



PHYTOCHEMICAL CHARACTERIZATION, ANTIMICROBIAL ACTIVITY, AND *IN SILICO* ANALYSIS OF *CASSIA OCCIDENTALIS* LEAF EXTRACTS AGAINST MULTIDRUG-RESISTANT BACTERIA AND *TRICHOPHYTON RUBRUM*

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Abstract:

Antimicrobial resistance poses a global health threat, prompting exploration of plant-derived alternatives. *Cassia occidentalis* is traditionally used for infections, yet its efficacy against resistant pathogens remains underexplored. In this research work, methanolic and ethanolic leaf extracts of *Cassia occidentalis* were prepared and subjected to qualitative phytochemical screening. An agar well diffusion antimicrobial assays (against MDR *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Trichophyton rubrum*) was conducted accordingly. These was followed by GC-MS analysis, SwissADME pharmacokinetic prediction, and molecular docking against resistance proteins. The results obtained shows that, both extracts contained flavonoids, alkaloids, saponins, steroids, and phenols. Concentration-dependent inhibition was observed, with methanolic extract showing zones up to 25 mm against *S. aureus* and bactericidal effects at 200 mg/mL against all bacteria (fungistatic against *T. rubrum*). GC-MS identified 32 compounds, including α -estradiol, norbuprenorphine, and fatty acid esters. Several compounds exhibited favorable ADMET profiles and strong docking affinities (e.g., -6.65 kcal/mol to MexA). In conclusion, *C. occidentalis* leaf extracts demonstrate promising broad-spectrum antimicrobial activity, supporting ethnomedicinal use and potential as resistance-modulating agents

Keywords: *Cassia occidentalis*, Antimicrobial Resistance, Phytochemical Screening, Molecular Docking, Multidrug-resistant Bacteria, *Trichophyton rubrum*.

Introduction

Antimicrobial resistance (AMR) compromises antibiotic efficacy, increasing morbidity, mortality, and healthcare costs globally, particularly in low-resource settings (Walsh *et al.*, 2023; Gulzar *et al.*, 2025). In Nigeria, multidrug resistance exceeds 58% in clinical isolates (Kayode *et al.*, 2020). Medicinal plants offer promising alternatives due to diverse secondary metabolites (e.g.,

alkaloids, flavonoids, tannins, anthraquinones) that disrupt bacterial membranes, efflux pumps, and resistance mechanisms (Khare *et al.*, 2021; El-Readi *et al.*, 2021). *Cassia occidentalis* (Fabaceae), known as coffee senna, is widely used in African ethnomedicine for infections, wounds, and inflammation (Yusuf *et al.*, 2018; Nde *et al.*, 2022). Previous studies

report its antibacterial and antifungal properties (Imon *et al.*, 2023)⁽⁵⁾, but data on multidrug-resistant (MDR) strains and dermatophytes like *Trichophyton rubrum* remain limited. This study aimed to: (1) characterize phytochemicals via screening and GC-MS, (2) evaluate antimicrobial activity against MDR bacteria and *T. rubrum*, (3) predict pharmacokinetics using SwissADME, and (4) perform molecular docking against key resistance proteins.

Materials and Methods

Plant Material Collection and Extraction

Fresh *Cassia occidentalis* leaves were collected from Maiduguri, Borno State, Nigeria, authenticated (Herbarium No. UMH-2507), shade-dried, powdered, and extracted (100 g in 500 mL methanol/95% ethanol, 72 h maceration). Filtrates were

concentrated via rotary evaporation at 40°C and stored at 4°C. Aqueous extracts were discarded due to spoilage.

Phytochemical Screening

Qualitative tests (Muhammad *et al.*, 2018) detected alkaloids (Wagner's), flavonoids (Shinoda), saponins (frothing), tannins/phenols (FeCl₃), steroids (Liebermann–Burchard), proteins (Biuret), and cardiac glycosides (Keller–Killiani)

Microbial Isolates and Resistance Profiles

MDR isolates (*S. aureus* NCTC 12493 [PBP2a], *E. coli* NCTC 13846 [mcr-1], *K. pneumoniae* NTCC BAA-1705 [blaKPC], *P. aeruginosa* NTCC 27853 [MexAB]) were from Microbiologics QC kits; *T. rubrum* from clinical samples. Resistance genes/profiles are summarized in **Table 1**

Table 1. Profiles of Quality Control Multi-drug Resistant Isolates

Organism	Resistance Gene	Gene Function	Known Resistance Profile	Resistant To	Sensitive To
<i>Klebsiella pneumoniae</i> (NTCC BAA-1705)	blaKPC	Encodes <i>K. pneumoniae</i> carbapenemase (KPC), hydrolyzing carbapenems and other β-lactams	Carbapenem-resistant	Imipenem, meropenem, ertapenem, many β-lactams	Possible susceptibility to colistin/polymyxins
<i>Pseudomonas aeruginosa</i> (NTCC 27853)	MexAB	Encodes multidrug efflux pump MexAB-OprM, exporting a wide range of antibiotics	Intrinsic efflux-mediated resistance	β-lactams, fluoroquinolones, tetracyclines (depending on expression level)	Aminoglycosides, ceftazidime, ciprofloxacin, carbapenems (wild-type profile)

<i>Staphylococcus aureus</i> (NCTC 12493)	Pdp2a	Encodes penicillin-binding protein PBP2a, conferring resistance to β -lactams	MRSA phenotype	Methicillin, oxacillin, ceftiofur, other β -lactams	Vancomycin, clindamycin, linezolid
<i>Escherichia coli</i> (NCTC 13846)	mcr-1	Encodes phosphoethanolamine transferase that modifies lipid A, conferring colistin resistance	Colistin-resistant; may carry blaTEM-1, CTX-M-27, aadA1	Colistin; possible aminoglycosides and certain penicillins	Cephalosporins, fluoroquinolones (if no additional genes active)
<i>Trichophyton rubrum</i>	ERG11	Encodes lanosterol 14 α -demethylase, a key enzyme in ergosterol biosynthesis; mutations confer azole resistance	Azole resistance (mechanism under investigation)	Fluconazole, itraconazole (depending on mutation)	Terbinafine, griseofulvin (if no resistance mechanisms active)

Antimicrobial Assays

Agar well diffusion (100 μ L of 100–400 mg/mL extracts in 10% DMSO) on Mueller-Hinton/Sabouraud agar; controls: 10% DMSO (negative); Ciprofloxacin against *Escherichia coli* and *Pseudomonas aeruginosa*, Vancomycin against *Staphylococcus aureus*, Colistin against *Klebsiella pneumoniae*, and Griseofulvin against *Trichophyton rubrum* (as positive controls respectively). Zones measured after 24h (bacteria, 37°C) or 7 days (*T. rubrum*, 28–30°C) on all four different concentrations. MBC/MFC determined by subculture. A stock concentration of 400 mg/mL was achieved by reconstituting the previously prepared and stored dry methanolic and ethanolic crude extracts in 10% dimethyl sulfoxide (DMSO).

Reconstitution and Serial Dilution of Extracts

To achieve concentrations of 400 mg/mL, 300 mg/mL, 200 mg/mL and 100 mg/mL, repeated two-fold dilutions were made from each stock. The dose-dependent antimicrobial activity of each extract was assessed using these concentrations.

Ligand Preparation

The main bioactive substances found by GC-MS (such as emodin, anthraquinones, methyl esters, etc.) were prepared by retrieving their 2D structures from PubChem in SMILES format, converting them to 3D using Open Babel or ChemSketch, minimizing energy using the MMFF94 force field using Avogadro or PyRx to obtain stable conformations, and converting the file format.

Target Protein Preparation

The three-dimensional structures of target proteins were obtained from the RCSB PDB database when available and modeled using SwissModel for proteins without experimentally determined structures. The protein structures were prepared for docking by removing water molecules, ligands and cofactors, adding polar hydrogens and assigning Kollman charges. Finally, the prepared protein structures were converted to PDBQT format using AutoDock Tools for subsequent molecular docking analysis.

GC-MS Analysis

Extracts (1 mg/mL) analyzed on Agilent 7890B/5977A system (DB-5MS column,

helium 1 mL/min, EI 70 eV). Compounds identified via NIST/Wiley libraries.

In Silico ADMET and Molecular Docking

SwissADME/pkCSM predicted Lipinski compliance, GI absorption, toxicity. AutoDock Vina docked major GC-MS compounds against PDB structures: 1VQQ (PBP2a), 5UJ4 (blaKPC), 5GRR (mcr-1), 6TA6 (MexA), and ERG11 homology model. Interactions visualized in PyMOL/Discovery Studio.

Results

Phytochemical Screening

Both extracts contained flavonoids, alkaloids, saponins, steroids, and phenols; cardiac glycosides negative; proteins absent in ethanolic extract.

Table 2. Phytochemical Properties of *C. occidentalis* Leaf Extracts

Phytochemical Test	Ethanol	Methanol
Flavonoid	+	+
Alkaloid	+	+
Saponin	+	+
Steroid	+	+
Cardiac glycoside	-	-
Phenol	+	+
Protein	-	+

Antimicrobial Activity

Ethanolic extract: highest zones against *S. aureus* and *P. aeruginosa* (22 mm at 400

mg/mL); methanolic: 25 mm and 23 mm, respectively (Tables 3–4). Bactericidal at 200 mg/mL for all bacteria; fungistatic against *T. rubrum* (8 mm at 400 mg/mL only).

Table 3. Zone of Inhibition by Ethanol Extract

S/NO	Organism	Concentration/Zone of Inhibition				
		400mg/ml	300mg/ml	200mg/ml	100mg/ml	Control (Antibiotics)
1	<i>Staphylococcus aureus</i>	22mm	12mm	2mm	0	25mm
3	<i>Escherichia coli</i>	8mm	0	0	0	20mm

4	<i>Pseudomonas aeruginosa</i>	22mm	9mm	2mm	0	26mm
5	<i>Klebsiella pneumoniae</i>	18mm	8mm	2mm	0	26mm
6	<i>Trichophyton rubrum</i>	8mm	0	0	0	19mm

Table 4. Zone of Inhibition by Methanol Extract

S/NO	Organism	Concentration/Zone of Inhibition				
		400mg/ml	300mg/ml	200mg/ml	100mg/ml	Control (Antibiotics)
1	<i>Staphylococcus aureus</i>	25mm	12mm	3mm	0	25mm
2	<i>Escherichia coli</i>	12mm	6mm	0	0	19mm
3	<i>Pseudomonasa eruginosa</i>	23mm	7mm	4mm	0	26mm
4	<i>Klebsiella pneumoniae</i>	10mm	3mm	0	0	26mm
5	<i>Trichophyton rubrum</i>	8mm	0	0	0	19mm

GC-MS Profile

33 compounds identified, including fatty acid esters, naphthalene derivatives, α -estradiol, norbuprenorphine.

Table 5. Gas Chromatography-Mass Spectrometry Characteristics of *Cassia occidentalis*

Peak	Compound Name	RT	Area	Area%	Area Sum%
1	2(1H)-Pyridinone,1-(2-deoxy- β -	2.8	246633.16	14.07	20201.17
2	o-Cymene	4.1	198706.68	11.34	20345.84
3	Octanoicacid,ethylester	4.4	1752641	100	204365.17
4	Naphthalene,1,2,3,4-tetrahydro-	5.1	67259.57	3.84	12180.54
5	Nonanoicacid,ethylester	5.3	319235.84	18.21	66009.29
6	2-Isopropylamino-4-	5.4	261798.83	14.94	40449.62
7	β -Estradiol	5.7	67799.94	3.87	10944.14
8	Decanoicacid,ethylester	6.2	1487016.56	84.84	493830.1
9	Dihydro-cis- α -copaene-8-ol	6.5	55091.01	3.14	9043.28
10	Quinoline,2-ethyl-	6.7	58818.75	3.36	13591.97
11	8-Chloro-1-octanol	7.1	17875.18	1.02	5995.4
12	Octadecanoicacid,ethylester	7.6	39620.37	2.26	10253.8

13	Octadecanoicacid,ethylester	7.9	258261.4	14.74	121430.72
14	Octadecanoicacid,2-oxo-	8.8	36675.83	2.09	11694.66
15	1,4-Naphthalenedione,5,8-	9.1	24681.37	1.41	9754.98
16	St-st-peroxide-I;	9.3	22455.38	1.28	10942.06
18	Ascorbicacid	9.8	85272.96	4.87	57021.5
21	Tropicacid	10.7	87810.48	5.01	25378.74
22	Ethyl9-hexadecenoate	10.9	121871.37	6.95	58083.5
25	1,2-Dihexylcyclopropene-3-	11.7	21552.61	1.23	11187.19
26	Elaidicacid	11.9	67310.53	3.84	34536.48
29	Glycerol1-myristate	12.7	31095.17	1.77	10977.89
30	Norbuprenorphine	17.7	117859.92	6.72	18130.15
31	Quinomethionate	17.9	175953.84	10.04	20011.14
32	Chlordiazepoxide	18.4	261539.76	14.92	57198.71
33	β -Amyrin	19.4	233858.05	13.34	16671.94

ADMET and Docking

Many compounds complied with Lipinski's Rule; low predicted toxicity. Strongest affinities:

MexA (-6.65 kcal/mol), blaKPC (-6.58 kcal/mol); moderate for PBP2a and ERG11.

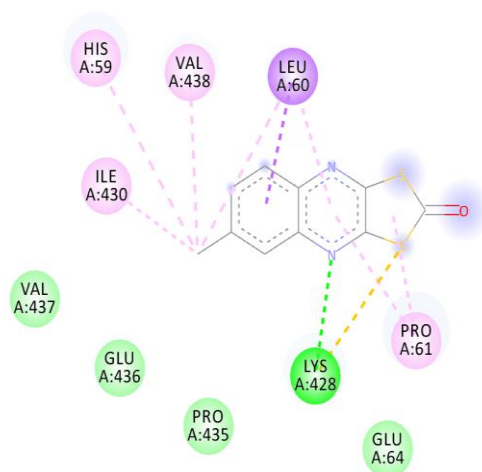


Figure 1: CID 17109 Quinomethionate

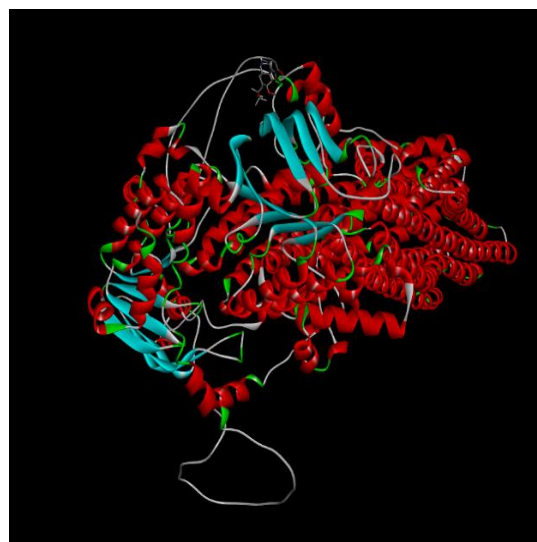
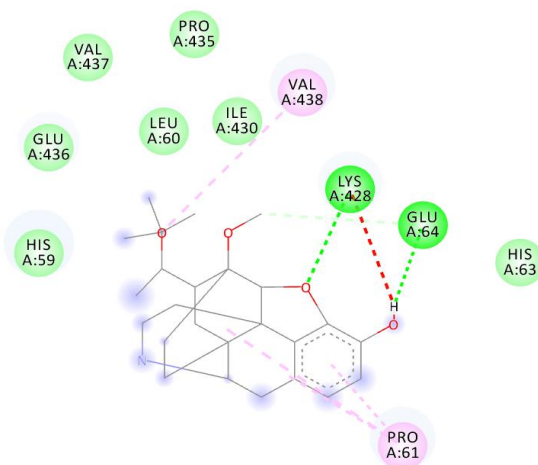


Figure 2 : CID114976 Norbuprenorphine

Discussion

The presence of flavonoids, alkaloids, saponins, phenols, and steroids aligns with prior reports (Saminu and Na'ala, 2025; Imon *et al.*, 2023) and explains broad-spectrum activity via membrane disruption, enzyme inhibition, and efflux modulation (El-Readi *et al.*, 2021; Suganya *et al.*, 2022). Methanolic extracts outperformed ethanolic ones, likely due to better extraction of polar phenolics (Baluchamy and Subramanian, 2023). Stronger activity against Gram-positive (*S. aureus*) than Gram-negative bacteria reflects cell wall differences and efflux pump activity (Singh and Sharma, 2021). Bactericidal effects at 200 mg/mL exceed many prior MICs (150–250 mg/mL) (Ahmed *et al.*, 2022; Imon *et al.*, 2023), suggesting synergistic metabolite action. Fungistatic effects against *T. rubrum* may stem from limited ERG11 inhibition or ergosterol/chitin barriers (Tanwar *et al.*, 2024; Rather *et al.*, 2022). GC-MS revealed membrane-active lipids (e.g., glycerol 1-

myristate) and enzyme-interacting compounds (e.g., Quinomethionate, norbuprenorphine), supporting observed bactericidal patterns. Docking showed favorable interactions with efflux (MexA) and β -lactamase (blaKPC) targets, indicating resistance modulation potential (Yakobi and Nwodo, 2025). ADMET profiles suggest drug-likeness for several hits, warranting further development. Limitations include seasonal phytochemical variation, limited strains, in vitro-only assays, and predictive (not experimental) ADMET/docking. Future work should isolate pure compounds, evaluate synergies with antibiotics, and conduct in vivo/toxicity studies.

Conclusion

Methanolic and ethanolic *C. occidentalis* leaf extracts exhibit good antibacterial (bactericidal) and moderate antifungal activity against MDR pathogens, validated by phytochemical, GC-MS, and in silico evidence. These findings support ethnomedicinal use and highlight potential

for novel resistance-modulating agents in resource-limited settings.

Authors Contribution: The first author comes with the research idea. The overall

work was supervised by the second and the third authors. The fourth author reviewed the work, as assisted by the last author. The laboratory work was carried out by the first author and funded collectively among authors.

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