Phytochemical Screening and Antimicrobial Activity of Ethyl Acetate Root Extract of *Senna italica*

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ABSTRACT

Senna italica Mill. is a diffuse perennial herb or small shrub belonging to the family Fabaceae. The roots of this plant are traditionally used by indigenous herbalists to treat certain sexually transmitted infections (STIs). The crude ethyl acetate root extract of *Senna italica* was obtained following defatting with n-hexane using the cold extraction method. Antimicrobial activity of the crude extract was evaluated using the agar well diffusion method against *Candida albicans* and *Staphylococcus aureus*. Phytochemical screening of the ethyl acetate extract revealed the presence of alkaloids, flavonoids, saponins, quinones, terpenoids, tannins, sterols, and phenols. The extract exhibited concentration-dependent inhibitory activity against *Candida albicans*, with a maximum zone of inhibition of 10.00 mm at the highest concentration, but showed no activity against *Staphylococcus aureus*. These findings suggest that the root of *Senna italica* possesses moderate antifungal activity against *Candida* species. Further biological studies are required to evaluate the sensitivity of other pathogenic organisms associated with sexually transmitted diseases to the plant extract.

Keywords: Phytochemicals, *Senna italic,* antimicrobial, ethyl acetate.

INTRODUCTION

The use of complementary and alternative medicine, particularly traditional herbal medicine, has increased significantly in both developed and developing countries [1]. This growing reliance on herbal remedies is largely attributed to their affordability, accessibility, perceived safety, and therapeutic efficacy. In many developing regions, medicinal plants remain the primary source of healthcare, while in developed countries they are increasingly used as complementary therapies alongside conventional medicine [2]. Repeated therapeutic failures and adverse effects associated with the management of chronic and severe diseases such as arteriosclerosis, cardiovascular and neurological disorders, and cancer have further encouraged the use of plant-based nutraceutical products. These products are now widely employed to improve general health and to prevent or manage various disease conditions [3].

Senna italica Mill. is a diffuse perennial herb or small shrub belonging to the family Fabaceae. The plant is also known by several common names, including Cassia italica, Italian senna, Senegal senna, and Port Royal senna [4]. It typically grows to a height of 50–75 cm and is characterized by 4–8 pairs of leaflets and flat, curved pods containing 5–8 seeds. The flowers are yellow, bisexual, and composed of five petals. Based on differences in inflorescence size and petiole length, *Senna italica* has been classified into three subspecies: italica, miracantha, and aracoides [5]. Ethnobotanical reports indicate that various parts of *Senna italica* are widely used in traditional medicine for the treatment of numerous ailments. The leaves, pods, and immature seeds are commonly employed as purgatives, while decoctions and macerations are used to treat stomach disorders, fever, jaundice, venereal diseases, and biliousness [6]. The plant is also reported to be used as an abortifacient and in the management of intestinal worm infestations. Fresh, dried, or pulverized leaves are applied topically for the treatment of skin diseases, burns, and ulcers [7]. The flowers are prepared as herbal tea and used both as a purgative and to induce labor. In addition, macerated roots are used in the treatment of colic and influenza, while boiled roots are applied externally to dress wounds. Root infusions are also used as eye drops for sore eyes and for treating indigestion, liver and gall bladder disorders, nausea, vomiting, and dysmenorrhoea [8]. Young seeds are sometimes consumed as snacks or cooked as vegetables [9].

Plants synthesize a wide range of secondary metabolites, commonly referred to as phytochemicals, which play vital roles in plant defense against insect predation, microbial infections, and environmental stress. Many of these phytochemicals including alkaloids, flavonoids, tannins, saponins, terpenoids, and phenolic compounds are biologically active in humans and have been associated with significant health benefits [10]. *Senna italica* has been traditionally used in many African communities for the treatment of infections, skin diseases, gastrointestinal disorders, and inflammatory conditions, there is still limited scientific information regarding the phytochemical constituents and antimicrobial potential of its root extracts, particularly those obtained using ethyl acetate as a solvent [11]. Most previous studies on *Senna italica* have focused mainly on the leaves and stem bark, while the roots remain underexplored despite their extensive use in traditional medicine [12]. Moreover, many existing studies have concentrated on crude aqueous or methanolic extracts, with little attention given to ethyl acetate extracts, which are important because ethyl acetate is capable of extracting moderately polar bioactive compounds such as flavonoids, phenolics, terpenoids, and alkaloids that may possess strong antimicrobial activities. Consequently, there is insufficient information on the specific phytochemicals present in the ethyl acetate root extract of *Senna italica* and their relationship with antimicrobial efficacy.

This study aimed to investigate the phytochemical constituents and antimicrobial activity of the ethyl acetate root extract of *Senna italica* against selected pathogenic microorganisms by collecting and preparing the plant roots for extraction, extracting bioactive compounds using ethyl acetate as the extraction solvent, screening the extract for the presence of phytochemical constituents such as alkaloids, flavonoids, tannins, saponins, terpenoids, glycosides, and phenolic compounds, evaluating the antimicrobial activity of the extract against selected bacterial and fungal pathogens, determining the effect of different extract concentrations on microbial growth inhibition

MATERIALS AND METHODS

Apparatus

Ultrasonicator (Model/AS3120) an ultra-sonic water bath, were purchased from Autoscience Ltd USA, analytical weighing balance (Model: PA214) was from Chaus Corporation, Pine Brook, USA; Blender (Model: HR 2815), China, Drying cabinet (Model: FSM 140), Japan, Genlab (Water bath) from Thermal Engineers (Model: WBH22/FL), United Kingdom, Vortex mixer Bibby Scientific Limited Stone, Staffordshire (Model: ST15 0SA), UK. Whatman No.1 filter paper was purchased from Whatman International Ltd, Maidstone, England.

Chemicals and reagents

All the chemicals and reagents used in this study were of analytical grade.

Sample collection

Fresh *Senna italic* roots were collected from Amshi town in Jakusko LGA of Yobe State, Nigeria [13]. The plant was identified by a botanist at the Biological Sciences Department of Yobe State University, Damaturu Nigeria, with voucher number YSUH_088.

Preparation of extraction

The sample was washed and dried for seven days, at room temperature and pulverized into a coarse powder using a pestle and mortar. The coarse particles (300 g) were successively extracted with 500 mL each of solvents n-hexane, ethyl acetate, and 70% methanol for three days with agitation on a mechanical shaker. It was drained using muslin cloth, then filtered with Whatman's No. 1 filter paper. The volume of the extracts was concentrated to dryness on a water bath and later kept in a drying cabinet. It was then weighed and labeled appropriately then stored for use in further analysis.

Phytochemical screening

Preliminary phytochemical analysis

The crude n-hexane, ethyl acetate and methanol extracts of root of *Senna italica*. were tested for the presence of phytochemicals using the following qualitative procedures [14].

Test for saponins

About 5 mL of the extract was shaken with 10 mL of water in a test tube. Frothing which persists was taken as preliminary evidence for the presence of saponin [2].

Test for tannins

To 2 mL of the extract, 10 mL of water was added and a drop of ferric chloride. Green precipitate indicates the presences of tannins [15].

Tests for flavonoids To 5 mL of the extract, 0.5 g of magnesium chips was added and a few drops of concentrated H_2SO4 down the side of the test-tube. Reddish coloration indicates the presence of flavonoids [9].

Test for essential oils

About 10 mL of the extract were dissolved in 90% alcohol and 3 drops of ferric chloride was added. Green coloration indicates the presences of essential oils [14].

Test for glycoside

About 5 mL of the extract was boiled with 25 mL of dilute tetraoxosulphate (VI) acid (2.5 cm^3) for 15 minutes. The resulting solution was cooled and neutralized with 10% potassium hydroxide and of Fehling's solution A and B were added. Formation of a brick red precipitate indicates the presence of glycosides [16].

Test for phenols

Equal volume of the extract was added to equal volume of ferric chloride. A deep blue bluish solution indicates the presence of phenols [10].

Test for alkaloids

The extract (0.5 g) was dissolved in 5mL of 2N HCl and filtered. The filtrate was treated with Dragendroff's reagent (Solution of potassium Iodide and bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids [14].

Test for oxalate

To 3 mL portion of the extract (0.2 g in 3 mL of methanol) were added a few drops of glacial acetic acid. A greenish black colouration indicates the presence of oxalates [6].

Test for sterols

To 1mL of the extracts was treated with few drops of chloroform, acetic anhydride and concentrated sulphuric acid. The formation dark pink or red colour indicates the presence of sterols [10].

Test for quinones

Few drops of concentrated, HCl were added to a small portion of extract, formation of yellow precipitate coloration indicates the presence of quinines [14].

Microorganisms

The strains of bacterium and fungus used in this study (*Staphylococcus aureus*, and C*andida albican*) were obtained from the Pathology Laboratory Yobe State University Teaching Hospital, Damaturu, Yobe State, Nigeria. They were tested on Nutrient Agar with ciprofloxacin and Ketoconazole as control. The antimicrobial activity screening was carried out using agar well diffusion and dilution techniques.

RESULTS AND DISCUSSION

Table 1 shows the results of the phytochemical screening of the ethyl acetate extract of *Senna italica* root. The results revealed the presence of some important secondary metabolites like alkaloid were strongly present (++), while flavonoids, saponins, quinones, terpenoids, tannins, phenols, and sterols were moderately present (+). Cardiac glycosides and oxalates were not detected in the *Senna italica* **roots.** The diversity of compounds observed suggests that the extract possesses significant pharmacological potential [14].

The strong presence of alkaloids is particularly noteworthy. Alkaloids are widely recognized for their potent biological activities, especially antimicrobial effects. They are known to interfere with microbial DNA replication, inhibit key enzymes, and disrupt cell membrane integrity [17]. Therefore, the marked presence of alkaloids in this extract may play a central role in any observed antimicrobial activity. Many medicinal plants rich in alkaloids demonstrate strong activity against Gram-negative bacteria, which may explain the traditional use of *S. italica* in treating infections.

Flavonoids were also detected in moderate amounts. These compounds are well known for their antioxidant and antimicrobial properties. Flavonoids are effective free radical scavengers [8]. Their presence suggests that the extract may not only inhibit microbial growth but also protect tissues from oxidative stress.

Saponins were present, as confirmed by the frothing test. Saponins are known for their ability to interact with membrane sterols, increasing membrane permeability and leading to cell lysis [15]. This property makes them important contributors to antimicrobial action.

The presence of terpenoids further strengthens the therapeutic potential of the extract. Terpenoids are known to disrupt microbial membranes and interfere with essential cellular functions [18]. Their detection is consistent with the semi-polar nature of ethyl acetate, which is capable of extracting moderately non-polar compounds.

Tannins and phenolic compounds were also identified. These polyphenolic substances are among the most studied plant metabolites due to their strong antimicrobial and antioxidant properties. Tannins can precipitate proteins and inhibit microbial enzymes, limiting bacterial growth [18]. Phenolic compounds, on the other hand, can denature proteins and disrupt cell membranes while also acting as powerful antioxidants [8]. Together, these compounds may significantly enhance the biological activity of the extract. Although sterols are not primarily known for antimicrobial activity, plant sterols contribute to anti-inflammatory effects and may support membrane-related interactions that enhance the activity of other compounds [19].

Importantly, cardiac glycosides and oxalates were absent. The absence of cardiac glycosides may be beneficial from a safety perspective, as these compounds can possess cardiotoxic effects at high concentrations. Similarly, the absence of oxalates reduces concerns related to mineral binding and kidney stone formation.

Table 1: Phytochemical Screening of Ethyl Acetate Root Extract of *Senna italic*

Phytochemicals	Type of Test	Ethyl acetate extract
Alkaloids	Mayer's	++
Flavonoids	Sodium hydroxide	+
Saponins	Foaming	+
Cardiac glycosides	Keller-kelliani's	-
Oxalates	Acetic acid	-
Quinones	Conc. HCl	+
Terpenoids	Salkowski's	+
Tannins	Braymer's	+
Phenols	Ferric chloride	+
Sterols	Libermann-Burchard's	+

Key: + = positive test - = negative test

Antimicrobial activity

The results presented in Table 2. indicate that the ethyl acetate extract of *Senna italica* roots xhibits selective antimicrobial activity, showing no inhibitory effect against *Staphylococcus aureus* but moderate antifungal activity against *Candida albicans*. This differential response is important in understanding both the phytochemical composition of the extract and its potential therapeutic applications. The extract showed zero inhibition (0.00 mm) against *Staphylococcus aureus for the* antibacterial activity against *Staphylococcus aureus* across all tested concentrations (2.50–20.00 µg/mL). This suggests that the bioactive compounds present in the ethyl acetate fraction are either absent in antibacterial constituents, or present but ineffective against Gram-positive bacteria such as *S. aureus*. This observation aligns with previous findings [17] that solvent polarity significantly influences the extraction of antibacterial phytochemicals. Ethyl acetate, being moderately polar, may not effectively extract compounds like tannins or certain alkaloids known for antibacterial activity [20].

Additionally, *S. aureus* possesses structural defences such as a thick peptidoglycan layer, which can reduce susceptibility to plant-derived compounds [11]. In contrast, the control (ciprofloxacin) exhibited a 7.00 mm zone of inhibition, confirming the sensitivity of the test organism and validating the experimental conditions. The antifungal activity against *Candida albicans*, unlike the antibacterial results revealed that the extract demonstrated a concentration-dependent antifungal effect against *Candida albicans*. The zone of inhibition increased progressively from 2.00 mm at 2.50 µg/mL to 10.00 mm at 15.00–20.00 µg/mL. This trend suggests the presence of bioactive antifungal compounds in the extract and dose-response relationship, typical of pharmacologically active plant extracts. The plateau observed at higher concentrations (15–20 µg/mL) may indicate saturation of antifungal activity, where increasing concentration no longer enhances efficacy. Such behaviour is common when the active compounds reach their maximum inhibitory potential [22]. Interestingly, at 20.00 µg/mL, the extract (10.00 mm) exhibited a greater antifungal effect than the control ketoconazole (6.00 mm). This suggests that the ethyl acetate extract may contain potent antifungal constituents, possibly flavonoids, anthraquinones, or phenolic compounds, which have been reported in *Senna* species [12].

Table 2: Antimicrobial Activities of Ethyl Acetate Extract of *Senna italic* Roots

			Zone of Inhibition	(mm)
Extract		Concentration (µg/mL)	*Staphylococcus aureus*	*Candida albicans*
		2.50	0.00	2.00
		5.00	0.00	4.00
Ethyl acetate		10.00	0.00	6.00
		15.00	0.00	10.00
		20.00	0.00	10.00
Control		20.00	7.00	6.00

Key: C =Control (Antibacterial =Ciprofloxacin) (Antifungal =Ketoconazole) 0=No activity

CONCLUSION

Senna italica root contains essential secondary metabolites. Based on the findings from the anti microbial activity, the root of *Senna italic* possesses moderate anti fungal activity against *Candida*. Further biological studies are required to prove the sensitivity of pathogenic organisms involved in sexually transmitted diseases to the plant extract

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