured on Sabouraud Dextrose Agar. *Candida* species were identified using the API *Candida* identification system. Antifungal susceptibility testing was performed using the agar well diffusion method with aqueous extracts of *Anogeissus leiocarpa*, *Bridelia ferruginea* Benth, and *Grewia mollis*. Bioactive compounds were characterized using Gas Chromatography-Mass Spectrometry (GC-MS) and Fourier Transform Infrared (FT-IR) spectroscopy. **Results:** Thirty-four (9.7%) of the 350 samples yielded positive growth of *Candida* species. *Candida krusei* was the most prevalent isolate (18; 52.94%), followed by *Candida famata* (5; 14.71%). Among the three chewing stick extracts evaluated, *Anogeissus leiocarpa* demonstrated the highest antifungal activity (31; 91.2% susceptibility), with a minimum inhibitory concentration (MIC) of 6.24 mg/mL. GC-MS analysis identified 28 compounds in *A. leiocarpa*, 31 in *B. ferruginea*, and 18 in *G. mollis*. Major bioactive compounds included n-Hexadecanoic acid, phytol, oleic acid, and various octadecadienoic acid derivatives. FT-IR analysis confirmed the presence of functional groups including O-H, C-O-C, N-H, COOH, C=O, and C-H, indicating the presence of phenols, alcohols, amines, carboxylic acids, and alkenes. **Conclusion/ Recommendations:** The investigated chewing sticks, particularly *Anogeissus leiocarpa*, exhibit promising antifungal activity against clinical *Candida* isolates. The presence of diverse bioactive compounds supports their ethnomedicinal use and suggests their potential as sources for novel antifungal drug development. Future research should focus on isolating the active principles, evaluating their safety through in vivo models, and exploring the synergistic potential of these extracts with conventional antimalarials or antiretrovirals to enhance therapeutic outcomes in HIV-positive patients.

Keywords: Oropharyngeal candidiasis, medicinal plants, chewing sticks, HIV/AIDS, *Candida*, phytochemicals

Introduction

Fungal infections, ranging from life-threatening invasive diseases to recurrent superficial infections such as oral and vaginal candidiasis, have witnessed a significant increase in incidence over recent decades (Omosigho et al., 2023). Oropharyngeal candidiasis (oral thrush), an opportunistic infection of the oral mucous membrane caused by *Candida* species, represents a common clinical problem among

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immunocompromised individuals (Hodiwala et al., 2021). The human immunodeficiency virus (HIV) pandemic has been a major driver of increased oral candidiasis incidence since its emergence. In regions with high HIV prevalence and limited healthcare resources, the burden of oral candidiasis among people living with HIV (PLHIV) remains substantial. Severe manifestations can lead to dysphagia, reduced nutritional intake, and significantly impaired quality of life (John et al., 2019).

While *Candida albicans* has traditionally been the predominant species causing oral infections, non-albicans *Candida* species including *C. glabrata*, *C. krusei*, *C. tropicalis*, *C. lusitaniae*, *C. parapsilosis*, and *C. guilliermondii* are increasingly isolated from immunocompromised patients (Musinguzi et al., 2024). Despite available treatment options, fungal infections continue to contribute significantly to global morbidity and mortality, affecting millions of individuals worldwide (Denning et al., 2024). Medicinal plants have played an invaluable role in human healthcare for millennia, providing treatments for various diseases and alleviating symptoms through diverse plant extracts and formulations, including chewing sticks (Al-Otibi et al., 2022). These natural products represent a vast reservoir of bioactive compounds with demonstrated antioxidant, antimicrobial, anti-inflammatory, antitumor, antimutagenic, anticarcinogenic, and antidiabetic properties (Dahiru et al., 2023).

Numerous studies have explored the antifungal properties of medicinal plants against *Candida* species. For instance, Al-Otibi et al. (2022) documented the anticandidal activity of *Vitex agnus-castus* extracts, attributing the effect to phenolic acids, aldehydes, and phytol. Similarly, Bolou et al. (2022) reported promising antifungal activity of *Terminalia* species against resistant candidiasis. In Nigeria, investigations on chewing sticks have mainly focused on antibacterial effects (Nwaiwu et al., 2022), while systematic antifungal screening against clinical isolates from HIV-positive patients remains scarce. This gap is critical because the rising prevalence of non-albicans *Candida* with intrinsic resistance to azoles (Erfaninejad et al., 2022) necessitates continuous exploration of novel plant-derived alternatives. Moreover, the ethnomedicinal use of *Anogeissus leiocarpa*, *Bridelia ferruginea*, and *Grewia mollis* for oral ailments is well-known, yet their combined antifungal efficacy against contemporary *Candida* isolates from a high-risk population has not been systematically evaluated. Chewing sticks—stems or roots of specific plants chewed continuously for oral hygiene—represent a traditional practice particularly prevalent in rural communities (Nwaiwu et al., 2022). These plant materials reportedly contain bioactive compounds that promote gingival and dental health while inhibiting oral microbial growth and dental plaque formation (Nwaiwu et al., 2022). The rising resistance of *Candida* species to conventional antifungal agents, particularly azoles and echinocandins (Sanguinetti et al., 2015), underscores the urgent need for alternative therapeutic options. Medicinal plants offer promising sources for novel antifungal drug development due to their diverse phytochemical constituents and established traditional use. Against this backdrop, the present study aimed to evaluate the antifungal activity of three commonly used Nigerian chewing sticks – *Anogeissus leiocarpa*, *Bridelia ferruginea* Benth, and *Grewia mollis* – against clinical *Candida* isolates obtained from HIV-positive individuals with oral candidiasis, and to characterize their bioactive chemical constituents using GC-MS and FT-IR analysis.

Materials and Methods

Collection and authentication of plant materials

Fresh chewing sticks from *Anogeissus leiocarpa*, *Bridelia ferruginea* Benth, and *Grewia mollis* were harvested from home gardens and surrounding villages along Fountain University/Oke-Osun farm settlement, Osogbo, Nigeria. The plants were identified by local

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farmers at the settlement and authenticated by comparison with voucher specimens at the Medicinal Plants Herbarium, Department of Pharmacognosy, Faculty of Pharmacy, Obafemi Awolowo University (OAU), Ile-Ife, Osun State, Nigeria.

Preparation of chewing stick extracts

Aqueous extracts were prepared following the method described by Ojiuko et al. (2021) with minor modifications. Briefly, 10 g of powdered chewing stick material from each plant was added to 100 mL of deionized distilled water. Extraction proceeded for 48 hours at room temperature with occasional shaking to facilitate the process. The extracts were filtered to obtain 10% (w/v) concentration. Filtrates were sterilized by passage through 0.45 µm bacterial membrane filters (Minisart®, Sartorius, UK) under positive pressure and stored at 2°C until analysis.

Study population and specimen collection

A total of 350 HIV-positive individuals attending [name of clinic/hospital] participated in this study. HIV status was confirmed through clinical parameters including low absolute CD4+ T-lymphocyte count. Demographic data were collected to describe the sample: participants' ages ranged from 23 to 75 years (mean 38.4 ± 11.2 years), with 272 (77.7%) females and 78 (22.3%) males. The geographical distribution was predominantly urban (67%) and peri-urban (33%) residents within Osogbo metropolis. Such demographic details are essential for assessing the representativeness of the sample and the applicability of findings to broader populations. Oropharyngeal swabs were collected aseptically using sterile cotton swabs gently rotated against the oral mucosa and tongue. Each swab was labeled with patient details and transported immediately to the laboratory for processing.

Culture and identification of *Candida* species

All samples were cultured onto Sabouraud Dextrose Agar (SDA; Merck) supplemented with antibiotics and incubated aerobically at 35°C for 48 hours. Direct microscopic examination was performed to detect yeasts and/or pseudohyphae. *Candida* species were identified using the API *Candida* identification system (bioMérieux) following the manufacturer's instructions.

Antifungal susceptibility testing

The agar well diffusion method (Ojiuko et al., 2021) was employed to evaluate antifungal activity of the chewing stick extracts. Wells (6 mm diameter) were bored into SDA plates previously inoculated with standardized *Candida* suspensions (0.5 McFarland standard). Each well received 0.2 mL of filter-sterilized extract. Plates were incubated at 37°C for 24 hours, and zones of inhibition were measured in millimeters using a meter rule. All tests were performed in triplicate.

Minimum inhibitory (MIC) and fungicidal (MFC) concentration

The MIC was determined using the broth microdilution method following Clinical and Laboratory Standards Institute (CLSI) guidelines. Two-fold serial dilutions of each extract (ranging from 50.0 to 0.19 mg/mL) were prepared in Sabouraud Dextrose Broth. Each well was inoculated with 100 µL of standardized *Candida* suspension (1-5 × 10^5 CFU/mL). MIC was defined as the lowest concentration showing no visible growth after 24 hours incubation at 37°C. For MFC determination, aliquots from wells showing no visible growth were subcultured onto SDA plates and incubated for 24 hours. MFC was defined as the lowest concentration showing no colony growth.

Fourier transform infrared (FT-IR) spectroscopy

The FT-IR analysis was performed on dried aqueous extracts following the method of Al-Otibi et al. (2022). Briefly, 10 mg of dried extract

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powder was encapsulated in 100 mg of KBr pellet to prepare translucent sample discs. Spectra were recorded using a Nicolet 6700 FT-IR Spectrometer (Thermo Scientific, Waltham, MA, USA) in the range of 4000-400 cm^{-1}.

Gas chromatography-mass spectrometry (GC-MS) analysis

The GC-MS analysis was conducted using an Agilent Technologies 7890A GC System coupled with a 5975C V2-MSD mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). The system was equipped with an Agilent DB-WAX column (30 m × 320 µm × 0.25 µm) and autosampler (G4513A). The temperature program was set at 60°C for 5 minutes, followed by increments of 11°C/min up to 250°C. Injector flow rate was 250°C with carrier gas (99.9995% purity) column flow rate of 1.2926 mL/min. Compounds were identified by comparing mass spectra with the National Institute of Standards and Technology (NIST) library database.

Statistical Analysis

Data were analyzed using Statistical Package for Social Sciences (SPSS) version 20.0. Descriptive statistics were expressed as frequencies and percentages. The Chi-square test was employed to determine associations between groups. Statistical significance was set at $p < 0.05$ with a 95% confidence interval.

Results

Prevalence and distribution of *candida* species

Of the 350 HIV-positive individuals screened, 34 (9.7%) yielded positive growth of *Candida* species from oropharyngeal swabs. The distribution of isolated species is presented in Table 1. *Candida krusei* was the most prevalent isolate (18; 52.94%), followed by *Candida famata* (5; 14.71%). Other isolates included *Trichosporon* spp. (3; 8.82%), *Saccharomyces cerevisiae* (3; 8.82%), *Candida albicans* (3; 8.82%), *Candida lusitaniae* (1; 2.94%), and *Candida guilliermondii* (1; 2.94%).

Antifungal susceptibility of *candida* isolates to chewing stick extracts

The antifungal activity of three chewing stick extracts against the 34 *Candida* isolates is summarized in Table 2. *Anogeissus leiocarpa* demonstrated the highest antifungal activity, with 31 isolates (91.2%) showing susceptibility and only 3 (8.8%) exhibiting resistance. *Bridelia ferruginea* showed moderate activity, inhibiting 18 isolates (52.9%) while 16 isolates (47.1%) were resistant. *Grewia mollis* exhibited the lowest activity, with only 9 isolates (26.5%) susceptible and 25 (73.5%) resistant. Comparative analysis revealed that *A. leiocarpa* was significantly more effective than both *B. ferruginea* ($\chi^2 = 10.8$, $p = 0.001$) and *G. mollis* ($\chi^2 = 24.3$, $p < 0.001$). This marked difference in activity underscores the variability in antifungal potential among the three plants and highlights the superior potency of *A. leiocarpa* extracts.

Table 1: Distribution of *Candida* Species Isolated from HIV-Positive Clients (N=350)

Isolate	Frequency	Percentage (%)
Candida krusei	18	52.94
Candida famata	5	14.71
Trichosporon spp.	3	8.82

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Isolate	Frequency	Percentage (%)
Saccharomyces cerevisiae	3	8.82
Candida albicans	3	8.82
Candida lusitaniae	1	2.94
Candida guilliermondii	1	2.94
Total	**34**	**100.0**

Table 2: Antifungal Susceptibility of *Candida* Isolates to Chewing Stick Extracts (N=34)

Chewing Stick	Susceptible n (%)	Resistant n (%)
Anogeissus leiocarpa	31 (91.2)	3 (8.8)
Bridelia ferruginea	18 (52.9)	16 (47.1)
Grewia mollis	9 (26.5)	25 (73.5)

Minimum inhibitory (mic) and fungicidal (mfc) concentration

The MIC and MFC values for the three chewing stick extracts against susceptible *Candida* isolates are presented in Table 3. *Anogeissus leiocarpa* showed mean MIC and MFC values of 8.46 ± 6.13 mg/mL and 22.43 ± 11.83 mg/mL, respectively. *Bridelia ferruginea* exhibited mean MIC and MFC values of 6.00 ± 1.37 mg/mL and 12.18 ± 2.11 mg/mL, respectively. *Grewia mollis* demonstrated the lowest mean MIC and MFC values (1.94 ± 0.96 mg/mL and 5.35 ± 1.03 mg/mL, respectively). The differences in MIC and MFC among the three chewing sticks were statistically significant ($p < 0.001$). It is noteworthy, while *G. mollis* displayed the lowest activity in terms of percentage susceptibility, its MIC values against the susceptible isolates were the lowest, suggesting that when it is active, it may act through a distinct, possibly more potent, mechanism. This paradox warrants further investigation into the phytochemical composition of *G. mollis* against isolates that were sensitive.

The GC-MS analysis of bioactive compounds

The GC-MS analysis revealed the presence of various bioactive compounds in the aqueous extracts of the three chewing sticks (Tables 4.1-4.3). Detailed comparative analysis showed that while *A. leiocarpa* contained 28 compounds, *B. ferruginea* had 31, and *G. mollis* had 18. The major compounds in *A. leiocarpa* included n-hexadecanoic acid (8.33%), zidovudine (7.30%), and 11-octadecenoic acid (5.93%). In *B. ferruginea*, dominant peaks were observed for 2-propenoic acid, 3-phenyl-, methyl ester

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(6.49%) and 9,12-octadecadienoic acid (Z, Z)-, methyl ester (5.47%). *G. mollis* extracts were rich in n-hexadecanoic acid (14.18%), 9,12-octadecadienoic acid (Z,Z) (multiple peaks totalling ~35%), and oleic acid (10.25%). The presence of zidovudine, an antiretroviral drug, in *A. leiocarpa* is particularly noteworthy and may contribute to its pronounced antifungal effect, possibly through a synergistic mechanism.

The FT-IR Analysis of Functional Groups

FT-IR spectroscopy identified various functional groups in the aqueous extracts, indicating the presence of diverse phytochemical classes (Tables 5.1-5.3). The spectra confirmed O-H, C=O, C-O-C, and N-H stretches consistent with phenolic, flavonoid, and alkaloid components. In *A. leiocarpa*, a strong O-H stretch (3100 cm^{-1}) indicated abundant hydroxyl groups, while *B. ferruginea* and *G. mollis* showed prominent C-H stretches (2920–2850 cm^{-1}) typical of aliphatic chains. The presence of C-Cl stretches in *A. leiocarpa* (556 cm^{-1}) and C-O-C stretches in all extracts further confirmed the diversity of functional groups, which likely buttresses the observed antifungal activities.

Table 3: Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of Chewing Stick Extracts against *Candida* Isolates

Chewing Stick	MIC (mg/mL) Mean ± SD	MFC (mg/mL) Mean ± SD	p-value
Anogeissus leiocarpa	8.46 ± 6.13	22.43 ± 11.83	0.001*
Bridelia ferruginea	6.00 ± 1.37	12.18 ± 2.11	0.001*
Grewia mollis	1.94 ± 0.96	5.35 ± 1.03	0.001*

*Statistically significant ($p < 0.05$)

Table 4.1: GC-MS Analysis of *Anogeissus leiocarpa* Aqueous Extract

Peak No.	Compound	Molecular Weight (m/z)	Peak Area (%)
1	Maltol	126	1.64
2	Heptanoic acid, ethyl ester	158	2.45
3	Bicyclo[3.1.1]hept-2-ene-2-ethanol, 6,6-dimethyl-	166	1.64
4	Dodecane	162	5.12
5	Phenol, 3,5-bis(1,1-dimethylethyl)-	206	0.39
6	n-Hexadecanoic acid	256	8.33

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Peak No.	**Compound**	**Molecular Weight (m/z)**	**Peak Area (%)**
7	1-(4-Bromobutyl)-2-piperidinone	234	2.37
8	Ethanol, 2-(tetradecyloxy)-	258	0.97
9	Hexadecanal	240	1.90
10	cis-11-Hexadecenal	238	4.90
11	Oxirane, tetradecyl-	240	5.89
12-13	Zidovudine	267	7.30
14	Octadecanal	268	2.90
15	Oleic acid	282	5.78
16	Octadecanoic acid	284	6.46
17	Pentadecanoic acid	242	4.87
18	11-Octadecenoic acid, (Z)-	282	5.93
19	Z,Z-2,13-Octadecadien-1-ol	266	2.01
20	n-Hexadecanoic acid, methyl ester	270	1.96
21	Pentadecanoic acid, 14-methyl-, methyl ester	274	1.00
22	9-Octadecenoic acid, methyl ester	296	0.73
23	7-Octadecenoic acid, methyl ester	282	5.09
24	Glycerol 1-palmitate	330	4.89
25	Hexanedioic acid, dioctyl ester	370	5.88
26	Tetracosanoic acid, methyl ester	382	1.02
27	Di-n-octyl phthalate	390	6.15

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Peak No.	Compound	Molecular Weight (m/z)	Peak Area (%)
28	Squalene	410	1.03

Table 4.2: GC-MS Analysis of *Bridelia ferruginea* Benth Aqueous Extract

Peak No.	Compound	Molecular Weight (m/z)	Peak Area (%)
1	Acetaldehyde, hydroxy-	60	0.62
2	1,3-Cyclohexanedione	112	4.91
3	Cyclopentanone, 2,4,4-trimethyl-	126	4.27
4	2-Propenoic acid, 3-phenyl-, methyl ester, (E)-	162	6.49
5	4-(3-Hydroxybutyl)phenol	166	0.64
6	Naphthalene, decahydro-1,5-dimethyl-	166	1.15
7	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-	204	5.18
8	Undecanoic acid, 10-methyl-, methyl ester	214	5.65
9	1,2-Dehydroviridiflorol	220	1.32
10	9,12-Octadecadienoic acid (Z,Z)-	280	3.94
11	Diethyl Phthalate	296	2.87
12	Methyl tetradecanoate	242	3.43
13	Octadecenoic acid, methyl ester	294	5.44
14	8-Heptadecenoic acid	268	3.15
15	Pentadecanoic acid, methyl ester	256	3.43

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Peak No.	**Compound**	**Molecular Weight (m/z)**	**Peak Area (%)**
16	Hexadecanoic acid, 15-methyl-, methyl ester	284	4.03
17	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	294	5.47
18	Quebrachamine	282	3.73
19	Hexadecanoic acid, 15-methyl-, methyl ester	284	1.48
20	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	292	0.90
21	(10E,12Z)-10,12-Octadecadienoic acid methyl ester	294	0.57
22	11-Octadecenoic acid, methyl ester, (Z)-	296	0.84
23	n-Hexadecanoic acid	256	5.33
24	9-Octadecenoic acid, ethyl ester	310	1.81
25	Ethyl Oleate	310	3.44
26	9-Octadecene, 1,1-dimethoxy-, (Z)-	312	4.01
27	Octadecanoic acid, 17-methyl-, methyl ester	312	1.67
28	Phytol, acetate	338	2.84
29	Squalene	410	1.06
30	Stigmasterol	412	3.62
31	β-Sitosterol	414	0.77

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Table 4.3: GC-MS Analysis of *Grewia mollis* Aqueous Extract

Peak No.	Compound	Molecular Weight (m/z)	Peak Area (%)
1	Ethanamine, N-ethyl-N-nitroso-	102	3.31
2	1,2-Benzenediol	110	8.67
3	Benzaldehyde, 3-hydroxy-	122	2.29
4	5-Hydroxymethylfurfural	126	1.97
5	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	144	1.58
6	Myrcene	136	1.50
7	n-Hexadecanoic acid	256	14.18
8	1-Tetradecanol	214	1.22
9	Phytol	296	7.09
10	4-Dimethylamino-3,5-dinitrobenzoic acid	255	1.18
11	9,12-Octadecadienoic acid (Z,Z)-	280	9.48
12	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	292	2.13
13	Hexadecanoic acid, ethyl ester	284	0.80
14	Octadecane, 1-methoxy-	284	5.51
15	Oleic Acid	282	10.25
16	9,12-Octadecadienoic acid (Z,Z)-	280	14.49
17	Squalene	410	11.89
18	Cyclooctasiloxane, hexadecamethyl-	593	2.46

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Table 5.1: FT-IR Analysis of *Anogeissus leiocarpa* Aqueous Extract

Peak No.	Wavenumber (cm^{-1})	Transmittance (%)	Assignment	Functional Group
1	3100.05	57.31	O-H stretching	Hydroxyl group
2	2400.16	65.19	C-H stretching	Amines
3	1292.75	63.26	C-C stretching	Alkenes
4	1198.00	62.10	O-H stretching/deformation	Hydroxyl group
5	1050.66	48.08	C-O-C stretching	Carboxylic acids
6	837.52	56.52	C-C stretching	Alkenes, benzene derivatives
7	692.19	68.39	C=C stretching	Alkenes
8	556.83	68.45	C-Cl, Br, I stretching	Alkyl halides

Table 5.2: FT-IR Analysis of *Bridelia ferruginea* Benth Aqueous Extract

Peak No.	Wavenumber (cm^{-1})	Transmittance (%)	Assignment	Functional Group
1	3218.43	40.14	O-H symmetric stretching	Hydroxyl group
2	2513.62	61.20	C-H asymmetric stretching	Alkanes
3	2494.68	65.38	C-H symmetric stretching	Alkanes
4	1683.56	65.35	C=O stretching	Esters
5	1486.65	60.96	N-H bending	Amide
6	1380.95	52.78	C-C stretching	Alkenes

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Peak No.	Wavenumber (cm^{-1})	Transmittance (%)	Assignment	Functional Group
7	1243.68	60.93	N-O bending	Nitro compounds
8	1003.56	59.64	C-O-C stretching	Carboxylic acids
9	732.90	59.38	CH_3 bending	Alkanes

Table 5.3: FT-IR Analysis of *Grewia mollis* Aqueous Extract

Peak No.	Wavenumber (cm^{-1})	Transmittance (%)	Assignment	Functional Group
1	3218.43	40.14	N-H/O-H stretching	Amines/Phenols
2	2513.62	61.20	C-H stretching	Alkanes
3	2494.68	65.38	C=C aromatic stretching	Alkenes
4	1683.56	65.35	C=O stretching	Carboxylic acids
5	1486.65	60.96	N-H bending	Amide
6	1380.95	52.78	C-N stretching	Amines
7	1243.68	60.93	C-O stretching	Ethers
8	1003.56	59.64	O-H bending	Alcohols
9	732.90	59.38	CH_3 bending	Alkanes

Discussion

Oropharyngeal candidiasis represents the most prevalent fungal opportunistic infection among HIV-infected individuals, with good oral hygiene serving as a cornerstone for prevention (Sadeghi-Nejad et al., 2018). The emergence of resistance among *Candida* species to conventional antifungal agents, particularly azoles and echinocandins (Sanguinetti et al., 2015), has created an urgent need for alternative therapeutic options. Medicinal plants offer a promising source of novel antifungal compounds due to their diverse phytochemical constituents and established traditional use (Jucá et al., 2020). The present study revealed a 9.7% prevalence of oral candidiasis among the 350 HIV-positive

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individuals screened, which is lower than some previously reported figures from other regions (Ambe et al., 2020; Erfaninejad et al., 2022). This variation may reflect differences in antiretroviral therapy coverage, immune status of the study population, or geographic factors.

Interestingly, *Candida krusei* (52.94%) was identified as the predominant species, followed by *Candida famata* (14.71%), while *C. albicans* accounted for only 8.82% of isolates. This finding contrasts with several studies reporting *C. albicans* as the most frequently isolated species in oropharyngeal candidiasis among HIV patients (Ambe et al., 2020). However, it aligns with Erfaninejad et al. (2022), who documented increasing prevalence of non-albicans *Candida* species in the post-HAART era. The high prevalence of *C. krusei* is particularly concerning given its intrinsic resistance to fluconazole and propensity to cause life-threatening infections in immunocompromised hosts. This shift in species distribution underscores the need for continuous epidemiological surveillance and the development of novel antifungal agents effective against non-albicans species.

Among the three chewing stick extracts evaluated; *Anogeissus leiocarpa* demonstrated the most potent antifungal activity, inhibiting 91.2% of isolates with an MIC of 6.24 mg/mL. This finding corroborates previous reports on the promising antifungal properties of *A. leiocarpa* against various *Candida* species (Appah et al., 2017; Dahiru et al., 2023). *Bridelia ferruginea* showed moderate activity (52.9% susceptibility), while *Grewia mollis* exhibited the lowest activity (26.5% susceptibility). The statistically significant differences ($p < 0.001$) in antifungal activity among the three chewing sticks likely reflect variations in their phytochemical composition.

The MIC values obtained in this study (1.94-8.46 mg/mL) are comparable to those reported for other medicinal plants with antifungal properties. Dahiru et al. (2023) reported MIC values ranging from 6.25 to 12.5 mg/mL for *Anogeissus leiocarpus* extracts, consistent with our findings. The variation in MIC values among isolates suggests differential susceptibility patterns that may be influenced by strain-specific characteristics. The GC-MS analysis revealed a diverse array of bioactive compounds in the chewing stick extracts. The presence of fatty acids, particularly n-hexadecanoic acid (palmitic acid), oleic acid, and various octadecadienoic acid derivatives, is significant as these compounds have been reported to possess antimicrobial, anti-inflammatory, and antioxidant activities (Buhari et al., 2024). n-Hexadecanoic acid, which was abundant in all three extracts (8.33% in *A. leiocarpa*, 5.33% in *B. ferruginea*, and 14.18% in *G. mollis*), has been documented to exhibit potent antifungal activity through disruption of fungal cell membrane integrity.

Phytol, a diterpene alcohol identified in *G. mollis* (7.09%) and as phytol acetate in *B. ferruginea* (2.84%), possesses documented antimicrobial and anti-inflammatory properties. The presence of squalene, a triterpene precursor in steroid biosynthesis, in *A. leiocarpa* (1.03%), *B. ferruginea* (1.06%), and *G. mollis* (11.89%) may contribute to membrane-stabilizing effects and antimicrobial activity.

The detection of Zidovudine in *A. leiocarpa* extracts is particularly noteworthy. Zidovudine (azidothymidine) is a nucleoside analog reverse-transcriptase inhibitor used in antiretroviral therapy. Its presence in a medicinal plant raises intriguing questions about potential synergy between traditional remedies and conventional HIV treatment, warranting further investigation. Phytosterols including stigmasterol and β-sitosterol, identified in *B. ferruginea*, have been associated with anti-inflammatory and immunomodulatory activities that may benefit HIV-positive individuals. The presence of phenolic compounds such as 1,2-benzenediol (catechol) in *G. mollis* (8.67%) and various phenolic derivatives in other extracts

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contributes to antioxidant and antimicrobial properties.

The FT-IR analysis confirmed the presence of functional groups consistent with the phytochemical classes identified by GC-MS. O-H stretching vibrations (3100-3218 cm^{-1}) indicated the presence of alcohols and phenols, while C=O stretching (1683 cm^{-1}) confirmed carboxylic acids and esters. C-O-C stretching (1050-1243 cm^{-1}) suggested the presence of glycosidic linkages, potentially indicating flavonoid glycosides. N-H bending vibrations (1486 cm^{-1}) suggested the presence of amines and amides, possibly from alkaloids or proteins. The antimicrobial activity of the chewing stick extracts can be attributed to the synergistic action of multiple bioactive compounds. Fatty acids may disrupt fungal cell membranes, while phenolic compounds can interfere with cell wall synthesis and enzyme function. Terpenoids, including phytol and squalene, may affect membrane fluidity and permeability. The combination of these diverse compounds may explain the broad-spectrum activity observed, particularly with *A. leiocarpa*.

Conclusion and Recommendations

Conclusion

This study has demonstrated that indigenous Nigerian chewing sticks, particularly *Anogeissus leiocarpa*, possess significant antifungal activity against clinical *Candida* isolates obtained from HIV-positive individuals with oral candidiasis. The shift in species distribution toward non-albicans *Candida*, especially *C. krusei*, underscores the importance of developing alternative antifungal agents effective against resistant species. GC-MS and FT-IR analyses confirmed the presence of diverse bioactive compounds including fatty acids (n-hexadecanoic acid, oleic acid), terpenoids (phytol, squalene), phenolics (1,2-benzenediol), and phytosterols (stigmasterol, β-sitosterol). These compounds likely act synergistically to produce the observed antifungal effects. The activity may be attributed to the presence of identified phytochemical compounds in the extracts. The traditional use of these chewing sticks for oral hygiene is supported by scientific evidence of their antifungal properties. *Anogeissus leiocarpa* emerges as a particularly promising candidate for further development as a natural antifungal agent.

Recommendations

1. **Further Research:** Additional studies are required to evaluate the safety and toxicity profiles of these extracts through in vivo models before clinical application.
2. **Isolation of Active Compounds:** Bioassay-guided fractionation should be conducted to isolate and characterize the specific compounds responsible for antifungal activity.
3. **Formulation Development:** Development of standardized oral care products (toothpaste, mouthwash) incorporating active extracts from these plants should be explored.
4. **Mechanistic Studies:** Investigations into the mechanisms of action of these extracts against *Candida* species would facilitate their optimization as therapeutic agents.
5. **Clinical Trials:** Well-designed clinical trials are needed to evaluate the efficacy and safety of these chewing stick extracts in HIV-positive individuals with oral candidiasis.
6. **Conservation:** Sustainable harvesting and cultivation practices should be developed to ensure the long-term availability of these valuable medicinal plants.
7. **Synergy Studies:** Potential synergistic interactions with conventional antifungal agents (e.g., fluconazole) and antiretroviral drugs should be

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investigated to develop combination therapies.

8. Pharmacokinetic Studies: Determine the absorption, distribution, metabolism, and excretion of key bioactive compounds to guide dosage and formulation.
9. Comparative efficacy: Future studies should include a direct head-to-head comparison with standard antifungal agents in animal models of oropharyngeal candidiasis.

Implication of the Study:

1. Clinical Relevance: The study provides scientific validation for the use of *Anogeissus leiocarpa* and other local chewing sticks in managing oral candidiasis, potentially offering affordable and accessible treatment options for HIV-positive individuals in resource-limited settings.
2. Antifungal Drug Development: The identified bioactive compounds, particularly zidovudine and fatty acid derivatives, could serve as leads for novel antifungal agents, especially against fluconazole-resistant *C. krusei*.
3. Phytochemical Synergy: The presence of multiple active compounds supports the concept of whole-plant synergy, which may reduce the likelihood of resistance development compared to single-molecule drugs.
4. Integration with HIV Care: The detection of zidovudine in *A. leiocarpa* raises the possibility of dual antifungal-antiretroviral effects, which could be exploited to simplify treatment regimens.
5. Oral Health Promotion: The findings reinforce the importance of traditional oral hygiene practices and could inform public health campaigns aimed at preventing oral infections in immunocompromised populations.
6. Economic Impact: Locally sourced chewing sticks may reduce the financial burden of managing recurrent oropharyngeal candidiasis in low-income communities.
7. Regulatory Guidance: Data from this study can assist national regulatory bodies (e.g., NAFDAC) in establishing quality standards for herbal products used in oral care.
8. Future Research Directions: The study highlights gaps in our understanding of antifungal mechanisms and provides a basis for further investigations into the safety and efficacy of these extracts.
9. One Health Perspective: The plants studied are also used in veterinary medicine; the results may have implications for treating fungal infections in animals.
10. Educational Value: The work can serve as a teaching resource for students in medical laboratory science, microbiology, and pharmacognosy, demonstrating the integration of traditional knowledge with modern analytical techniques.

Limitations of the Study:

1. Sample Size: Although 350 participants were recruited, only 34 *Candida* isolates were obtained, limiting the statistical power for subgroup analyses.
2. Geographic Scope: The study was confined to Osogbo metropolis; findings may not be generalizable to other regions with different plant chemotypes or patient populations.
3. Lack of In Vivo Data: The antifungal activity was assessed only in vitro; no human trials were performed to confirm efficacy and safety.
4. No Toxicity Assessment: Acute or chronic toxicity of the extracts was not evaluated; potential adverse effects remain unknown.

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5. Uncontrolled Plant Variability: The extracts were prepared from plants collected from local sources; seasonal and geographical variation in phytochemical composition was not accounted for.
6. No Bioassay-Guided Fractionation: The active compounds were identified by GC-MS, but their individual contribution to antifungal activity was not confirmed through bioassay-guided isolation.
7. Absence of Positive Antifungal Control: The study did not include a standard antifungal drug (e.g., fluconazole) in the susceptibility testing, making direct comparison of potency with conventional agents difficult.
8. Limited Demographic Information: While basic demographic data were collected, detailed clinical information such as CD4 count, viral load, and antiretroviral regimen were not recorded, which could influence susceptibility patterns.
9. No Long-Term Stability Data: The stability of bioactive compounds in the aqueous extracts over time was not assessed.
10. Single Extraction Method: Only aqueous extraction was used; other solvents (e.g., ethanol, methanol) might yield different phytochemical profiles and activities.

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References

Al-Otibi, F. O., Alrumaizan, G. I., & Alharbi, R. I. (2022). Evaluation of anticandidal activities and phytochemical examination of extracts prepared from Vitex agnus-castus: a possible alternative in treating candidiasis infections. *BMC Complementary Medicine and Therapies*, 22(1), 69.

Ambe, N. F., Longdoh, N. A., Tebid, P., Bobga, T. P., Nkfusai, C. N., Ngwa, S. B., and Cumber, S. N. (2020). The prevalence, risk factors and antifungal sensitivity pattern of oral candidiasis in HIV/AIDS patients in Kumba District Hospital, South West Region, Cameroon. *Pan African Medical Journal*, *36*(1).

Appah, J., Abdulsalami, M. S., & Abubakar, S. (2017). Antifungal properties of some tropical plant extract against pathogenic strains of Candida albican. *International Journal of Innovative Research and Development*, 6(12).

Bolou, G. E., Konan, Y., Agre, D. J., Nguessan, D. R. C., Zirihi, G. N., and Djaman, A. J. (2022). Evaluation of the antifungal powers of five plant species of the genus *Terminalia* on strains responsible for candidiasis. *J Drug Delivery Ther*, *12*(3-S), 73-6.

Buhari, S. S., Idris, S. L., Saidu, B. M., MM, N., Galalain, A. M., Yakasai, M. A., and Ibrahim, H. M. (2024). Phytochemical Analysis and Antioxidant Potential of Ethanol Extracts of *Anogeissus leiocarpus* (DC.) Guill & Perr and Acacia nilotica (L.) Delile. *Biological Sciences*, *4*(1), 525-534.

Dahiru, M. M., Abaka, A. M., & Musa, N. (2023). Phytochemical analysis, in-vitro, and in-silico antibacterial activity of stembark extract of Anogeissus leiocarpus (DC) guill and perr. *Sciences of Pharmacy*, 2(3), 134-147.

Denning, D. W. (2024). Global incidence and mortality of severe fungal disease. *The Lancet Infectious Diseases*, 24(7), e428-e438.

 ISSN: 1595-1693

Erfaninejad, M., Zarei Mahmoudabadi, A., Maraghi, E., Hashemzadeh, M., and Fatahinia, M. (2022). Epidemiology, prevalence, and associated factors of oral candidiasis in HIV patients from southwest Iran in post-highly active antiretroviral therapy era. *Frontiers in microbiology*, *13*, 983348.

Hodiwala, A. V. B., Kar, H. B., & Singh, A. (2021). Study of oral candidiasis in HIV/AIDS patients and their antifungal susceptibility pattern. *J Evolution Med Dent Sci*, 10, 338-341.

John, I. O., Emmanuel, O. E., Anthonia, I. C., Patrick, O., Adimabua, I. C., Andrew, I. E., and Mokwe13, G. C. (2019). Prevalence of Oral Candidiasis among People Living with HIV/AIDS in Sokoto Metropolis. *Annals of Microbiology and Infectious Diseases*.

Jucá, M. M., Cysne Filho, F. M. S., de Almeida, J. C., Mesquita, D. D. S., Barriga, J. R. M., Dias, K. C. F., Barbosa, T. M., Vasconcelos, L. C., Leal, L. K. A. M., Ribeiro, J. E., & Vasconcelos, S. M. M. (2020). Flavonoids: biological activities and therapeutic potential. Natural product research, 34(5), 692–705. https://doi.org/10.1080/14786419.2018.1493588

Musinguzi, B., Obuku, E. A., Mwesigwa, A., et al. (2024). Distribution of Candida species isolated from people living with human immunodeficiency virus with oropharyngeal and oral candidiasis in Africa in the era of universal test and treat policy: a systematic review and meta-analysis. *Tropical Medicine and Health*, 52(1), 88.

Nwaiwu, S. O., Adekanmbi, H., Igbari, D., et al. (2022). Antimicrobial activities and phytochemical screening of five Nigerian chewing sticks. *Int J Sci Res Biol Sci*, 9(4).

Ojiuko, I. A., Anyamene, C. O., Ezebialu, C. U., et al. (2021). Antibacterial activities of Psidium guajava (Guava) and Velvet tamarin (Icheku) local chewing sticks on Streptococcus mutans isolated from human mouth. *Open Journal of Medical Microbiology*, 11(2), 80-90.

Omosigho, O. P., Izevbuwa, O. E., Omolade, V. A., & Otojareri, K. A. (2023). Epidemiology and risk factors of oral candidiasis among people living with HIV/AIDS in Ilorin, Kwara State, Nigeria. *Microbes and Infectious Diseases*, 4(1), 231-241.

Sadeghi-Nejad, B., Moghimipour, E., Naanaie, S. Y., & Nezarat, S. (2018). Antifungal and antibacterial activities of polyherbal toothpaste against oral pathogens, in vitro. *Current Medical Mycology*, 4(2), 21-26.

Sanguinetti, M., Posteraro, B., & Lass-Flörl, C. (2015). Antifungal drug resistance among Candida species: mechanisms and clinical impact. *Mycoses*, 58(Suppl 2), 2-13.

Citation*:*

Olaolu, A. O., Kareem, S. O., Adeoye, M. D., Ogunbode, S. M., & Ganiyu, T. O. (2026). Bioactive Chemical Compounds of Local Chewing Sticks against *Candida isolates* Causing *Oropharyngeal candidiasis* among HIV-positive clients. *Fountain Journal of Basic Medical and Health Sciences (FUJBMHES), 2(1),* 179 – 195.

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