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Phytochemical and Antimicrobial Assessment of the Leaves of *Senna occidentalis* Against Selected Pathogenic Microorganisms

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ABSTRACT

This study examined the phytochemical constituents and antimicrobial potential of the methanolic leaf extract of *Senna occidentalis* against selected pathogenic microorganisms. Leaves were shade-dried, pulverized, and extracted with methanol using a Soxhlet apparatus. Screening identified the major secondary metabolites present, while antimicrobial activity was assessed by agar well diffusion, and Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration/ Minimum Fungicidal Concentration (MBC/MFC) values were determined through broth dilution. Flavonoids, phenols, glycosides, saponins, steroids, and anthraquinones were detected, whereas tannins, alkaloids, and phlobatannins were absent. *Escherichia coli* proved completely resistant, while *Candida stellatoidea* and *Staphylococcus aureus* were highly susceptible, with inhibition zones of 26 mm and 24 mm, respectively. Both organisms shared an MIC of 5 mg/mL and MBC/MFC values of 10 mg/mL. The resistance of *E. coli* may reflect its protective outer membrane. Overall, *S. occidentalis* leaves show notable antimicrobial activity against Gram-positive bacteria and fungi, supporting their traditional use in treating infections.

Keywords: *Senna occidentalis*, methanolic extract, phytochemicals, antimicrobial activity, MIC, MBC/MFC

INTRODUCTION

For centuries, medicinal plants have served as a primary source of treatment for various ailments, and today they remain central to pharmaceutical research and drug discovery [1]. Beyond their

therapeutic value, these plants sustain rural economies traditional healers rely on them directly, while countless others earn a living collecting and trading them. In much of Nigeria, as elsewhere, they are still prepared the old way: as decoctions, infusions, or tinctures used to manage everyday infections and long-term illnesses alike [2].

Meanwhile, the problem of drug resistance keeps growing. Multi-drug resistant strains are becoming more common, and even microbes once considered easy to treat are showing reduced sensitivity to antibiotics. Much of this has been traced to the indiscriminate use of broad-spectrum antibiotics, immunosuppressive therapy, intravenous catheters, organ transplantation, and the lingering effects of the HIV epidemic. Given this, finding new ways to fight infection has become less of an option and more of a necessity [3]. Herbal medicine has stepped into this gap for several reasons it is inexpensive, aligns with the broader 'green' movement's emphasis on natural products, and fits comfortably within a cultural preference for self-medication in many Western societies [4]. Plants continue to matter in drug development precisely because of this: they offer real biological activity against infectious disease, often with minimal side effects [5]. It is this promise that has kept natural products research alive as a viable path toward discovering new bioactive agents. Ultimately, though, a plant's usefulness against disease comes down to its phytochemistry, the secondary metabolites that make up the active ingredients of countless traditional remedies.

Senna occidentalis, known in English as coffee senna [6], goes by different names across Nigeria, Sanga sanga or Rai ndori to the Hausa, Akidi agbara to the Igbo, and Abo rere to the Yoruba [7]. Native to tropical and subtropical America, it has since spread and naturalized across Australia, eastern Africa, and parts of the southern and eastern United States [8]. It tends to thrive in disturbed, moisture-rich environments abandoned fields, waste ground, open woodland, pastures, roadsides and often turns up mixed in among cultivated cereals like soybean, corn, and sorghum, making it a stubborn presence at harvest time [9].

Nearly every part of the plant roots, leaves, flowers, stems, seeds finds some uses in traditional medicine. The roots, for instance, are used against diabetes, though this application still lacks solid scientific backing [10]. The leaves, meanwhile, are said to help with typhoid fever when taken as an infusion, a practice reported in northern Nigeria. Applied externally as a paste, they've also been used on wounds, sores, itches, cutaneous conditions, fractures, fever, ringworm, and throat infections [10]. Yet for all this traditional use, rigorous scientific validation particularly

regarding antimicrobial activity against clinically relevant pathogens remains thin on the ground. Most existing work has stayed at the level of general phytochemical or pharmacological profiling [11-12], leaving a real gap when it comes to testing the leaf extract against a defined set of pathogenic bacteria and fungi, complete with proper MIC and MBC/MFC data. That gap matters more now than ever, given how quickly antibiotic resistance is spreading and how urgently alternative antimicrobial agents are needed.

This study aims to evaluate both the phytochemical composition and antimicrobial activity of *S. occidentalis* leaves against selected pathogens partly to validate its traditional use scientifically, and partly to contribute toward the broader search for plant-based antimicrobial agents.

The specific objectives are to:

i. identify the phytochemical constituents present in the leaves of *Senna occidentalis* using standard analytical techniques;
ii. assess the antimicrobial activity of the leaf extract against a range of pathogenic bacteria and fungi.
iii. determine the minimum inhibitory, bactericidal, and fungicidal concentrations of the extract against the selected organisms.

MATERIALS AND METHODS

Fresh *Senna occidentalis* leaves formed the basis of this study, extracted using methanol in a Soxhlet apparatus set up for continuous extraction. Three test organisms were used throughout: two bacteria *Escherichia coli* and *Staphylococcus aureus* and a single fungal species, *Candida stellatoidea*. Mueller Hinton agar and Nutrient broth provided the culture media, while a range of reagents (1% hydrochloric acid, Wagner's reagent, 10% ferric chloride, benzene, 12% ammonia solution, sodium hydroxide, glacial acetic acid, concentrated sulfuric acid, and acetic anhydride) supported the phytochemical screening work. A weighing balance, autoclave, incubator, refrigerator, water bath, Petri dishes, and inoculation loop rounded out the equipment needed for media preparation, sterilization, and microbial culturing.

Collection and Preparation of the Sample

Leaves were collected fresh around Tudun Wada, Zaria, then washed thoroughly to remove any soil and debris clinging to them. After shade-drying at room temperature, they were ground into a fine powder, and 100 g of this powder was weighed out for extraction.

Extraction

The 100 g of powdered sample was wrapped in filter paper to form a thimble sealed at one end with a stapler and tied tightly at the other to prevent any spillage. A Soxhlet apparatus was then set up on a heating mantle connected to an electric power source, with the condenser linked to a cold-water circulation system via two rubber hoses (one for water in, one for water out) and a submersible pump running in a bucket to keep the cooling constant. With the thimble in place inside the extraction chamber, 900 ml of methanol was poured into the round-bottom flask below to act as the solvent. Heating at 220 volts caused the methanol to evaporate, condense in the upper chamber, and steadily drip down onto the sample; once the chamber filled, it siphoned back into the flask below, carrying the dissolved phytochemicals along with it. This cycle ran for about 6 h. Afterward, roughly 550 ml of methanol was recovered, and the remaining extract was placed in a desiccator near an open window to let any leftover solvent evaporate off, leaving behind the dried crude extract [13].

Phytochemical Analysis

Standard methods guided the qualitative phytochemical screening carried out in this study [14-16].

Test for Tannins

A 2.0 ml portion of the extract was combined with a few drops of 10% ferric chloride solution; no bluish-black coloration developed, pointing to the absence of tannins.

Test for Flavonoids

Adding 2.0 ml of dilute sodium hydroxide to an equal volume of the extract produced a yellow coloration, confirming the presence of flavonoids.

Test for Phenols

Mixing equal volumes (2.0 ml each) of the extract and ferric chloride solution produced a deep

bluish-green colour, confirming the presence of phenols.

Test for Glycosides

To 2.0 ml of the extract was added 2.0 ml of glacial acetic acid containing a drop of ferric chloride solution, followed carefully by 2.0 ml of concentrated sulfuric acid. A brown ring forming at the interface confirmed the presence of glycosides (deoxy sugars).

Test for Saponins

One millilitre of distilled water was added to an equal volume of extract and shaken vigorously; a stable, persistent froth formed, confirming the presence of saponins.

Test for Steroids

Mixing 2.0 ml of the extract with 1.0 ml of acetic acid produced a colour change to blue, confirming the presence of steroids.

Test for Anthraquinones

A 0.5 g portion of the extract was weighed and shaken with 2.0 ml of benzene, then filtered. Adding 12% ammonia solution to the filtrate produced colour development in the ammoniacal layer, confirming the presence of anthraquinones.

Test for Alkaloids

A 0.5 ml portion of the extract was combined with 2.0 ml of 1% hydrochloric acid, heated on a water bath, and filtered while still hot. A few drops of distilled water followed by Wagner's reagent were then added to the filtrate; no reddish-brown precipitate formed, indicating the absence of alkaloids.

Test for Phlobatannins

Boiling 2.0 ml of the extract with 1% aqueous hydrochloric acid over a table gas heat source produced no red precipitate, indicating the absence of phlobatannins.

Antimicrobial Activity of the Extract against Test Organisms

Antimicrobial testing was carried out against the selected pathogens, which had been obtained from the research laboratory of the Research and Development Studies Department at NILEST, Zaria. Dissolving 0.2 g of the extract in 10 ml of methanol gave a starting concentration of 20 mg/ml, which was then used to test antimicrobial activity via the agar well diffusion method [17].

Mueller Hinton agar served as the growth medium prepared per the manufacturer's instructions, sterilized at 121°C for 15 min, poured into sterile Petri dishes, and left to cool and set. Each plate was seeded with 0.1 ml of the standardized inoculum for a given test organism, spread evenly across the surface with a sterile wire loop. A well was then cut into the centre of each inoculated plate using a sterile 6 mm cork borer, and 0.1 ml of the 20 mg/ml extract solution was introduced into it. Plates were incubated upside-down at 37 °C for 24 h, after which the resulting zones of inhibition were measured with a transparent ruler and recorded in millimetres.

Preparation of Media

Both media used in this study Mueller Hinton agar and Nutrient broth were prepared according to the manufacturer's instructions. The agar was autoclaved at 121°C for 15 min, then poured into sterile Petri dishes and left to cool and solidify under aseptic conditions.

Minimum Inhibitory Concentration

MIC values were determined using the broth dilution method [18-19]. Ten millilitres of nutrient broth was dispensed into sterile test tubes, autoclaved at 121°C for 15 min, and left to cool. A 0.5 McFarland turbidity standard was prepared separately, and the test organisms were inoculated into broth and incubated at 37 °C for 6 h. The resulting microbial suspension was then diluted with normal saline until its turbidity visually matched the 0.5 McFarland standard corresponding to roughly 1.5×10^8 CFU/ml. From there, two-fold serial dilutions of the extract were prepared in sterile nutrient broth to give concentrations of 20, 10, 5, 2.5, and 1.25 mg/ml, starting from an initial solution made by dissolving 0.2 g of extract in 10 ml of sterile broth. Each test organism was inoculated into its own tube using a freshly flame-sterilized loop; bacterial tubes went into incubation at 37 °C, while the fungal tubes were kept at 25 °C. Afterward, each tube was checked for turbidity as a sign of microbial growth relative to the control.

Minimum Bactericidal and Fungicidal Concentration

To determine whether the extract was actually killing the organisms or simply holding them back, MBC and MFC values were established next [18-19]. Mueller Hinton agar was prepared as before autoclaved at 121 °C for 15 min, poured into sterile plates, and left to solidify. Samples taken from the MIC tubes that showed no visible growth were then subcultured onto these agar plates and incubated at 37 °C for 24 h. Whichever concentration produced no visible colony growth on

subculture was taken as the MBC or MFC the point at which the extract was killing the organism outright, not just suppressing it.

RESULTS AND DISCUSSION

Qualitative Phytochemical Assessment of the Methanolic Leaf Extract of *Senna occidentalis*

Table 1 summarizes the results of the qualitative phytochemical screening.

Table 1: Qualitative phytochemical assessment of methanolic leaf extract of *Senna occidentalis*

	Phytochemical Test	Result
1	Tannins	-
2	Flavonoids	+
3	Phenols	+
4	Glycosides	+
5	Saponins	+
6	Steroids	+
7	Anthraquinones	+
8	Alkaloids	-
9	Phlobatannis	-

Keys: - = Not detected, + = Detected

As Table 1 shows, the methanolic leaf extract turned out to contain several noteworthy secondary metabolites flavonoids, phenols, glycosides, saponins, steroids, and anthraquinones all tested positive. This tracks with earlier work showing that *Senna* species tend to be rich in bioactive constituents with a wide range of pharmacological effects [11-12]. Flavonoids and phenolics, in particular, are well known for their antioxidant punch, neutralizing free radicals before they can trigger oxidative damage at the cellular level [20]. Glycosides and saponins bring their own value too, having been linked to antimicrobial, anti-inflammatory, and immunomodulatory effects [21-22], while steroids and anthraquinones carry analgesic, laxative, antimicrobial, and even cytotoxic properties that help explain their long history in traditional medicine [23-24]. Tannins, alkaloids, and phlobatannins, on the other hand, didn't show up at all likely a reflection of solvent selectivity, since methanol tends to favour polar to moderately polar compounds [13]. That absence doesn't

really take anything away from the extract's overall bioactivity, though, given how much else was present.

Antimicrobial Activities of *Senna occidentalis* Leaf Extract and Control Against the Test Organisms

Table 2 compares the antimicrobial activity of the extract against the methanol control.

Table 2: Antimicrobial activities of *Senna occidentalis* leaf extract and control against the test organisms

	Test Organism	Extract	Control (Methanol)
1	*Escherichia coli*	R	R
2	*Candida stellatoidea*	S	R
3	*Staphylococcus aureus*	S	R

Keys: R = Resistant, S = Sensitive

What stands out here is how selective the extract's inhibitory effect really was. *Escherichia coli* showed no response to it whatsoever not altogether surprising, given that Gram-negative bacteria are shielded by a lipopolysaccharide-rich outer membrane known to block many plant-derived compounds from getting through [25]. *Candida stellatoidea* and *Staphylococcus aureus* told a different story, both showing clear sensitivity to the extract. In the case of *S. aureus*, being Gram-positive likely worked in the extract's favour, since its more permeable peptidoglycan wall makes it easier for phytochemicals to get inside [26]. All of this fits with what's already documented about flavonoids, phenols, glycosides, and saponins compounds well known for disrupting microbial membranes and interfering with metabolic processes [27-28].

Zone of Inhibition of the Extract and Control Against the Test Organisms

Table 3 presents the zones of inhibition recorded for both the extract and the control.

Table 3: Zone of inhibition of the extract and control against the test organisms

	Test Organism	Inhibition Zone (mm)	Control (mm)
1	*Escherichia coli*	0	0
2	*Candida stellatoidea*	26	0
3	*Staphylococcus aureus*	24	0

These numbers back up what Table 2 already suggested. *E. coli* produced no inhibition zone at all (0 mm) again pointing to the reduced permeability that comes with being Gram-negative [29-30]. *Candida stellatoidea*, meanwhile, produced a notably large zone of 26 mm, and *Staphylococcus aureus* wasn't far behind at 24 mm, both results reflecting genuinely strong antimicrobial action. The pattern lines up well with existing reports that fungal and Gram-positive cell walls tend to let phenolics, saponins, and flavonoids through far more easily [31-32].

Minimum Inhibitory Concentration of the Extract Against the Test Organisms

Table 4 sets out the MIC results obtained for each organism.

Table 4: Minimum inhibitory concentration of the extract against the test organisms

	Test Ogranism	Extract Concentration				
		20 mg/ml	10 mg/ml	5 mg/ml	2.5 mg/ml	1.25 mg/ml
1	*Escherichia coli*	-	-	O*	+	++
2	*Candida stellatoidea*	-	-	O*	+	++
3	*Staphylococcus aureus*	-	-	O*	+	++

Keys: - = No turbidity (no growth), O* = MIC, + = Light turbidity, ++ = Moderate turbidity

These figures reinforce the earlier results. *E. coli* showed no turbidity at any tested concentration further evidence of the intrinsic impermeability tied to its Gram-negative structure [29-30]. Both *Candida stellatoidea* and *Staphylococcus aureus*, on the other hand, hit their MIC at 5 mg/ml, with turbidity increasing steadily as the concentration dropped below that point.

Minimum Bactericidal/Fungicidal Concentration of the Extract Against the Test Organisms

Table 5 shows the corresponding MBC/MFC results.

Table 5: Minimum bactericidal/fungicidal concentration of the extract against the test organisms

	Test Ogranism	Extract Concentration				
		20 mg/ml	10 mg/ml	5 mg/ml	2.5 mg/ml	1.25 mg/ml
1	*Escherichia coli*					
2	*Candida stellatoidea*	-	O*	+	++	+++
3	*Staphylococcus aureus*	-	O*	+	++	+++

Keys: - = No colony, O* = MBC/MFC, + = Scanty colony growth, ++ = Moderate colony growth, +++ = Heavy colony growth

Here we see the concentrations actually needed to kill the organisms outright, rather than just hold them in check. *E. coli* remained unaffected across the board, as expected by now, while both *C. stellatoidea* and *S. aureus* showed no colony growth at 10 mg/ml their respective MFC and MBC. Below that threshold, colonies began reappearing at 5 mg/ml and grew more pronounced as concentration decreased further to 1.25 mg/ml, confirming that the extract's killing power tapers off at lower doses. This tracks with other work showing that fungi and Gram-positive bacteria tend to be particularly sensitive to plant metabolites like flavonoids, phenolics, and saponins [31-32].

CONCLUSION

This study set out to examine the phytochemical makeup and antimicrobial activity of the methanolic leaf extract of *Senna occidentalis* against a selected panel of pathogenic microorganisms, and the results speak fairly clearly: these leaves carry a range of biologically active compounds and show real, if selective, antimicrobial potential. On the phytochemical side, screening turned up flavonoids, phenols, glycosides, saponins, steroids, and anthraquinones, while tannins, alkaloids, and phlobatannins were nowhere to be found. Given how often these detected compounds are linked to antimicrobial, antioxidant, and broader therapeutic effects, their presence goes some way toward explaining why *Senna occidentalis* has held such a longstanding place in traditional treatment of infections. The antimicrobial results tell a similarly clear story.

Both *Staphylococcus aureus* and *Candida stellatoidea* responded strongly to the extract visible in their inhibition zones, a shared MIC of 5 mg/ml, and bactericidal/fungicidal activity kicking in at 10 mg/ml. *Escherichia coli*, though, remained entirely unaffected no matter the concentration tested, which suggests the extract's antimicrobial reach doesn't extend evenly across the board it favours Gram-positive bacteria and fungi far more than it does Gram-negative organisms. Taken together, these findings make a reasonably strong case that *Senna occidentalis* leaves hold genuine antimicrobial value, rooted in their phytochemical makeup. That, in turn, lends scientific weight to the plant's traditional use and points to its potential as a natural antimicrobial resource worth developing further.

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