REVIEW ARTICLE

Methodological Heterogeneity and the Need for Standardization in Evaluating the Antifungal Activity of Polyisoprenylated Benzophenones Against Candida: A Systematic Review

[1]B. O. Akinboboye, [2]F. O. Nwaokorie, [3]R. O. Oladele

[1]Department of Restorative Dentistry, Faculty of Dental Sciences, College of Medicine, University of Lagos.
[2]Department of Medical Laboratory, Faculty of Basic Medical Sciences, College of Medicine, University of Lagos.
[3]Department of Medical Microbiology, Faculty of Basic Clinical Sciences, College of Medicine, University of Lagos

ABSTRACT

Background: The rising incidence of Candida infections and increasing antifungal resistance highlight the urgent need for novel therapeutic agents and robust assessment methodologies. Polyisoprenylated benzophenones, a class of naturally derived compounds, have shown promising antifungal properties. However, variability in assessment methods complicates comparative analysis and evidence synthesis.

Objective: This systematic review comprehensively evaluated and synthesized the methodological approaches employed in studies assessing the antifungal efficacy of polyisoprenylated benzophenones against Candida species, following PRISMA guidelines.

Methods: A systematic literature search was conducted across major electronic databases (PubMed/MEDLINE, Scopus,Web of Science, google scholar, Cochrane Library) for studies published between January 2020 and March 2026. Eligible studies included in in vitro and in vivo investigations reporting on the antifungal activity of polyisoprenylated benzophenones against Candida spp. Data extraction and quality assessment were performed independently by three reviewers and discrepancies were resolved by consensus. Methodological variables abstracted included the extracted part of garcinia kola, the method of extraction, antifungal approach, and outcomes. The review process adhered to PRISMA 2020 standards.

Results: A total of 470 results were screened, from which only 10 studies were selected for review. In these studies, the most commonly used part of Garcinia kola for extraction was the seed or fruit (60%), and ethanol was the most frequent solvent (40%). Agar diffusion was the primary method of antifungal testing (60%), with most studies (90%) assessing efficacy against Candida albicans. Of the publications reviewed, seven (70%) reported antifungal activity of Garcinia kola. The antifungal testing methods reported included agar diffusion (70%), tube dilution (10%), disc diffusion (10%), and Potato Dextrose Agar (PDA) (10%). Outcomes were typically reported as zones of inhibition or minimal inhibitory concentrations, with considerable heterogeneity in results across the studies reviewed.

Conclusion: This review highlighted the diversity and limitations of current methodological approaches for evaluating polyisoprenylated benzophenones' antifungal efficacy against Candida species. Recommendations for methodological standardization and improved reporting are proposed to enhance future research comparability and clinical translation.

Keywords: Candida, Benzophenones, Garcinia, Microbial Sensitivity Test.

Online Access

Corresponding author: Akinboboye BO.
Department of Restorative Dentistry, Faculty of Dental Sciences, College of Medicine, University of Lagos.
Email address: bakinboboye@unilag.edu.ng.
Tel: +234-7057251024

doi: 10.67250/zs9wbm03 ***Received:*** *21/04/2026* ***Revised:*** *08/05/2026* ***Accepted: 02/06/2026***

INTRODUCTION

Evaluating the antifungal efficacy of polyisoprenylated benzophenones against Candida species necessitates a multifaceted approach that combines microbiological, biochemical, and molecular techniques. Approximately 95% of invasive Candida infections are attributed to five species: C. *albicans*, C. *glabrata*, C. *parapsilosis*, C. *tropicalis*, and C. *krusei.* These candida species have been implicated as the major aetiological agent in the onset and persistence of denture stomatitis, especially candida albicans (Fifolato *et al.*, 2025; Qui *et al.*, 2023). This denture stomatitis is a chronic inflammatory condition that affects the oral mucosa, and globally prevalent among denture wearers (Fifolato *et al.*, 2025). These infections significantly increase morbidity and mortality, particularly among immunocompromised individuals (da Costa *et al.*, 2024). The current global rise of antibiotic resistance has heightened concerns and underscored the urgent need to identify new drugs and elucidate their mechanisms of action. In response, there has been a renewed focus on exploring indigenous herbal remedies to address the growing challenge of antibiotic-resistant infections (Nwobodo *et al.*, 2022; Yarahmadi *et al.*, 2025).

Polyisoprenylated benzophenones (PIBs) are biological secondary metabolites found in Garcinia fruits (Guttiferae), which are natural products with a long history of medicinal use and extensive research (Pasaribu *et al.*, 2021; Kumar et al., 2013; Gao et al., 2010).

Garcinia kola Heckel (Clusiaceae) is a tree native to West and Central Africa. Garcinia kola has long been used to treat a wide range of ailments, has demonstrated a broad spectrum of pharmacological activities, such as analgesic, anticancer, antidiabetic, anti-inflammatory, antimalarial, antimicrobial, hepatoprotective, and neuroprotective effects. A previous study conducted at Lagos University Teaching Hospital on patients with oral candidiasis reported a 96.4% clearance of lesions and better clinical outcomes with the use of Garcinia kola extract compared to chlorhexidine mouthwash (Abah *et al.*, 2014).

Recently, it has attracted significant scientific interest as a potential source of pharmaceutically important drugs (Tauchen *et al.*, 2023). Several unique classes of compounds (polyisoprenylated benzophenones) have been isolated from G. kola, such as biflavonoids, benzophenones, Kolaviron, benzofurans, benzopyrans, vitamin E derivatives, xanthones, and phytosterols (Kayinu *et al.*, 2024). Many of these compounds appear to be unique to this species, including garcinianin (found in the seeds and roots), kolanone (in the fruit pulp, seeds, and roots), gakolanone (in the stem bark), garcinoic acid and garcinal (both in the seeds), as well as garcifuran A and B, and garcipyran (all in the roots) (Tauchen *et al.*, 2023).

Kolaviron is the most extensively studied compound and is often considered the main active principle of G. kola (Olatoye et al., 2024; Tauchen et al., 2023). However, much of the research on kolaviron has notable limitations, such as the use of excessively high doses and inadequate positive controls. By contrast, garcinol has been investigated under more rigorous conditions and has shown particularly promising results, especially in anticancer, antimicrobial, and neuroprotective research (Tauchen *et al.*, 2023). To fully understand the therapeutic potential of G. kola, human clinical trials and detailed studies on the mechanisms of action are needed to determine whether any of its compounds could serve as leads for drug development.

Other secondary metabolites in garcinia Kola have various documented derivatives, such as Gakolanone (3',5'-digeranyl-2',4',6',3-tetrahydroxybenzophenone (a novel benzophenone derivative) isolated from the hexane extract of Garcinia kola Heckel stem bark. In addition, there are also three known 3-8'' linked biflavonoids—3'',4',4''',5,5'',7,7''-heptahydroxy-3,8''-biflavanone (2); 3'',4',5,5'',5''',7,7''-heptahydroxy-4-methoxy-3,8''-biflavanone; and 4',4''',5,5'',7,7''-hexahydroxy-3,8''-biflavanone. These are isolated isolated from ethanol extract (Akoro et al., 2020). Garcinoic acid dimers, δ,δ-bigarcinoic acid, δ,δ-bi-*O*-garcinoic acid, and γ,δ-bi-*O*-garcinoic acid, and a new benzophenone derivative, (8*E*)-4-geranyl-3,5-dihydroxybenzophenone, have also been isolated from the seeds of Garcinia kola.

There has been a rise in fungal infections and a global increase in antimicrobial resistance. These trends have heightened concerns and underscored the urgent need to identify new drugs and understand their mechanisms of action. In response, there is renewed interest in exploring indigenous herbal remedies to address the growing challenge of antibiotic-resistant infections (Oyegabmi *et al.*, 2016). Reviewing this topic could reveal new

approaches to indigenous drug formulation that may help in treating antimicrobial-resistant infections.

The objective of this review is to evaluate the methodological approaches for assessing antifungal activity or efficacy of polyisoprenylated benzophenones against Candida species.

METHODS

This systematic review was conducted and reported in accordance with the PRISMA 2020 guidelines. The review protocol was prospectively registered with PROSPERO (CRD420261368455).(Chan et al., 2024).

The method for this review was developed using the Population/participants, intervention, comparison, and output (PICO) framework (Chan et al., 2024). In the population element, the population of interest is Candida Species, especially in denture wearers. The intervention is polyisoprenylated benzophenone, comparison is method of testing antifungal activity, and outcome is the inhibition of candida species. The context is globally with emphasis on low-, and middle-income countries.

Research Question: What are the experimental and analytical methods used to evaluate the efficacy of polyisoprenylated benzophenones against Candida species? What is the efficacy of Polysisoprenylated benzophenones compared to standard antifungal agents in treating Candida species?

A systematic search of the literature using search engines such as, PubMed/MEDLINE, Scopus,Web of Science, google scholar, Cochrane Library. The Concepts are Candida species, polyisoprenylated benzophenones, and antifungal activity. The search terms for candida species are "candida", "candida species", "candida albicans", "candida glabrata", "candida tropicalis", "candida krusei", "non-albicans candida". Search terms for Polyisoprenylated benzophenone, were "Polyisoprenylated diaryl ketones", "Isoprenylated benzophenone derivatives", "Prenylated benzophenones","Polyprenylated benzophenones", "Polyisoprene-substituted benzophenones", and "Garcinia Kola". The search term for antifungal activity includes Fungicidal activity "Fungistatic effect", "Antimycotic activity", "Fungal growth inhibition", "Fungal suppressive effect", "Antifungal efficacy", "Fungus-inhibiting property", "Antimycotic effect", "Fungal inhibitory action". Location is global.

"Candida Albican" OR "Candida Species" OR "Candida" AND "Polyisoprenylated benzophenone" OR "Polyisoprenylated diaryl ketones" OR "Isoprenylated benzophenone derivatives" AND " Fungicidal activity" OR "Antifungal efficacy" OR "Antimycotic effect" OR "Fungicidal activity"

1. "Candida Species" AND "Polyisoprenylated benzophenone" AND "Antifungal activity"
2. "Candida Species" AND "Garcinia Kola" AND "Antifungal activity"

Risk of bias/ study quality is assessed using the GRADE assessment tool. The tool was also used to assess the risk of bias due to missing results, and certainty of findings. Outcomes were analyzed.

Relevant Studies

A systematic search of the literature, from April to June 2026, on MEDLINE [PubMed], African Journals Online, Google Scholar, and Cochrane Library [CENTRAL] was conducted using the terms above. A search of related citations and references was carried out. Non-English articles were excluded if no English translation is available.

Study Selection

Publications identified through the search strategy was downloaded into Endnote, imported into Rayyan, and duplicates were removed. Three researcher performed the title and abstract screening independently using pre-defined inclusion and exclusion criteria. Studies were included if there was agreement between three researchers. The researchers completed the full-text review. Uncertainty regarding whether publications met the inclusion criteria was resolved via consensus among the researchers.

Inclusion Criteria:

Studies published in English Language between 2020 and 2026, involving antifungal testing of candida species, studies reporting methodological approach in the use of polyisoprenylated benzophenone as anti-fungal agent,

Table 1: Assessment of Quality

S/N	Author	Were the criteria for inclusion in the sample clearly defined?	Were the study subjects and the setting described in details?	Was the exposure measured in a valid and reliable way?	Were objective, standard criteria used for measurement of the condition?	Were confounding factors identified?	Were strategies to deal with confounding factors were stated?	Were the outcomes measured in a valid and reliable way?	Was appropriate statistical analysis used?	Overall comment on quality
1	Doh et al., 2023 experimental	1	2	2	2	1	1	2	2	13 (High quality)
2	Falana, 2021 experimental	1	2	2	2	1	1	2	1	12 (High quality)
3	Nirasha et al., 2020 Experimen	1	2	2	2	1	1	2	1	12 (High quality)
4	Akintelu et al., 2022 Case - control	1	2	2	2	1	1	2	1	12 (High quality)
5	Yahaya et al., 2021. Experimental	1	2	2	2	1	1	2	1	12 (High quality)
6	Winful, et al., 2021 Experimental	1	2	2	2	1	1	2	1	12 (High quality)
7	Sakirigui et al., 2020 Experimental	1	2	2	2	1	1	2	2	13 (High quality)
8	Adenrinsola et al., 2025 Experimental	1	2	1	1	1	1	1	1	9 (Moderate quality)
9	Amala et al., 2021 Experimental	1	2	2	2	1	1	2	2	13 (High quality)
10	Ewelike et al., 2021 Experimental	1	2	2	2	1	1	2	1	12 (High quality)

Table 2: Garcinia Extraction Method, and Antifungal testing Approach

S/N	Author &Date	Extract Type	Extraction Medium, and Method	Antifungal testing method	Antifungal activity	Phytochemical analysis Secondary metabolites
1	Doh et al., 2023 Experimental	Garcinia kola seed oil	Dried, pulverised, and macerated in n - hexane.	agar diffusion method	Inhibit *Candida albicans* (20 ± 0.07 to 18 ± 0.01 mm) at concentration ranging from 12.5% to 100%.	polyphenol content flavonoid content
2	Falana, 2021 Experimental	G. Kola seed	Dried Pulverized, and extracted with n - hexane,50ml honey, and vinegar	Kirby Bauer disc diffusion method	The highest (16.00 mm) inhibition zone was exhibited against *C. albicans by combination of honey, and vinegar* at 100 mg/mL concentration,while the least inhibition was by the honey extract at 7mm with concentration of 25gm/mL.	Alkaloids, anthraquinine glycosides, cardiac glycosides, flavonoids, saponins, cardenolide, phenol, tannins, carbohydrate
3	Nirasha et al., 2020 Experimental	G. zeylanica	Sun-dried fruit rinds (50 g), and dried leaves (50 g) of G. zeylanica was extracted using Soxhlet extraction apparatus separately with methanol (200 mL) as the solvent.	Agar well diffusion	C. albicans was inhibited by G. zeylanica dried fruit rind extract (MIC= 18.200 (± 0.447), but not inhibited by leaf extract MIC=0.00mm compared with standard antibiotic fluconazole (MIC = 25.000 (± 0.000). Statistical evalua tions done using turkey test. This demonstrated a significant different among inhibition zone's diameters of *G. zeylanica* leaf extract, *G. zeylanica* dried fruit rind extract and standard anti biotic against selected bacteria species.	No Phytochemical analysis
4	Akintelu et al., 2022 Case - control	Garcinia Kola Seed	Dried Pulverized, and Aqueous extract. Imm aqueous silver nitrate following necessary procedure to form AgNPs	Agar	The synthesized AgNPs show good activity against tested fungi strains with inhibition zones ranging from 6 to 17 mm which is comparable to the inhibition zones demonstrated by the control in the range of 18 to 24 mm. The concentration of 75 microgram against candida tropicalis, and lowest against candida albicans at 25 microgram.	Terpenoids, steroids iso flavonoids, and neoflavonoids

Table 2 (Contd): Garcinia Extraction Method, and Antifungal testing Approach

S/N	Author &Date	Extract Type	Extraction Medium, and Method	Antifungal testing method	Antifungal activity	Phytochemical analysis Secondary metabolites
5	Yahaya et al., 2021.	Garcinia kola seeds tannins, saponins, flavonoids, alkaloids and glycosides	Dried Pulverized, and aqueous extraction. Ethanol extraction	Tube dilution method	Zone of inhibition for candida 4mm, for both aqueous, and ethanol. Not active	alkaloids, saponins, cardiac glycoside, reducingsugar, steroids, terpenes, tannins, flavonoids, phenols and anthronoids
6	Winful, et al., 2021	Garcinia kola seeds	Dried Pulverized, and aqueous extraction. Ethanol extraction	disc diffusion and agar well diffusion sensitivity tests.	The ethanol extract produced zones of inhibition of about 7.3 mm for *Candida albicans* only at a concentration of 800mg/ml for the disc diffusion test. For the agar well diffusion test, the aqueous extract produced zones of inhibition of about 9.5 mm, while the ethanol extract produced zone of inhibition of 19 mm against Candida albicans at a concentration of 800 mg/ml	Tannins, saponins, flavonoids, alkaloids and glycosides
7	Sakirigui et al., 2020 Experimental	Peeled and Unpeeled Garcinia Kola alkaloids, flavonoids, saponins and phenolic compounds consisting of catechic and gallic tannins in the two extracts Anthocyanins and o - heterosides were present in the unpeeled seeds and absent in the peeled seeds. Other compounds such as leuco-anthocyanins, quinonics compound, coumarin, terpenoids, mucilages, cartenoids, free anthracenics are not present in the two extract	Dried Pulverized, and aqueous extraction. Ethanol extraction The qualitative phytochemical screening was performed based on colouring or precipitation reaction	Agar disc method	The MIC has been determined by macrodilution method with visual assessment of the growth of microorganisms. the smallest minimum inhibitory concentrations is (1.25 mg / ml).	Alkaloids, saponins, flavonoids and phenolic compounds consisting of catechic and gallic tannins . anthocyanins and O-heterosides had been identified in unpeeled seeds extract.

Table 2 (Contd): Garcinia Extraction Method, and Antifungal testing Approach

S/N	Author &Date	Extract Type	Extraction Medium, and Method	Antifungal testing method	Antifungal activity	Phytochemical analysis Secondary metabolites
8	Adenrinsola et al., 2025 Experimental	Garcinia Kola alkaloids, tannins, flavonoids, steroids, saponins, cardiac glycosides, phenolic flavonoids, anthraquinones, and anthocyanins Column chromatography was employed to fractionate the ethanol and methanol extracts using a 1:1 solvent mixture of ethanol and methanol as the mobile phase. Thin-layer chromatography (TLC) was used to monitor the separation of compounds. Fourier-transform infrared (FTIR) spectroscopy was used to identify functional groups Gas chromatography-mass spectrometry (GC-MS) was used to determine the chemical composition of the extracts using a Shimadzu GCMS-QP2010 Plus system. Retention times and fragmentation patterns were compared with entries in the NIST library for compound identification	The leaves were washed with distilled water, air-dried at ambient room temperature, and pulverized using a mechanical grinder. The powdered material was stored in clean airtight containers for subsequent analyses. Cold maceration was used to obtain crude extracts. Exactly 100 g of powdered leaf sample was soaked in 700 mL of each of the following solvents: ethanol, methanol, distilled water, and n-hexane.	antifungal testing was done on Potato Dextrose Agar (PDA) using the Poison Plate Method.	Zones of inhibition were measured in millimeters No inhibition zone against *Candida albican*.	Alkaloids, tannins, flavonoids, steroids, saponins, cardiac glycosides, phenolic flavonoids, anthraquinones, and anthocyanins

studies reporting use of other anti-fungal agents against candida species, studies reporting inhibitory effect of anti-fungal agent against candida species.

Exclusion Criteria:

Articles not published in English including case report, case series, editorial report, dissertation, and thesis.

PubMed/MEDLINE, Scopus, Web of Science, google scholar, Cochrane Library.

Other search include searching trial or study registers, looking through all the articles that cite the papers included in the review ("snowballing" or forward citation searching).

Study Quality Assessment

The quality of the included studies was evaluated using the Joanna Briggs Institute (JBI) Critical Appraisal Checklist 9 Munn et al., 2014). This checklist consists of eight items, each with response options of 'Yes', 'No', 'Unclear', and 'Not applicable'. Responses of 'Unclear' or 'Not applicable' were assigned 0 points, 'No' was assigned 1 point, and 'Yes' was assigned 2 points. The total possible score ranged from 0 to 16. Studies scoring between 12 and 16 points were categorized as 'high quality', those scoring between 9 and 11 as 'moderate quality', and those scoring 8 or below as 'poor quality'. The results of the quality assessments were tabulated to enable comparison across studies, ensuring that only relevant, high-quality research contributed to the synthesis.

DISCUSSION

Extraction and Purification of Polyisoprenylated Benzophenones can be from natural Sources or through Chemical Synthesis. These compounds are typically found in various plant species, particularly within the Clusiaceae (Guttiferae) family, or the Garcinia kola species, as seen in Table 1. Extraction from Natural Sources such as Garcinia Kola (9 publications), and Garcinia Zeylanica species (Nirasha et al., 2020).

The Sample Collection and preparation involved harvesting plant materials, such as fruits, dried fruit rinds, leaves, or bark, which were then dried and pulverized to

Table 2 (Contd): Garcinia Extraction Method, and Antifungal testing Approach

S/N	Author &Date	Extract Type	Extraction Medium, and Method	Antifungal testing method	Antifungal activity	Phytochemical analysis Secondary metabolites
9.	Amala, et al., 2021	G. kola , Peeled Seeds. Alkaloids, flavonoids, tannins, anthraquinone, triterpenoid, steroids, carbohydrates, cardenolide, cyanogenic glycosides and saponin	G. kola , the brown covers of the seeds were peeled using table knife, the seeds were chopped into smaller pieces. The pieces were further ground using a mechanical blender. The crushed G. kola was placed in a fine sieve clot and sieved -squeezed to obtain sticky, milky juice. This was decanted into a bottle and preserved in the refrigerator Aqueous extraction	Agar well diffusion method	*C. albicans* isolates were 60% sensitive to G. kola juice C. albicans isolates were 100% sensitive to the mixed juice G. kola + Allum Sativum (garlic) at 100% concentration	Alkaloids, flavonoids, tannins, anthraquinone,triterpenoid, steroids, carbohydrates, cardenolide, cyanogenic glycosides, and saponin
10.	Ewelike et al., 2021	Leaf extract of Garcinia kola	Methanol extraction Aqueous extraction	Disc diffusion method	No inhibition against *candida albican.*	No Phytochemical analysis

increase the surface area for extraction. The majority 60% (6 out of 10 reviewed publication) was from the Garcinia Seed. One publication reported the use of the seed oil (Doh et al., 2023).

The powdered plant materials were subjected to solvent extraction using solvents like methanol, ethanol, aqueous, and n-hexane, as reported in Table 1. One publication reported solvent such as vinegar, and honey. Ethanol was the most common solvent used, as documented by 40% (4 out of 10 publication reviewed) of the publication reviewed (Yahaya et al., 2021;Winful et al., 2021; Aderinola et al., 2025; Sakiguiri st al., 2020; Ewelike et al., 2021. The choice of solvent depends on the polarity of the target benzophenones. In most of the publications reviewed, the solvent extracts were filtered to remove plant debris and then concentrated under reduced pressure using rotary evaporation.

The concentrated extract may be partitioned with solvents of varying polarities (e.g., hexane, ethyl acetate, water) to separate different classes of compounds (Liquid-Liquid Partitioning*) (*Doh *et al.,* 2023*;* Falana *et al.,* 2021). The target polyisoprenylated benzophenones are further purified by chromatographic techniques such as column chromatography, high-performance liquid chromatography (HPLC), or preparative thin-layer chromatography (TLC) (Chromatographic Purification) (Mwankuna et al., 2023).

Some of the publication (Nirasha *et al.,* 2020*;* Sakirigui *et al.*, 2020) used Structural Elucidation: The purified compounds are characterized using spectroscopic methods like NMR, MS, and IR to confirm their identity and purity (Table 1). The metabolites identified in the reviewed studies included alkaloids, tannins, steroids, saponins, cardiac glycosides, phenolic flavonoids, anthraquinones, and anthocyanins (Aderinsola et al., 2025). These compounds were detected using column chromatography, gas chromatography, and Fourier transform infrared (FTI) analysis. Sakirigui et al. (2020) also reported the isolation of anthocyanins and o-heterosides from unpeeled Garcinia kola seeds, noting that these metabolites were absent in peeled seeds. Additionally, Amala et al. (2021) documented the presence of triterpenoids, steroids, carbohydrates, cardenolides, cyanogenic compounds, and glycosides, expanding on the metabolites previously identified by Aderinsola et al. (2025). The 3 publication reported aqueous extraction, and only Amala et al, 2021 reported using Garcinia Kola leaves.

Most (80%) of the publication reviewed reported secondary metabolites of garcinia kola, and the constant active agents in the reviewed publications reporting phytochemical analysis was flavanoids (100%).

The report showed inhibitory activity against *Candida tropicalis*, and lesser inhibitory activity against *Candida albican*. It was the only paper reviewed that reported antimicrobial activity against non-*candida albican* species. This can suggest that both natural extraction and chemical synthesis are vital for obtaining polyisoprenylated benzophenones, each offering unique advantages. Extraction from natural sources is often more environmentally friendly and can yield naturally occurring derivatives, while chemical synthesis allows for the creation of novel analogues and facilitates structure-activity relationship studies. The choice of method depends on the availability of natural material, desired yield, and specific research objectives.

The tested candida strain selection of clinical relevance in most of these studies was *Candida albicans*. Cultivation under standardized conditions to ensure reproducibility.

Preparation of standardized inocula, typically adjusted to a specific cell density (e.g., 1 × 10 CFU/mL) were used in most of the studies.

The in vitro anti-fungal susceptibility testing were done using assay such as the:

- *Tube dilution or Broth Microdilution Assay:* Standardized according to CLSI or EUCAST guidelines to determine minimum inhibitory concentrations (MICs).
- *Agar Diffusion Methods:* Disk diffusion or well diffusion assays to qualitatively assess anti- fungal activity via zones of inhibition.

Six publications (60%) made use of agar diffusion methods. The studies reporting the use of potato dextrose agar (PDA) (Aderinsola *et al.*, 2025), disc diffusion (Ewelike *et al.*, 2021), tube dilution (Yahaya et al, 2021), recorded little or no anti-fungal inhibitory activity against candida albicans.

None of the publications reviewed used the Time-Kill Kinetics (Öz et al., 2016). This method assesses the

fungistatic versus fungicidal effects by quantifying viable cells over time in the presence of test compounds.

None of the studies reviewed were mechanistic Studies (Kauser et al., 2024), such as

- *Cell Membrane Integrity Assays:* Using propidium iodide or similar dyes to evaluate membrane disruption.
- *Ergosterol Binding / Quantification Assays:* Investigating effects on fungal membrane sterol content.

There was also no report of Biofilm Inhibition and Eradication Assays (Kamimura et al., 2022) such as

- Assessing the impact of polyisoprenylated benzophenones on biofilm formation and on preformed biofilms using crystal violet staining or XTT reduction assays.

Data Analysis

Only two publication reported details of the percentage inhibition or eradication calculated by comparing treated wells to untreated controls. Out of these 2 publications (Amala *et al.*, 2021;Nirasha *et al.*, 2020), only one (Nirasha *et al.*, 2020) reported mean of repeated assay performance, expressing result as mean ± standard deviation, and also reporting statistical analysis using Turkey t test in evaluating significance of observed effects, by determining statistical significance between case , and control test. This methodology allows for comprehensive assessment of the anti-fungi effect of polyisoprenylated benzophenones.

None of the publication reported Molecular and Microscopic Analyses such as:

- *Gene Expression Studies:* RT-qPCR to evaluate changes in expression of key virulence or drug-resistance genes.
- *Microscopy:* SEM or fluorescence microscopy to visualize morphological changes or compound localization.

Molecular and Microscopic Analyses includes;

Gene Expression Studies: Reverse transcription quantitative polymerase chain reaction (RT-qPCR) was used to measure the expression levels of key virulence and drug-resistance genes. This approach enables precise quantification of gene expression changes in response to experimental treatments or conditions, providing detailed insights into the molecular mechanisms underlying observed phenotypes (Bong *et al.*, 2024).

Microscopy: Scanning electron microscopy (SEM) and fluorescence microscopy was used to visualize morphological changes and to localize compounds within cells or tissues. SEM produced high-resolution images of surface structures and reveal any morphological alterations, while fluorescence microscopy detect and track specific compounds or cellular components, offering complementary information on cellular dynamics and interactions (Fischer *et al.*, 2024).

Seven publications reviewed (70%) reported antifungal activity of garcinia kola against candida species. The 3 publication reporting non -inhibitory activity used different antifungal testing such as tube dilution, disc diffusion, and potato dextrose agar method. Tube dilution, disc diffusion, and Potato Dextrose Agar (PDA) antifungal testing all share the same fundamental goal: evaluating how effectively an antifungal agent inhibits fungal growth. Tube dilution and disc diffusion are widely standardized methods, often making use of RPMI 1640 medium. In contrast, PDA is a specialized agar medium suitable for similar susceptibility testing, especially when screening new antifungal agents or assessing filamentous fungi (Berkow et al., 2020). The primary method of anti-fungal testing was agar diffusion (70%), with most studies assessing efficacy against *Candida albicans*. Outcomes were typically reported as zones of inhibition or minimal inhibitory concentrations. However, there was considerable heterogeneity in results across the studies reviewed. All (100%) reviewed publications reported biological approach of anti-fungal testing, highlighting the need for molecular approaches for assessing anti-fungal activity or efficacy of polyisoprenylated benzophenones against *Candida species*.

The publications reviewed were of moderate to high quality, resulting in moderate to high evidence of certainty. Most of the publications reported inhibitory effect on *Candida albicans,* and presence of flavanoids as active agents. None of the publications used molecular approach of anti-fungal testing, highlighting the need for higher-quality research, and clinical research to more robustly address the research questions.

CONCLUSION

A systematic review in which most included studies are at very low to moderate risk of bias underscores the need for comprehensive and multidisciplinary investigations. Robust assessment of the anti-fungal efficacy of polyisoprenylated benzophenones against *Candida species* should integrate traditional susceptibility assays with mechanistic and molecular analyses. This combined approach allows for a thorough evaluation of both the potency and mechanisms of action of candidate compounds.

REFERENCES

1. da Costa PC, Santos TLB, Ramos JF, Santos JAM, de Medeiros FD, Freitas JCR, et al. Synthesis and antifungal evaluation against Candida spp. of (E)-3-(furan-2-yl)acrylic acid. Braz J Microbiol. 2024;55:133-142.
2. Doh AA, Adjouman YD, Nindjin C, Kouamé KA, Solange AG, Kolo BO, et al. Antioxidant activity and efficacy of Garcinia kola (bitter kola) oil on pathogenic and alteration microorganisms of attiéké. Heliyon. 2023;9:e21152.
3. Akoro SM, Aiyelaagbe OO, Onocha PA, Gloer JB. Gakolanone: a new benzophenone derivative from Garcinia kola Heckel stem-bark. Nat Prod Res. 2020;34:241-250.
4. Chinemerem ND, Ugwu MC, Oliseloke AC, Al-Ouqaili MTS, Chinedu IJ, Victor CU, et al. Antibiotic resistance: the challenges and some emerging strategies for tackling a global menace. J Clin Lab Anal. 2022;36:e24655.
5. Yarahmadi A, Najafiyan H, Yousefi MH, Khosravi E, Shabani E, Afkhami H, et al. Beyond antibiotics: exploring multifaceted approaches to combat bacterial resistance in the modern era: a comprehensive review. Front Cell Infect Microbiol. 2025;15:1493915.
6. Pasaribu YP, Fadlan A, Fatmawati S, Ersam T. Biological activity evaluation and in silico studies of polyprenylated benzophenones from Garcinia celebica. Biomedicines. 2021;9:1654.
7. Tauchen J, Frankova A, Manourova A, Valterova I, Lojka B, Leuner O. Garcinia kola: a critical review on chemistry and pharmacology of an important West African medicinal plant. Phytochem Rev. 2023;23:1-47.
8. Kayinu N, Adele BI, Ige A. Garcinia kola and kolaviron attenuate bisphenol A-induced memory impairment and hippocampal neuroinflammation in male Wistar rats. J Biosci Med. 2024;12:111-130.
9. Oyagbemi AA, Omobowale TO, Adedapo AA, Yakubu MA. Kolaviron, biflavonoid complex from the seed of Garcinia kola attenuated angiotensin II- and lipopolysaccharide-induced vascular smooth muscle cell proliferation and nitric oxide production. Pharmacogn Res. 2016;8(Suppl 1):S50-S55.
10. Abah AA, Agbelusi GA, Odukoya OA, Ayanbadejo PO, Adefule-Ositelu AO, Adebiyi KE. Garcinia kola extracts as antifungal therapy for oral Candida infections: a comparative study. IOSR J Dent Med Sci. 2014;13:20-24.
11. Falana. Estimation of phytochemical compounds and antimicrobial studies of different solvent extracts of Garcinia kola against some pathogens. Niger J Microbiol. 2021;35:5721-5734.
12. Nirasha WD, Chandani K, Sanjeewani DMR, Tharanga PR, Tharindra W, Hemal S. Screening Garcinia zeylanica for in vitro antimicrobial activity and antioxidant activity. Aust J Tech Nat Sci. 2020;8:3-10.
13. Akintelu SA, Olugbeko SC, Folorunso AS. Green synthesis, characterization, and antifungal activity of synthesized silver nanoparticles (AgNPs) from Garcinia kola pulp extract. BioNanoScience. 2022;12:105-115.
14. Yahaya MA, Mohammed BA, Abubakar AN, Muhammad FM, Abdulkadir A. Antibacterial and antifungal activities of aqueous and ethanol extracts of Phyllanthus niruri and Garcinia kola. Int J Appl Biol Res. 2021;12:17-26.
15. Winful E, Idowu O, Fasanya OO, Egbe NE. Comparative studies of the antimicrobial effect of ethanolic and aqueous extracts of Garcinia kola on Klebsiella pneumoniae and Candida albicans in vitro. Niger J Technol Res. 2021;16:52-58.
16. Sakirigui A, Nonviho G, Hounkpatin ASY, Sika KC. Effects of teguments on phytochemistry and antimicrobial activities of Garcinia kola seeds. J Pharmacogn Phytochem. 2020;9:21-26.
17. Adenrinsola N, Zakari B, Adebisi AO. Comprehensive profiling of secondary metabolites and bio-activity of Garcinia kola (Heckel) leaf. J Environ Sci Agric Res. 2025;3:1-6.
18. Amala SE, Nweke SN, Nwalozie R, Monsi TP. Antimicrobial properties and phytochemical composition of Garcinia kola, Bryophyllum pinnatum, and Allium sativum juices on some clinical pathogens. Adv Biosci Biotechnol. 2021;12:388-406.
19. Ewelike NC, Okammadu JC, Ogwudire VE, Nnadozie RI. In vitro antimicrobial activity of methanolic and aqueous leaf extracts of Chrysophyllum albidum (African star apple) and Garcinia kola (bitter kola). GSC Biol Pharm Sci. 2021;14:249-253.
20. Bong D, Sohn J, Lee SV. Brief guide to RT-qPCR. Mol Cells. 2024;47:100141.
21. Fischer ER, Hansen BT, Nair V, Hoyt FH, Schwartz CL, Dorward DW. Scanning electron microscopy. Curr Protoc. 2024;4(5):e1034.

22. Chan A. Can AI be used for risk assessments? [Internet]. ISACA; 2023 [cited 2026 Apr 10]. Available from: https://www.isaca.org/resources/news-and-trends/industry-news/2023/can-ai-be-used-for-risk-assessments
23. Fifolato MA, Clemente LM, Ribeiro AB, Oliveira VC, Salgado HC, Watanabe E, et al. Candida spp. in denture stomatitis: prevalence, microbial load, and antifungal resistance across severity levels. Microorganisms. 2025;13:2057.
24. Qiu F, Zhang CH, Wang JD, Fan YM. Scrotal tinea caused by Nannizzia incurvata in two men using molecular identification. J Eur Acad Dermatol Venereol. 2022;36:e957-e959.
25. Munn Z, Moola S, Riitano D, Lisy K. The development of a critical appraisal tool for use in systematic reviews addressing questions of prevalence. Int J Health Policy Manag. 2014;3:123-128.
26. Tauchen J, Frankova A, Manourova A, Valterova I, Lojka B, Leuner O. Garcinia kola: a critical review on chemistry and pharmacology of an important West African medicinal plant. Phytochem Rev. 2023;23:1-47.
27. Mwankuna CJ, Mariki EE, Mabiki FP, Malebo HM, Styrishave B, Mdegela RH. Thin layer chromatographic method for detection of conventional drug adulterants in herbal products. Separations. 2023;10(1):23.
28. Kauser S, Raj N, Ahmedi S, Manzoor N. Mechanistic insight into the membrane disrupting properties of thymol in Candida species. Microbe. 2024;2:100045.
29. Öz Y, Özdemir HG, Gökbolat E, Kiraz N, Ilkit M, Seyedmousavi S. Time-kill kinetics and in vitro antifungal susceptibility of non-fumigatus Aspergillus species isolated from patients with ocular mycoses. Mycopathologia. 2016;181(3-4):225-233.
30. Kamimura R, Kanematsu H, Ogawa A, Kogo T, Miura H, Kawai R, et al. Quantitative analyses of biofilm by using crystal violet staining and optical reflection. Materials (Basel). 2022;15(19):6727.
31. Berkow EL, Lockhart SR, Ostrosky-Zeichner L. Antifungal susceptibility testing: current approaches. Clin Microbiol Rev. 2020;33(3):e00069-19.
32. Kumar S, Sharma S, Chattopadhyay SK. The potential health benefit of polyisoprenylated benzophenones from Garcinia and related genera: ethnobotanical and therapeutic importance. Fitoterapia. 2013;89:86-125.
33. Gao X, Yu T, Lai FSF, Pu J, Qiao C, Zhou Y, et al. Novel polyisoprenylated benzophenone derivatives from Garcinia paucinervis. Tetrahedron Lett. 2010;51:2442-2446.
34. Olatoye FJ, Akindele AJ, Awodele O. The role of kolaviron, a bioflavonoid from Garcinia kola, in the management of cardiovascular diseases: a systematic review. Heliyon. 2024;10:e27333.