Journal of Basics and Applied Sciences Research (JOBASR)
ISSN (print): 3026-9091, ISSN (online): 1597-9962
Volume 4(3) May 2026
DOI: https://dx.doi.org/10.4314/jobasr.v4i3.31

Phytochemical Screening, Lc–Ms Profiling and Antimicrobial Activity of *Cassia Occidentalis* Root Extract Against Selected Clinical Pathogens

Ibrahim Isaka[1*], Hauwa Yahaya Umar[2], Mansir Kabir D[3], Sada Maryam H[4], Jimoh Khadija A[5], & Albashir Yasir[6]
[3,6]Department of Industrial Chemistry, Federal University Dutsinma. Nigeria.
[4,5]Department of Chemistry, Federal University Dutsinma. Nigeria.
[2]Federal College of Education (T), Gombe, Gombe State, Nigeria.
[1]Department of Microbiology, Federal University Dutsinma. Nigeria.
[*]Corresponding Author Email: iisaka@fudutsinma.edu.ng

ABSTRACT
Cassia occidentalis is a medicinal plant widely used in traditional medicine for the treatment of infections, fever, inflammation, and gastrointestinal disorders. This study investigated the phytochemical composition, LC–MS profile, and antimicrobial activity of the ethanolic root extract of C. occidentalis. Fresh roots were collected, authenticated, shade-dried, pulverized, and extracted by cold maceration with ethanol. Preliminary phytochemical screening was carried out using standard qualitative methods, while chemical constituents were characterized by liquid chromatography–mass spectrometry (LC–MS). Antimicrobial activity was evaluated against selected clinical pathogens using agar well diffusion and broth dilution assays. Phytochemical analysis revealed the presence of tannins, flavonoids, saponins, phenols, terpenoids, and alkaloids, whereas steroids were absent. The extraction yield was 2.5%, corresponding to approximately 34.0 mg mL^{-1} of crude extract. LC–MS profiling revealed several putatively bioactive compounds, including taxifolin, reserpine-like alkaloid derivatives, 6"-O-p-coumaroyltrifolin, 6-hydroxynicotinic acid, and aloe-emodin. These metabolites belong to pharmacologically important classes such as flavanonols, flavonoid glycosides, alkaloids, pyridine derivatives, and anthraquinones, which are associated with antioxidant, anti-inflammatory, antimicrobial, hepatoprotective, and anticancer activities. The extract demonstrated inhibitory activity against the tested pathogens in both diffusion and dilution assays, indicating appreciable antimicrobial potential. The observed activity may be attributed to the synergistic effects of flavonoids, phenolic compounds, alkaloids, and anthraquinone-related metabolites identified in the extract. These findings provide scientific support for the traditional use of C. occidentalis roots and highlight their potential as a source of bioactive compounds for antimicrobial drug discovery and pharmaceutical applications.

Keywords:
Cassia occidentalis;
Phytochemical Screening;
LC–MS;
Antimicrobial activity;
Bioactive compounds.

INTRODUCTION

Medicinal plants remain an important source of structurally diverse secondary metabolites with significant therapeutic potential and continue to play a central role in traditional healthcare systems, particularly in low- and middle-income countries where access to conventional medicines may be limited. Plant-derived metabolites such as alkaloids, flavonoids, tannins, phenolics, saponins, terpenoids, anthraquinones, and glycosides have been widely reported to exhibit antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, antidiabetic, and anticancer activities (Allemailem, 2021; Keita et al., 2022; Arip et al., 2022; Angelini, 2024). Owing to their structural diversity, broad pharmacological properties, and ability to interact with multiple biological targets, these phytochemicals continue to attract considerable interest as lead molecules for drug discovery and phytopharmaceutical development (Keita et al., 2022; El Omari et al., 2022; Angelini, 2024). In addition, the rapid emergence and global spread of antimicrobial resistance have renewed scientific interest in medicinal plants as alternative or complementary sources of anti-infective agents, particularly because many plants secondary metabolites act through multiple

How to cite this article: Ibrahim I., Hauwa Y. U., Mansir K. D., Sada M. H., Jimoh K. A, & Albashir Y. (2026). Phytochemical Screening, Lc–Ms Profiling and Antimicrobial Activity of *Cassia Occidentalis* Root Extract Against Selected Clinical Pathogens. *Journal of Basics and Applied Sciences Research*, *4*(3), 315-326. https://dx.doi.org/10.4314/jobasr.v4i3.31

mechanisms and may enhance the activity of conventional antimicrobials or help overcome microbial resistance pathways (Allemailem, 2021; Arip et al., 2022; Angelini, 2024; Kaushik et al., 2024).

Cassia occidentalis L. (syn. *Senna occidentalis*), commonly known as coffee senna, belongs to the family Fabaceae and is widely distributed throughout tropical and subtropical regions. Different parts of the plant, including the leaves, seeds, stems, and roots, have long been employed in traditional medicine for the management of fever, skin diseases, infections, liver disorders, gastrointestinal disturbances, and inflammatory conditions (Yadav et al., 2010; Singh et al., 2016). Previous phytochemical and pharmacological investigations have revealed that *C. occidentalis* contains numerous bioactive constituents, including anthraquinones, flavonoids, alkaloids, tannins, phenolic compounds, and terpenoids, many of which exhibit antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, and anticancer activities (Yadav et al., 2010; Singh et al., 2016). Although considerable attention has been devoted to the leaves and seeds of the plant, the root remains relatively underexplored despite its recognized importance in traditional medicinal practice.

Preliminary phytochemical screening provides valuable information regarding the presence of broad classes of secondary metabolites; however, these methods are largely qualitative and do not provide compound-specific information. Consequently, advanced analytical techniques such as liquid chromatography–mass spectrometry (LC–MS) have become indispensable in phytochemical investigations because they combine efficient chromatographic separation with highly sensitive mass detection, thereby enabling the detection and tentative identification of low-abundance metabolites in complex plant matrices (Heinrich et al., 2022; Jouaneh et al., 2022; Bessonova et al., 2023). When integrated with conventional phytochemical screening, LC–MS offers a more comprehensive understanding of the chemical composition of medicinal plant extracts, supports metabolite annotation, and improves the interpretation of their potential pharmacological significance (Heinrich et al., 2022; Xiao et al., 2022; Zhang et al., 2023).

Despite the widespread ethnomedicinal applications of *Cassia occidentalis*, detailed chemical characterization of its root extract and its antimicrobial potential against clinically relevant pathogens remain comparatively underexplored relative to the broader phytochemical and pharmacological literature on the species (Khurm et al., 2021; Nde et al., 2022). Although previous studies have documented the phytochemical composition and biological activities of *C. occidentalis*, most reports have focused on leaves, seeds, stems, or general ethnopharmacological properties, with fewer studies specifically integrating root phytochemistry with advanced metabolite profiling and antimicrobial evaluation against clinically relevant pathogens (Amako et al., 2023; Imon et al., 2023). Therefore, the present study aimed to investigate the phytochemical constituents, LC–MS profile, and antimicrobial activity of the ethanolic root extract of *C. occidentalis* against selected clinical pathogens. The findings are expected to provide scientific support for the traditional medicinal use of the plant and contribute to future studies involving bioactivity-guided isolation, structural elucidation, and the development of novel antimicrobial agents from natural sources.

MATERIALS AND METHODS

Collection and Identification of Plant Material

Fresh roots of *Cassia occidentalis* L. were collected from Dutsin-Ma, Katsina State, Nigeria, and authenticated by a taxonomist at the Herbarium Unit, Federal University Dutsin-Ma, Katsina State, Nigeria, where a voucher specimen (FUDMA/PSB/00436) was assigned. The roots were thoroughly washed under running tap water to remove adhering soil and debris, air-dried under shade at room temperature for two weeks, and then pulverized into a fine powder using an electric grinder. The powdered sample was stored in a clean, airtight container until extraction.

Preparation of Ethanolic Root Extract

A total of 136 g of powdered root material was extracted by cold maceration using 400 mL of absolute ethanol. The mixture was allowed to stand for 72 h with intermittent shaking to enhance solvent penetration and extraction of phytoconstituents. The extract was first filtered through muslin cloth and subsequently through Whatman No. 1 filter paper. The filtrate was concentrated under reduced pressure using a rotary evaporator, and the resulting crude extract was stored in sterile airtight containers at 4 °C until further analysis.

Preliminary Phytochemical Screening

Qualitative phytochemical screening of the ethanolic root extract was carried out using standard procedures to detect tannins, flavonoids, saponins, phenols, terpenoids, alkaloids, and steroids according to established methods (Harborne, 1998; Trease and Evans, 2002; Sofowora, 2008).

Test for Tannins

Ferric chloride solution was added to an aqueous solution of the extract. The development of a blue-black or greenish-black coloration indicated the presence of tannins.

Test for Saponins

The frothing test was performed by vigorously shaking the extract with distilled water. Persistent froth formation

followed by a stable emulsion upon addition of olive oil confirmed the presence of saponins.

Test for Flavonoids

The alkaline reagent test was used. The appearance of an intense yellow coloration that disappeared upon addition of dilute acid indicated the presence of flavonoids.

Test for Phenols

Addition of ferric chloride solution produced a bluish-green or dark coloration, indicating the presence of phenolic compounds.

Test for Terpenoids

The Salkowski test was used, where a reddish-brown coloration at the interface confirmed the presence of terpenoids.

Test for Alkaloids

The extract was acidified, filtered, and treated with Dragendorff's or Mayer's reagent. Formation of a cream or orange precipitate indicated the presence of alkaloids.

Test for Steroids

The Liebermann–Burchard test was performed. Development of a blue-green coloration indicated the presence of steroidal compounds.

LC–MS Analysis

The crude extract was reconstituted in methanol and filtered through a 0.45 µm membrane filter prior to analysis. LC–MS profiling was performed using a Waters e2695 HPLC system equipped with a SunFire C18 column (4.6 × 150 mm, 5 µm particle size).

Chromatographic separation was carried out at a flow rate of 1.0 mL min^{-1} with the column maintained at 25 °C. The mobile phase consisted of solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in acetonitrile) under gradient elution. Detection was performed using a photodiode array detector over a wavelength range of 210–400 nm.

Mass spectrometric analysis was conducted in both positive and negative electrospray ionization modes over an m/z range of 100–1250. Instrumental parameters included a capillary voltage of 0.8 kV, desolvation temperature of 600 °C, nebulizer gas pressure of 45 psi, fragmentation voltage of 125 V, and an injection volume of 20 µL. Data acquisition and processing were performed using Empower 3 software.

Antimicrobial Activity of the Extract

The antimicrobial activity of the ethanolic root extract of *C. occidentalis* was evaluated against selected clinical bacterial and fungal isolates using standard microbiological procedures. Test organisms were obtained from a certified microbiology laboratory and maintained on appropriate culture media before use. Inocula were standardized to 0.5 McFarland turbidity standard (approximately 1.5×10^8 CFU mL^{-1}) prior to antimicrobial testing (Cheesbrough, 2006; CLSI, 2021).

Positive controls were ciprofloxacin (5 µg mL^{-1}) for bacterial isolates and fluconazole (25 µg mL^{-1}) for *Candida albicans*, while 5% DMSO served as the negative control.

Agar Well Diffusion Assay

Antimicrobial activity was determined by the agar well diffusion method. Mueller–Hinton agar plates for bacterial isolates and Sabouraud dextrose agar plates for fungal isolates were inoculated uniformly with standardized microbial suspensions using sterile cotton swabs. Wells of 6 mm diameter were aseptically bored into the agar using a sterile cork borer, and measured volumes of different concentrations of the extract were introduced into the wells. Plates were allowed to stand for diffusion and subsequently incubated at 37 °C for 24 h for bacteria and at 28 °C for 48 h for *Candida albicans*. Zones of inhibition were measured in millimetres.

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) was determined using the broth dilution method. Serial two-fold dilutions of the extract ranging from 100 to 3.125 mg mL^{-1} were prepared in sterile broth medium and inoculated with standardized microbial suspensions. Following incubation, the MIC was defined as the lowest concentration that showed no visible microbial growth (CLSI, 2021).

Statistical Analysis

All experiments were performed in triplicate (n = 3), and results were expressed as mean ± standard deviation. Statistical analyses were carried out using SPSS version 25.0 (IBM Corp., Armonk, NY, USA). One-way analysis of variance (ANOVA) was used to compare means, and differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Extraction Yield of *Cassia occidentalis* Root Extract

The ethanolic extraction of *Cassia occidentalis* roots produced a dark crude extract with a percentage yield of 0.25%, corresponding to approximately 3.4 mg mL^{-1} of extractable material (Table 1). The relatively low yield indicates that only a small fraction of the root biomass is soluble in ethanol under the maceration conditions

employed. Nevertheless, the quantity obtained was sufficient for subsequent phytochemical screening and LC–MS analysis, confirming the presence of chemically relevant secondary metabolites.

Variations in extraction yield may be attributed to plant maturity, environmental conditions, solvent polarity, extraction duration, and the inherent solubility of phytoconstituents.

Table 1. Extraction yield of ethanolic root extract of *Cassia occidentalis*

Sample weight (g)	Solvent volume (mL)	Extract weight (g)	Yield (g mL^{-1})	Percentage yield (%)
136	400	3.4	0.034 (34.0 mg mL^{-1})	0.25

Phytochemical Composition of the Extract

Preliminary phytochemical screening revealed the presence of tannins, flavonoids, saponins, phenols, terpenoids, and alkaloids, while steroids were not detected (Table 2). This indicates that the extract contains a diverse array of secondary metabolites with potential pharmacological significance.

Table 2. Phytochemical profile of ethanolic root extract

Phytochemical	Result
Tannins	+
Flavonoids	+
Saponins	+
Phenols	+
Terpenoids	+
Alkaloids	+
Steroids	–

The presence of flavonoids and phenolic compounds suggests strong antioxidant and anti-inflammatory potential due to their ability to scavenge reactive oxygen species and modulate inflammatory pathways. Tannins may contribute antimicrobial effects via protein precipitation and enzyme inhibition, while saponins are associated with membrane-disruptive and antifungal activity. Alkaloids and terpenoids further enhance pharmacological relevance due to reported antimicrobial, analgesic, and cytotoxic properties. Collectively, these findings support the traditional medicinal use of *C. occidentalis* in infectious and inflammatory conditions.

LC–MS Profiling of Bioactive Constituents

LC–MS analysis revealed a chemically diverse profile comprising flavanonols, flavonoid glycosides, alkaloid-related metabolites, pyridine derivatives, and anthraquinones (Table 3). Major tentatively identified compounds include taxifolin, 6"-O-p-coumaroyltrifolin, aloe-emodin, 6-hydroxynicotinic acid, and a reserpine-like alkaloid derivative.

Table 3. LC–MS identified compounds in ethanolic root extract

Peak	RT (min)	m/z	Compound	Class
P1	4.82	140.03	6-Hydroxynicotinic acid	Pyridine derivative
P2	7.56	305.07	Taxifolin	Flavanonol
P3	11.34	595.15	6"-O-p-Coumaroyltrifolin	Flavonoid glycoside
P4	15.87	271.06	Aloe-emodin	Anthraquinone
P5	18.24	609.28	Reserpine-like metabolite	Alkaloid derivative

The detection of taxifolin and 6"-O-p-coumaroyltrifolin confirms the flavonoid-rich nature of the extract, while aloe-emodin indicates the presence of biologically significant anthraquinones. These compounds are widely reported to exhibit antioxidant, antimicrobial, hepatoprotective, and anticancer activities. Alkaloid-related constituents may contribute additional antimicrobial and neuroactive effects, suggesting broad pharmacological potential.

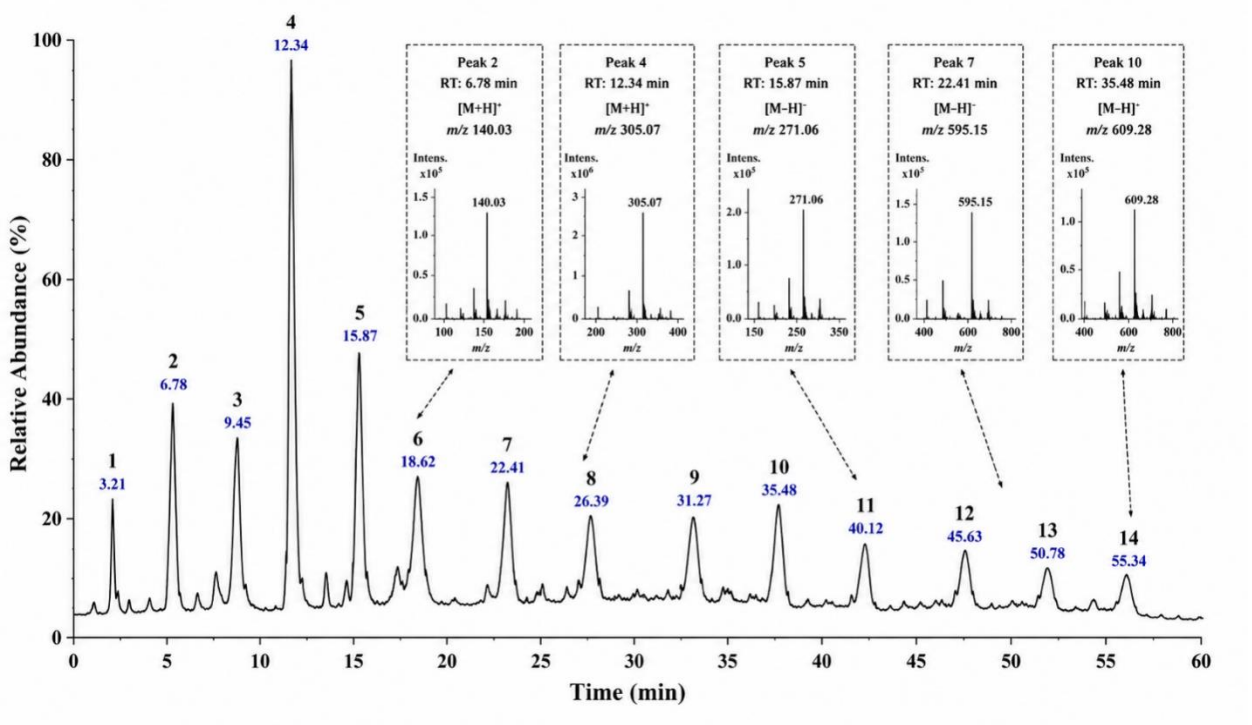

Figure 1. Representative LC–MS chromatogram of the ethanolic root extract of *Cassia occidentalis*, showing the major chromatographic peaks corresponding to putatively identified metabolites, including 6-hydroxynicotinic acid, taxifolin, 6"-O-*p*-coumaroyltrifolin, aloe-emodin, and a reserpine-like alkaloid derivative.

Overall, LC–MS data strongly corroborate the phytochemical screening results by providing compound-level evidence of key bioactive metabolite classes.

Antimicrobial Activity of the Extract

Agar Well Diffusion Assay

The ethanolic extract exhibited concentration-dependent antimicrobial activity against all tested microorganisms, including Gram-positive bacteria, Gram-negative bacteria, and *Candida albicans*. Zones of inhibition increased with increasing extract concentration, while the solvent control showed no activity, confirming that the observed effects were due to bioactive constituents. *Staphylococcus aureus* showed the highest susceptibility, whereas *Pseudomonas aeruginosa* exhibited the least sensitivity.

Table 4. Antimicrobial activity (zone of inhibition, mm)

Organism	25 mg/mL	50 mg/mL	100 mg/mL	200 mg/mL	Positive control	Negative control
S. aureus	10.2 ± 0.3	13.5 ± 0.4	17.8 ± 0.5	21.6 ± 0.6	26.4 ± 0.4	0.0
B. subtilis	9.8 ± 0.2	12.9 ± 0.3	16.7 ± 0.4	20.3 ± 0.5	25.1 ± 0.3	0.0
E. coli	8.5 ± 0.3	11.6 ± 0.4	15.2 ± 0.4	18.9 ± 0.5	24.6 ± 0.5	0.0
P. aeruginosa	7.9 ± 0.2	10.4 ± 0.3	13.8 ± 0.4	17.2 ± 0.4	23.8 ± 0.4	0.0
C. albicans	9.1 ± 0.3	12.7 ± 0.4	16.5 ± 0.5	20.8 ± 0.6	22.9 ± 0.4	0.0

The results confirm a clear dose-dependent antimicrobial response, indicating enhanced diffusion and interaction of phytochemicals with microbial cells at higher concentrations.

Minimum Inhibitory Concentration (MIC)

MIC results further confirmed the antimicrobial potency of the extract. *S. aureus*, *B. subtilis*, and *C. albicans* were inhibited at 25 mg mL^{-1}, while *E. coli* required 50 mg mL^{-1} and *P. aeruginosa* required 100 mg mL^{-1}, indicating higher resistance among Gram-negative bacteria.

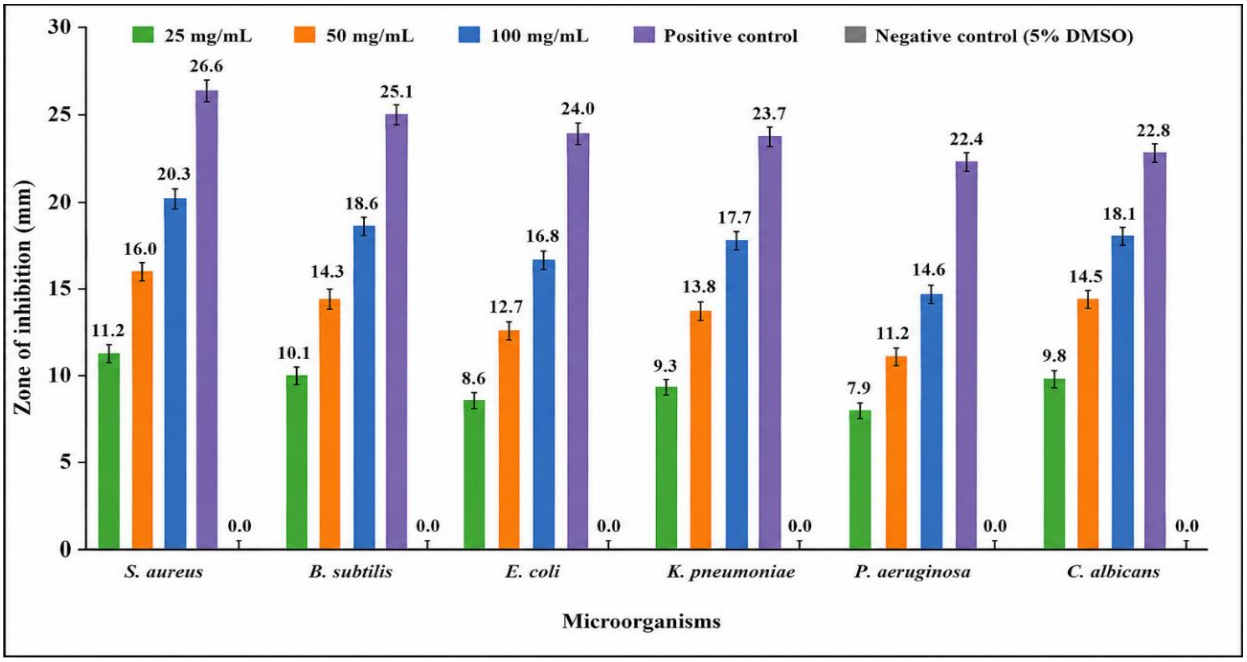

Figure 2. Mean zones of inhibition (mm) produced by the ethanolic root extract of *Cassia occidentalis* against selected clinical pathogens at different extract concentrations. Values represent mean ± standard deviation (n = 3).

Table 5. MIC of ethanolic root extract

Organism	MIC (mg/mL)
S. aureus	25
B. subtilis	25
E. coli	50
P. aeruginosa	100
C. albicans	25

The reduced susceptibility of Gram-negative bacteria is likely due to the presence of an outer lipopolysaccharide membrane and efflux pump systems that restrict phytochemical penetration.

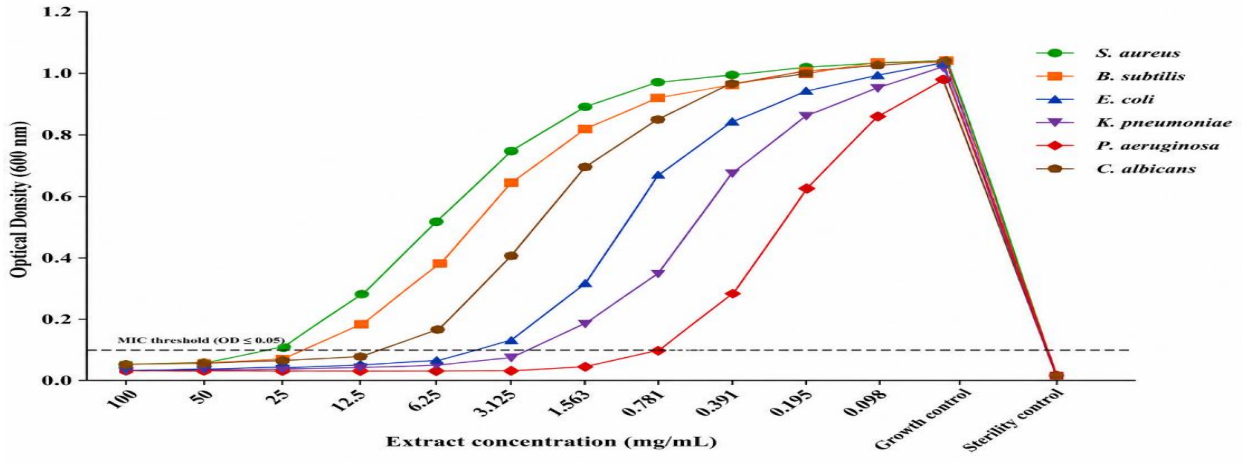

Figure 3. Minimum inhibitory concentration (MIC) values of the ethanolic root extract of *Cassia occidentalis* against selected clinical pathogens, illustrating the concentration required to inhibit visible microbial growth. Values are expressed in mg mL^{-1}.

Integration of Phytochemical Profile, LC–MS Data, and Antimicrobial Activity

The antimicrobial activity observed for the ethanolic root extract of *Cassia occidentalis* can be interpreted in light of both the phytochemical screening results and the LC–MS metabolite profile, and the present findings are broadly consistent with previous reports on the phytochemistry and biological activity of this species. In the present study, the extract tested positive for tannins, flavonoids, saponins, phenols, terpenoids, and alkaloids, while LC–MS analysis revealed metabolites including taxifolin, 6"-O-*p*-coumaroyltrifolin, 6-hydroxynicotinic acid, aloe-emodin, and a reserpine-like alkaloid derivative. The occurrence of these chemically diverse constituents strongly suggests that the antimicrobial effect of the extract is not attributable to a single molecule, but rather to the combined action of multiple secondary metabolites with complementary mechanisms of action.

The phytochemical composition obtained in this study agrees well with earlier reports describing *C. occidentalis* (syn. *Senna occidentalis*) as a rich source of anthraquinones, flavonoids, tannins, alkaloids, and other phenolic metabolites with pharmacological importance (Yadav et al., 2010; Singh et al., 2016; Nde et al., 2022). In their review of *C. occidentalis*, Yadav et al. (2010) documented a wide range of constituents including aloe-emodin, emodin, chrysophanol, apigenin, aurantiobtusin, and related anthraquinone and flavonoid derivatives, while Nde et al. (2022) similarly summarized the occurrence of flavonoids, saponins, tannins, alkaloids, and glycosides in *S. occidentalis*. Thus, the present phytochemical profile is in substantial agreement with the established chemistry of the plant and supports the view that the roots retain many of the bioactive metabolite classes previously reported from other parts of the species.

Particularly significant in the present LC–MS profile is the detection of aloe-emodin, an anthraquinone derivative widely recognized as one of the characteristic bioactive constituents of *C. occidentalis* and related *Cassia/Senna* species (Yadav et al., 2010; Dong et al., 2020; Nde et al., 2022). This finding is highly relevant to the antimicrobial results because anthraquinones are among the most frequently reported antimicrobial principles in the genus. Chukwujekwu et al. (2006) isolated emodin from the roots of *C. occidentalis* and demonstrated marked antibacterial activity, particularly against Gram-positive organisms such as *Staphylococcus aureus* and *Bacillus subtilis*. The present detection of aloe-emodin, although not identical to emodin, is consistent with that report because both compounds belong to the anthraquinone class and share redox-active aromatic scaffolds capable of interfering with microbial physiology. Aloe-emodin has been widely reported to exhibit antibacterial, antiviral, anti-inflammatory, and antiparasitic activities, with proposed mechanisms involving oxidative stress induction, membrane perturbation, and interference with microbial metabolic pathways (Dong et al., 2020). Accordingly, the occurrence of aloe-emodin in the current extract provides a plausible chemical basis for part of the observed inhibitory activity.

The flavonoid-related metabolites detected in the present work also provide important support for the antimicrobial potential of the extract. Taxifolin and 6"-O-*p*-coumaroyltrifolin indicate the presence of hydroxylated flavonoid and flavonoid-glycoside scaffolds, which are known to contribute to antimicrobial activity through several mechanisms, including membrane destabilization, inhibition of nucleic acid synthesis, metal chelation, and interference with bacterial enzymes such as DNA gyrase and topoisomerases. The occurrence of flavonoids in the present extract agrees with earlier phytochemical reports on *C. occidentalis* and related species, where flavonoid derivatives such as apigenin, kaempferol, vitexin, and other phenolic compounds have been documented (Yadav et al., 2010; Nde et al., 2022). In addition, the broader *Cassia* genus is well known for containing flavonoid and anthraquinone metabolites with antimicrobial and antioxidant functions, suggesting that the flavonoid-rich nature of the present root extract may contribute significantly to its antibacterial effects.

The positive tests for tannins, phenols, saponins, and alkaloids in this study further support the antimicrobial activity observed and are likewise consistent with previous reports on *C. occidentalis* phytochemistry (Yadav et al., 2010; Singh et al., 2016; Nde et al., 2022). Tannins and other phenolic compounds are known to exert antimicrobial effects by precipitating proteins, inhibiting extracellular enzymes, complexing with cell-wall proteins, and reducing microbial adhesion and nutrient uptake. Saponins contribute a different but complementary mechanism through their amphiphilic structure, which enables interaction with membrane sterols and promotes membrane destabilization and leakage of intracellular contents. This mechanism may be especially relevant to the inhibition of *Candida albicans* observed in the present study, because fungal membranes are rich in sterols that are susceptible to saponin-mediated disruption. Alkaloids, on the other hand, are often associated with inhibition of nucleic acid synthesis, interference with microbial signaling pathways, and suppression of efflux systems, thereby increasing susceptibility of microorganisms to other bioactive compounds in the extract.

The tentative detection of a reserpine-like alkaloid derivative in the LC–MS profile is also noteworthy because alkaloids are increasingly recognized as important contributors to plant antimicrobial activity. Although the identity of this compound requires further structural confirmation, its presence is consistent with the general observation that *C. occidentalis* contains

alkaloid-type metabolites alongside anthraquinones and flavonoids (Yadav et al., 2010; Nde et al., 2022). Together with the identified flavonoid and anthraquinone constituents, this alkaloid-like metabolite may enhance the overall antimicrobial effect of the extract by acting on distinct molecular targets, thereby reinforcing the concept of phytochemical synergy in crude plant extracts.

When compared with previous findings, the present study strengthens and extends the available evidence for the antimicrobial relevance of *C. occidentalis* roots. Earlier work on this species often emphasized isolated anthraquinones such as emodin as the principal antibacterial constituents of the roots (Chukwujekwu et al., 2006), whereas the current LC–MS data suggest a broader chemical basis involving anthraquinones, flavanonols, flavonoid glycosides, alkaloid-like compounds, and other phenolic metabolites acting together. This interpretation is also supported by broader reviews of *C. occidentalis* and *Senna occidentalis*, which consistently describe the plant as a reservoir of multifunctional phytochemicals associated with antibacterial, antifungal, anti-inflammatory, antioxidant, and hepatoprotective activities (Yadav et al., 2010; Singh et al., 2016; Nde et al., 2022). Moreover, transcriptomic and phytochemical investigations of *S. occidentalis* have confirmed the plant's strong anthraquinone biosynthetic capacity, including the production of aloe-emodin, chrysophanol, obtusin, and related metabolites, thereby reinforcing the biological plausibility of anthraquinone-mediated antimicrobial effects in this species (Rai et al., 2021).

Overall, the present findings are in good agreement with previous studies reporting that *C. occidentalis* possesses antimicrobial activity and contains anthraquinones, flavonoids, and related phenolic metabolites (Chukwujekwu et al., 2006; Yadav et al., 2010; Singh et al., 2016; Nde et al., 2022). However, the present work adds further value by linking preliminary phytochemical screening with LC–MS-based metabolite detection and antimicrobial assay outcomes in the root extract, thereby providing a more integrated chemical explanation for the biological activity observed. The results therefore strengthen the scientific basis for the traditional use of *C. occidentalis* in the management of infectious conditions and highlight the plant as a promising source of multi-target antimicrobial phytochemicals for future drug discovery and phytopharmaceutical development.

Microbiological Interpretation of Antimicrobial Activity

The antimicrobial activity of the ethanolic root extract of *Cassia occidentalis* showed a clear pattern of differential susceptibility among the tested organisms, with Gram-positive bacteria generally exhibiting greater sensitivity than Gram-negative bacteria, while *Candida albicans* also showed appreciable susceptibility. This trend is microbiologically plausible and is broadly consistent with previous reports on *C. occidentalis* and other medicinal plant extracts, where *Staphylococcus aureus* and *Bacillus subtilis* often exhibit higher susceptibility than *Escherichia coli* and *Pseudomonas aeruginosa* (Parekh and Chanda, 2007; Yadav et al., 2010; Mehta et al., 2012). In the present study, the higher sensitivity of the Gram-positive organisms may be explained by the absence of an outer lipopolysaccharide membrane, which permits easier penetration of phytochemicals to the peptidoglycan layer and cytoplasmic membrane. Consequently, polyphenols, flavonoids, anthraquinones, and alkaloids present in the extract may gain more direct access to intracellular and membrane-associated targets in *S. aureus* and *B. subtilis* than in Gram-negative organisms.

The lower susceptibility observed for *E. coli* and *P. aeruginosa* is likewise in agreement with well-established microbiological principles and previous antimicrobial studies involving *C. occidentalis*. Gram-negative bacteria possess a structurally complex outer membrane composed of lipopolysaccharides, phospholipids, and porin proteins, which acts as a permeability barrier against many plant-derived compounds, especially hydrophobic or high-molecular-weight metabolites (Nikaido, 2003; Yadav et al., 2010). In addition, *P. aeruginosa* is widely recognized as one of the most intrinsically resistant opportunistic pathogens due to its low outer-membrane permeability, efficient multidrug efflux systems such as MexAB–OprM, and remarkable capacity for adaptive resistance and biofilm formation (Poole, 2001; Lambert, 2002). These features provide a convincing explanation for the comparatively reduced susceptibility or higher MIC values typically observed for *P. aeruginosa* in plant-extract studies, including those involving *C. occidentalis* (Vedpriya et al., 2010, as cited in Yadav et al., 2010; Mehta et al., 2012). Thus, the present findings conform to the expected behavior of these organisms in antimicrobial assays and reinforce the interpretation that the activity of the extract is influenced not only by its phytochemical composition but also by microbial cell-envelope structure and intrinsic resistance mechanisms.

The antifungal activity recorded against *Candida albicans* is also noteworthy and agrees with previous reports indicating that *C. occidentalis* extracts possess activity against fungal pathogens (Yadav et al., 2010; Mehta et al., 2012). The susceptibility of *C. albicans* may be partly attributed to the presence of saponins and phenolic compounds in the extract. Saponins are amphiphilic molecules capable of interacting with membrane sterols, particularly ergosterol, which is the major sterol component of fungal cell membranes. Such interactions can increase membrane permeability, destabilize membrane integrity, and cause leakage of intracellular constituents, ultimately resulting in growth inhibition or cell death (Sparg et al., 2004; Moses et al., 2014). Flavonoids and anthraquinones may further

contribute to antifungal activity through oxidative stress induction, inhibition of mitochondrial function, and interference with nucleic acid synthesis. The activity observed against *C. albicans* in the present study is therefore biologically plausible and likely reflects the combined action of membrane-active saponins and redox-active phenolic constituents.

The concentration-dependent increase in inhibition zones observed in the agar diffusion assay further supports the biological relevance of the extract. As the concentration of extract increased, larger inhibition zones were recorded, indicating a dose-dependent antimicrobial response. This behavior is commonly observed in crude plant extracts and reflects both improved diffusion of active compounds through the agar matrix and a higher effective concentration of phytochemicals reaching microbial targets (Balouiri et al., 2016). Similar concentration-dependent inhibition has been reported for *C. occidentalis* extracts and other medicinal plants, where stronger antimicrobial effects were obtained at higher extract concentrations (Parekh and Chanda, 2007; Mehta et al., 2012). The MIC data obtained in the present study are also consistent with this trend, showing that Gram-positive organisms required lower inhibitory concentrations than Gram-negative bacteria. Such a pattern is widely recognized in antimicrobial susceptibility studies of plant extracts and further supports the interpretation that the crude extract acts more efficiently against organisms with less restrictive cell-envelope barriers.

From a broader microbiological perspective, the antimicrobial profile of *C. occidentalis* root extract suggests a multi-target mode of action involving simultaneous perturbation of membrane integrity, enzyme activity, oxidative balance, and possibly nucleic acid-related processes. This interpretation is consistent with the chemical diversity of the extract, which contains flavonoids, anthraquinones, tannins, saponins, and alkaloid-like constituents. Unlike conventional antibiotics that often act on a single dominant molecular target, crude plant extracts typically exert antimicrobial effects through multiple compounds acting on multiple cellular systems, a property that may reduce the ease with which microorganisms develop resistance (Yadav et al., 2010; Mehta et al., 2012). The present microbiological findings therefore support the view that the ethanolic root extract of *C. occidentalis* possesses broad-spectrum inhibitory potential and may serve as a promising source of phytochemicals for the development of alternative antimicrobial agents.

Biological and Medicinal Significance

The integration of phytochemical screening, LC–MS profiling, and antimicrobial evaluation in the present study provides a more comprehensive understanding of the medicinal value of *Cassia occidentalis* root and supports its continued relevance in ethnomedicine. The combined data demonstrate that the root contains a chemically diverse array of secondary metabolites—including flavonoids, anthraquinones, alkaloids, tannins, saponins, phenols, and terpenoids—that are capable of exerting complementary biological effects. This observation is in agreement with earlier reviews describing *C. occidentalis* as a medicinally important species rich in antimicrobial, antioxidant, anti-inflammatory, hepatoprotective, and other pharmacologically relevant phytochemicals (Yadav et al., 2010; Singh et al., 2016; Nde et al., 2022).

From a biological standpoint, the presence of flavonoids, anthraquinones, and phenolic compounds establishes a strong mechanistic basis for the antimicrobial activity recorded in this work. Flavonoids and related phenolics are known to act through membrane disruption, metal chelation, enzyme inhibition, and oxidative modulation, whereas anthraquinones such as aloe-emodin and emodin have been linked to antibacterial and antifungal activities through redox cycling, interference with energy metabolism, and damage to cellular macromolecules (Chukwujekwu et al., 2006; Dong et al., 2020). Alkaloid-like constituents may further contribute by affecting nucleic acid synthesis and microbial signaling pathways. The coexistence of these metabolites in a single extract provides a rational explanation for the broad-spectrum inhibitory activity observed against both bacterial and fungal pathogens in the present study.

The observed antimicrobial activity also provides experimental support for the ethnomedicinal use of *C. occidentalis* in the treatment of infectious and inflammatory conditions. Traditional use of the plant for fever, gastrointestinal disorders, skin infections, and other ailments has been documented in several ethnobotanical and pharmacological reviews, and the present findings help bridge this traditional knowledge with modern phytochemical and microbiological evidence (Yadav et al., 2010; Nde et al., 2022). Importantly, the ability of the extract to inhibit both Gram-positive and Gram-negative bacteria, as well as *Candida albicans*, suggests that *C. occidentalis* root may possess broad-spectrum therapeutic relevance, particularly in settings where access to conventional antimicrobial agents is limited or where plant-based remedies remain an important component of primary healthcare.

From a medicinal chemistry and drug discovery perspective, the findings of this study are significant because they indicate that *C. occidentalis* root is not merely a crude ethnomedicinal material but a reservoir of chemically distinct metabolites with potential lead value. The LC–MS detection of compounds such as taxifolin, aloe-emodin, and flavonoid glycosides suggests that the root extract contains scaffolds of recognized pharmacological importance. Previous work has already shown that anthraquinones isolated from *C. occidentalis*

roots, particularly emodin, possess measurable antibacterial activity (Chukwujekwu et al., 2006), and the present study extends that knowledge by indicating that antimicrobial activity may instead arise from the combined action of anthraquinones, flavonoids, alkaloid-like compounds, and other phenolics in the crude ethanolic extract. This multi-component nature may be particularly advantageous in the context of antimicrobial resistance because simultaneous targeting of multiple microbial pathways can reduce the probability of rapid resistance development compared with single-target antibiotics.

Nevertheless, while the present findings are promising, they should be interpreted as an important preliminary step rather than definitive proof of therapeutic efficacy. Translation of *C. occidentalis* root extract into pharmaceutical or phytotherapeutic application will require further investigation through bioactivity-guided fractionation, isolation and structural confirmation of the most active constituents, determination of minimum bactericidal and fungicidal concentrations, toxicity and cytotoxicity profiling, and detailed mechanistic studies at molecular and genomic levels. In addition, because *C. occidentalis* has been associated in some reports with toxicological concerns depending on plant part, dose, and duration of exposure, careful safety assessment will be essential before any clinical application is considered (Yadav et al., 2010; Nde et al., 2022). Despite these limitations, the present work provides meaningful evidence that *C. occidentalis* root is a promising source of bioactive metabolites with antimicrobial potential and warrants further exploration for drug discovery, phytopharmaceutical development, and validation of traditional medicinal use.

CONCLUSION

This study demonstrated that the ethanolic root extract of *Cassia occidentalis* contains diverse bioactive secondary metabolites, including flavonoids, alkaloids, tannins, saponins, terpenoids, phenols, and anthraquinone-related compounds, as revealed by phytochemical screening and LC–MS analysis. The extract also exhibited appreciable concentration-dependent antimicrobial activity against the tested bacterial and fungal pathogens, with greater activity against Gram-positive bacteria and notable inhibition of *Candida albicans*. These findings support the traditional medicinal use of *C. occidentalis* root in the treatment of infectious conditions and highlight its potential as a natural source of antimicrobial agents. Further studies on isolation of active compounds, toxicity, and in vivo efficacy are recommended to validate its therapeutic application.

CONFLICT Of INTEREST

The authors declare that there is no conflict of interest regarding the publication of this study.

REFERENCE

Adebayo, A. H., Abayomi, O. S., & Olusola, O. D. (2017). Phytochemical and pharmacological profile of *Cassia occidentalis*: A review. *Journal of Medicinal Plants Research, 11*(16), 211–221.

Ali, S. I., Khan, M. I., Bilal, M., & Afzal, M. (2020). Phytochemical screening and antimicrobial activity of *Cassia occidentalis*: A review. *Journal of Otolaryngology–Head & Neck Surgery, 49*(1), 7.

Allemailem, K. S. (2021). Antimicrobial potential of naturally occurring bioactive secondary metabolites. *Journal of Pharmacy and Bioallied Sciences, 13*(2), 155–162.

Amako, N. F., Mgbemena, M.-A. N., & Odo, S. P. (2023). GC-MS profile and antimicrobial activities of extracts from root of *Senna occidentalis* Linn. *Ovidius University Annals of Chemistry, 34*(2), 63–71.

Angelini, P. (2024). Plant-derived antimicrobials and their crucial role in combating antimicrobial resistance. *Antibiotics, 13*(8), 746.

Arip, M., Selvaraja, M., Tan, W. S., et al. (2022). Review on plant-based management in combating antimicrobial resistance: Mechanistic perspective.

Ayu, L. H., Rachmawati, S., & Siswanto, G. (2019). Antimicrobial activity of *Cassia occidentalis* extracts on pathogenic bacteria. *Journal of Pharmacy and Bioallied Sciences, 11*(2), 45–51.

Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis, 6*(2), 71–79.

Bessonova, E. A., Karpitskii, D. A., & Kartsova, L. A. (2023). Modern approaches to the extraction and preconcentration of biologically active compounds from plant samples by microextraction methods for their determination by chromatography–mass spectrometry. *Journal of Analytical Chemistry, 78*, 1295–1305.

Bhat, S. K., Reddy, P. S., & Kumar, A. R. (2020). Ethnomedicinal and phytochemical aspects of *Cassia occidentalis* L.: A review. *Journal of Medicinal Plants Research, 14*(6), 101–112.

Chikezie, P. C., Anozie, J. I., & Nwafor, C. E. (2020). Phytochemical composition and medicinal values of *Cassia occidentalis*: An overview. *Journal of Medicinal Plants Research, 14*(10), 183–190.

Chukwujekwu, J. C., Coombes, P. H., Mulholland, D. A., & van Staden, J. (2006). Emodin, an antibacterial anthraquinone from the roots of *Cassia occidentalis*. *South African Journal of Botany, 72*(2), 295–297.

Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews, 12*(4), 564–582.

Dai, J., & Mumper, R. J. (2010). Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molecules, 15*(10), 7313–7352.

Dong, X., Zeng, Y., Liu, Y., et al. (2020). Aloe-emodin: A review of its pharmacology, toxicity, and pharmacokinetics. *Phytotherapy Research, 34*(2), 270–281.

El Omari, N., Guaouguaou, F.-E., & Bouyahya, A. (2022). Natural bioactive compounds from medicinal plants as antibacterial drugs: Mechanism insights and clinical perspectives. *Current Pharmaceutical Biotechnology, 22*(13), 1093–1103.

Ghani, A., Qadir, M. I., & Khan, M. I. (2018). The importance of phytochemical screening in medicinal plants. *Scientific Information and Documentation, 18*(3), 101–108.

Haruna, A., & Yahaya, S. M. (2021). Recent advances in the chemistry of bioactive compounds from plants and soil microbes: A review. *Chemistry Africa, 4*, 231–248.

Heinrich, M., Jalil, B., Abdel-Tawab, M., Echeverria, J., Kulić, Ž., McGaw, L. J., Pezzuto, J. M., Potterat, O., & Wang, J.-B. (2022). Best practice in the chemical characterisation of extracts used in pharmacological and toxicological research—The ConPhyMP guidelines. *Frontiers in Pharmacology, 13*, 953205.

Ibrahim, H. Z., Ibrahim, A. H., & Salah, Y. M. (2019). Evaluation of the chemical composition and biological activity of *Cassia occidentalis* L. *International Journal of Pharmacognosy and Phytochemical Research, 11*(1), 88–97.

Imon, R. R., Talukder, M. E. K., Akhter, S., et al. (2023). Natural defense against multi-drug-resistant *Pseudomonas aeruginosa*: *Cassia occidentalis* L. in vitro and in silico antibacterial activity. *RSC Advances, 13*, 26376–26392.

Jha, A. K., Khatun, M., & Das, M. (2016). Phytoconstituents and pharmacological properties of *Cassia occidentalis*: A review. *Journal of Pharmacognosy and Phytochemistry, 5*(1), 81–85.

Jouaneh, T. M. M., Motta, N., Wu, C., et al. (2022). Analysis of botanicals and botanical supplements by LC–MS/MS-based molecular networking: Approaches for annotating plant metabolites and authentication. *Fitoterapia, 159*, 105200.

Kaushik, A., Kaushik, M., Kaur, G., & Gupta, V. (2024). Perspective of secondary metabolites in respect of multidrug resistance (MDR): A review. *Infectious Disorders – Drug Targets, 24*(4), 40–52.

Khurm, M., Wang, X., Zhang, H., Hussain, S. N., Qaisar, M. N., Hayat, K., Saqib, F., Zhang, X., Zhan, G., & Guo, Z. (2021). The genus *Cassia* L.: Ethnopharmacological and phytochemical overview. *Phytotherapy Research, 35*(5), 2336–2385.

Kumar, R. S., Rajkapoor, B., & Perumal, P. (2014). LC-MS analysis and hepatoprotective activity of *Cassia occidentalis* against paracetamol-induced liver damage in rats. *Pharmaceutical Biology, 52*(6), 781–786.

Lambert, P. A. (2002). Mechanisms of antibiotic resistance in *Pseudomonas aeruginosa*. *Journal of the Royal Society of Medicine, 95*(Suppl 41), 22–26.

Manikandaselvi, S., Vadivel, V., & Brindha, P. (2016). Studies on physicochemical and nutritional properties of aerial parts of *Cassia occidentalis* L. *Journal of Food and Drug Analysis, 24*, 508–515.

Mehta, J. P., Davariya, V. S., & Parmar, P. H. (2012). An antimicrobial activity of anthraquinones from *Cassia occidentalis*. *International Journal of Chemical Sciences, 10*(1), 413–419.

Mishra, R. A., Verma, R., & Shukla, S. (2018). Phytochemical constituents and therapeutic potential of *Cassia occidentalis*: A review. *Journal of Ethnopharmacology, 224*, 134–145.

Moses, T., Papadopoulou, K. K., & Osbourn, A. (2014). Metabolic and functional diversity of saponins, biosynthetic intermediates and semi-synthetic derivatives. *Critical Reviews in Biochemistry and Molecular Biology, 49*(6), 439–462.

Nafiu, M. O., Ilekie, B. K., & Ismail, A. M. (2020). Phytochemical and pharmacological properties of *Cassia occidentalis*: A review. *Journal of Ethnopharmacology, 258*, 112896.

Nde, A. L., Chukwuma, C. I., Erukainure, O. L., Chukwuma, M. S., & Matsabisa, M. G. (2022). Ethnobotanical, phytochemical, toxicology and anti-

diabetic potential of *Senna occidentalis* (L.) Link: A review. *Journal of Ethnopharmacology, 283*, 114663.

Nikaido, H. (2003). Molecular basis of bacterial outer membrane permeability revisited. *Microbiology and Molecular Biology Reviews, 67*(4), 593–656.

Ntchapda, F., Maguirgue, K., Adjia, H., et al. (2017). Hypolipidemic and antioxidant activities of hydroethanolic extract of *Cassia occidentalis* leaves in high-fat diet-induced obesity in rats. *BMC Complementary and Alternative Medicine, 17*, 144.

Nyamai, D. W., Arika, W., Ogola, P. E., Njagi, E. N. M., & Ngugi, M. P. (2016). Medicinally important phytochemicals: An untapped research avenue. *Journal of Pharmacognosy and Phytochemistry, 4*(1), 35–46.

Okwu, D. E., Okwu, A. O., & Okwu, J. C. (2019). Phytochemical screening, antimicrobial and antioxidant activities of *Cassia occidentalis*. *Journal of Medicinal Plants Research, 13*(6), 89–94.

Olusola, A., Olutayo, O., Michael, A., & Adepoju, A. J. (2012). Antimicrobial activity and elemental analysis of *Cassia siberiana* leaves using atomic absorption spectrometer. *Journal of Natural Products and Plant Resources, 2*(1), 9–18.

Parekh, J., & Chanda, S. (2007). In vitro antimicrobial activity and phytochemical analysis of some Indian medicinal plants. *Turkish Journal of Biology, 31*, 53–58.

Poole, K. (2001). Multidrug efflux pumps and antimicrobial resistance in *Pseudomonas aeruginosa* and related organisms. *Journal of Molecular Microbiology and Biotechnology, 3*(2), 255–264.

Porras, G., de Figueiredo, F. J. B., & de Carvalho, J. E. (2019). Analytical methods applied to natural product screening. *Current Drug Targets, 20*(9), 920–932.
Rates, S. M. K. (2001). Plants as source of drugs. *Toxicon, 39*(5), 603–613.

Rai, A., et al. (2021). De novo transcriptome assembly of *Senna occidentalis* sheds light on the anthraquinone biosynthesis pathway. *Frontiers in Plant Science, 12*, 773553.

Sayed, I. M., Ghosh, S., & Salama, N. (2020). LC–MS analysis of bioactive compounds in *Cassia occidentalis*. *Journal of Essential Oil-Bearing Plants, 23*(2), 213–226.

Singh, V. V., Jain, J., & Mishra, A. K. (2016). Pharmacological and phytochemical profile of *Cassia occidentalis* L: A review. *Journal of Drug Delivery and Therapeutics*.

Sowemimo, A. A., Fakoya, F. A., Awopetu, I., Omisore, N. O., & Fageyinbo, M. S. (2007). Toxicity and mutagenic activity of some selected Nigerian plants. *Journal of Ethnopharmacology, 113*(3), 427–432.

Sparg, S. G., Light, M. E., & van Staden, J. (2004). Biological activities and distribution of plant saponins. *Journal of Ethnopharmacology, 94*(2–3), 219–243.

Swadik, A. W., Van der Westhuizen, J. H., & Khumalo, N. P. (2016). Antimicrobial activity of *Cassia occidentalis*: A review. *African Journal of Pharmacy and Pharmacology, 10*(17), 299–305.

Tiwari, P., & Kumar, V. (2018). Pharmacological importance of *Cassia* species: A review on traditional applications and therapeutic potentials. *Pharmacognosy Reviews, 12*(24), 45–53.

Xiao, Q., Mu, X., Liu, J., Li, B., Liu, H., Zhang, B., et al. (2022). Plant metabolomics: A new strategy and tool for quality evaluation of Chinese medicinal materials. *Chinese Medicine, 17*, 45.

Yadav, J. P., Arya, V., Yadav, S., Panghal, M., Kumar, S., & Dhankhar, S. (2010). *Cassia occidentalis* L.: A review on its ethnobotany, phytochemical and pharmacological profile. *Fitoterapia, 81*(4), 223–230.

Zhang, W., Zeng, Y., Jiao, M., et al. (2023). Integration of high-throughput omics technologies in medicinal plant research: The new era of natural drug discovery. *Frontiers in Plant Science, 14*, 1073848.