



## RESEARCH ARTICLE

### Exploring the antioxidant performance and identification of bioactive phytochemicals of leaf extracts of *Senna alata* (L.) Roxb.

A. Bansa<sup>1\*</sup>, S. Dachi<sup>2</sup>, J. I. Usman<sup>3</sup>, A.E. Ajewole<sup>1</sup>, & N. Etsu-Musa<sup>1</sup>

<sup>1</sup>Department of Biological Sciences, Federal Polytechnic, Bida Niger State, Nigeria.

<sup>2</sup>Department of Agriculture, University of Jos, Jos Plateau State, Nigeria.

<sup>3</sup>Department of Biological Sciences, Federal Polytechnic, Nasarawa, Nasarawa State Nigeria.

#### Edited by:

Dr. Nusaibah Syd Ali,  
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#### Reviewed by:

Dr. P. Arjun, Saveetha University, Chennai,  
Tamil Nadu, India.

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\*Corresponding author e-mail address:  
[drbansa@yahoo.com](mailto:drbansa@yahoo.com) (Bansa, A).

#### ABSTRACT

*Senna* is a highly-producing Leguminosae herb known as a ringworm plant. Antioxidants protect the body against reactive oxygen species-induced oxidative damage, which is linked to cardiovascular disease and cancer. Naturally occurring plant antioxidants are being considered as preventive medicine. *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, and *Bacillus subtilis* were examined for *Senna alata* extract secondary metabolites, antioxidant, and antibacterial activity. The plant leaf was phytochemically examined for alkaloids, tannins, general glycosides, steroids, and terpenoids. A quantitative study of leaf extract secondary metabolites was done. Results showed that alkaloids, tannins, steroids, and terpenoids had concentrations of 8.5±2.0mg/100g, 12.5±1.0mg/100g, 17.0±2.0mg/100g, 11.5±3.0mg/100g, and 16.0±3.1mg/100g, respectively. The study discovered that increasing the quantities of leaf extract directly enhanced the scavenging action of DPPH (2, 2-diphenyl-1-1-picrylhydrazyl). The antioxidant activity of the leaf extract was inferior to that of ascorbic acid. The test organisms were susceptible to the plant extract due to the presence of zones of inhibition. At the lowest concentration, the *Senna alata* leaf extract combined with Ethanol exhibited the highest level of inhibition against *Escherichia coli* and *Staphylococcus aureus*. The findings of this study suggest that the chemical compounds found in *Senna alata* leaf extract will have potential applications in chemotherapy.

**Keywords:** herb, leguminosae, inhibition, natural, susceptibility, *Senna alata*

## INTRODUCTION

Antioxidants play a crucial role in safeguarding the body against oxidative harm induced by reactive oxygen species (ROS), which are associated with ailments like cardiovascular, cancer, neurological, and Alzheimer's diseases (Di-Matteo and Esposito, 2003). The promise of plant-based antioxidants in preventative medicine is being more acknowledged. Phenolic compounds and flavonoids, previously regarded as non-essential nutrients, are currently attracting interest due to their potential pharmacological properties on human health (Ashokkumar et al., 2020, 2021, & 2022). Flavonoids have been discovered to offer various beneficial qualities, including antioxidant, anti-inflammatory, hepatoprotective, neuroprotective, and anticarcinogenic effects (Araceli et al., 2003; Ambiga et al., 2020; Arjun et al., 2023). Phenolic chemicals provide antioxidant activity due to their potent hydrogen ion capabilities. These chemicals, derived from plants, exert an antioxidant effect by multiple mechanisms, including scavenging free radicals, binding to metal ions, catalysing the generation of free radicals, stimulating antioxidant enzymes, and inhibiting oxidases (Kulkarni et al., 2004; Arjun et al., 2019).

Plant-derived pharmaceuticals are abundant and provide substantial benefits compared to synthetic compounds, cheers to their wide variety, large-scale production, and safety characteristics (Laldinsangi, 2022). Some important medications commonly used in modern medicine are vinca alkaloids, artemisinin, chloroquine, and morphine. The ongoing exploration of novel bioactive chemicals derived from plants remains a vital means of discovering potent pharmaceutical molecules (Saradha Devi et al., 2016; Wu et al., 2021).

*Senna alata*, also called *Cassia alata*, is a flowering shrub that belongs to the Leguminosae family. *S. alata* has demonstrated clinical effectiveness in preventing diseases in Africa. *S. alata* is employed in northern Nigeria for the treatment of skin problems, wounds, diarrhoea, and constipation (Adebayo et al., 2001). The leaves are infused to alleviate tension, toothache, bodily pain, and gastrointestinal discomfort in Southwestern Nigeria (Benjamin et al., 1981). It is also acknowledged for its medicinal capabilities in the treatment of skin illnesses and epilepsy, as well as its ability to stimulate the bowels and limit water absorption in the colon to relieve constipation. The leaves, bark, and stem of plants in Cameroon are used to cure ringworm, hepatitis, and gastroenteritis (Owoyale et al., 2005). Study

conducted by Saginaw et al. (2014) have revealed that the plant exhibits antioxidant and wound-healing qualities, as well as anti-inflammatory benefits. A study conducted by Oladejo et al. (2020) found that quinones and terpenes extracted from *S. alata* showed notable antiplasmodial efficacy in laboratory tests. The pharmacological effects of these substances include their existence of phenolics, steroids, terpenoids, anthraquinones, and fatty acids. The increasing problem of drug-resistant microbes in the era following the effectiveness of antibiotics, due to the improper use of antibiotics, presents a substantial obstacle to public health (Goud, 2016). The exponential rise in antibiotic resistance underscores the imperative necessity for vigilant surveillance of resistant strains, responsible utilisation of antibiotics, and exploration of innovative antibacterial medicines.

## MATERIALS AND METHODS

### Collection and extraction of plant material

The International Committee for Botanical Nomenclature (ICBN) approved *Senna alata* fresh leaves from the University of Ilorin Botanical Garden and IITA Ibadan for authenticity. Leaves were tagged and put in polythene bags for future use. After air-drying in the shade at room temperature, the plant leaves were extracted using a method identical to Banso & Banso, (2023) and Banso et al. (2024a).

### Qualitative phytochemical screening

Quantitative phytochemical screening was done on the plant's ethanol leaf extract to analyze secondary metabolites.

### Test for general glycosides

1g of crudely powdered leaf sample was placed in two beakers to test for general glycosides. In one beaker, 5 ml of weak sulphuric acid was added. Both beakers were heated for 5 minutes and then filtered the contents into test tubes. 5% sodium hydroxide was added, and Fehling's solution was heated for 3 minutes to alkalize the filtrate. A reddish-brown precipitate in the aqueous filtrate suggested generic glycosides (Banso et al., 2024b).

### Test for saponins

To measure saponins, 0.5g of extract was dissolved in 10 ml of distilled water. The solution was then heated in a water bath for 5 minutes. Saponins are present when foam forms during this technique.

### Assay for alkaloids

To test for alkaloids, a 15 g leaf extract sample was dissolved in 6 ml of 1% HCl and water bathed for 5 minutes. This divided the solution into two equal pieces.

1. Dragendroff's test: 1 ml of  $\text{BiI}_7\text{K}_4$  solution was added to 2 ml of the solution above. Alkaloids appeared as an orange precipitate.
2. Mayer's test: 1ml of Mayer's reagent (mercuric chloride and potassium iodide) was added to 2ml of the solution. A cream-coloured precipitate indicated alkaloids.
3. Wagner's test: A limited drops of Wagner's reagent (Mixture of Iodine and Potassium iodide) were added to 2ml of the solution. The presence of dark precipitate indicates alkaloids.

#### **Test for steroids and Terpenoids**

Drying a 10ml chloroform extract from the leaf sample and dissolving the residue in 0.5ml chloroform tested for steroids. Using the Leibermann-Buchard reaction, 0.5 ml acetic anhydride and 2 ml concentrated sulphuric acid were added. Blue-green or similar colors indicated steroidal chemical presence (Banso et al., 2024c). Terpenoids were tested using the same manner as steroids, but a red, pink, or violet hue indicated terpenoids.

#### **Test for tannins and sesquiterpenoids**

To test for tannins, 0.5g extract was combined with 10 ml distilled water. Several drops of 5% ferric chloride solution were added. Black or blue-green precipitates indicated tannins. To test sesquiterpenes, 0.5 ml of aqueous leaf extract was shaken with 0.5 ml of methanol. After that, 0.4 ml of 5% sulphuric acid and 0.5% ferric chloride were added and agitated with a glass rod. The mixture was heated for 1 minute in a Grant model water bath. According to Banso et al. (2024c), ferric chloride caused a green-to-black color change, indicating sesquiterpenes.

#### **Quantitative analysis**

##### **Determination of total phenolic content (TPC) and tannin content**

The Folin-Ciocalteu technique and UV-Vis spectrophotometer measured the TPC and tannin content of the ethanolic leaf extract using the method of Chakraborty et al., 2002, Geetha, 2010, and Hullatti and Murthy, 2010).

##### **Determination of total flavonoid content**

The 1 ml of a 1mg/ml extract was combined with 4 ml of distilled water. Then, 0.3 ml of 5%  $\text{AlCl}_3$  was

added to a 10 ml volumetric flask with the mixture. Add 2 ml of 1M NaOH and mix well. The total volume was 10ml after adding 2.4ml distilled water. The 1ml extract was replaced with distilled water to create a blank. Additionally, standard quercetin solutions (20, 40, 60, 80, and 100 $\mu\text{g}/\text{ml}$ ) were dissolved in methanol. A UV-Vis spectrophotometer measured absorbance at 510nm for the reference and test solutions after 30 minutes.

#### **Antioxidant activity**

##### **DPPH radical scavenging assay**

The stock solution was made by dissolving 2.4 milligrams of DPPH in 100 ml methanol. The plant extract was prepared at concentrations of 50, 100, 150, 200, and 250 $\mu\text{g}/\text{ml}$  in ethanol. In a 25 ml volumetric flask, 100 $\mu\text{l}$  of each leaf extract concentration was combined with 3 ml of DPPH. The same process was used to prepare standard ascorbic acid solutions (50, 100, 150, 200, and 250 $\mu\text{g}/\text{ml}$  in distilled water). DPPH and methanol were used as a blank. After a 30-minute incubation, UV-Vis spectrophotometers evaluated standard and test solution absorbance at 515nm. Leaf extract radical scavenging activity was estimated using formula of Priya and Krishna (2007),

$$\text{DPPH \%} = \frac{\text{Ab}(\text{control}) - \text{Ab}(\text{sample})}{\text{Ab}(\text{control})} \times 100$$

(Ab(control)) = Absorbance of the control sample (DPPH only)

(Ab(sample)) = Absorbance of the test sample (DPPH + leaf extract)

##### **Antibacterial bioassay**

The leaf extract was tested on *Escherichia coli* (ATCC28923), *Staphylococcus aureus* (ATCC28923), *Enterococcus faecalis* (ATCC29212), and *Bacillus subtilis* (ATCC6051) using the agar diffusion method proposed by Banso & Banso (2023). The bacterium suspension's turbidity was standardized at  $1 \times 10^6$  bacteria cells/ml (0.5 McFarland standards) throughout the study. The bacteria cells were equally disseminated on nutrient agar plates with a sterile glass spreader. After creating wells on the agar surface, 200 $\mu\text{l}$  of a 50mg/ml leaf extract solution in DMSO was added using a sterilized pipette (Rashna et al., 2001). After one hour of diffusion at room temperature, the plates were kept at 37°C for 24 hours to develop bacteria. The zones of inhibition were quantified and compared to antibiotic-containing positive control wells. The zone of inhibition diameter was compared to Amoxicillin to determine antibacterial activity.

The MIC (minimum inhibitory concentration) was determined using the EUCAST-approved broth microdilution method. The extract was dissolved in 10% DMSO and serially half-diluted in Mueller-Hibton broth in a microtiter plate. Assessment wells had  $5 \times 10^6$  cfu/ml concentrations after this treatment. A  $10 \mu\text{g/ml}$  amoxicillin standard concentration was employed as a positive control (Islam et al., 2011).

### Data analysis

**Table 1.** Phytochemical components of leaf extract of *Senna alata*

Active principle	Leaf extract
Alkaloids	Positive
Tannins	Positive
General glycoside	Positive
Saponins	Negative
Steroids	Positive
Terpenoids	Positive
Sesquiterpenes	Negative

### Total secondary metabolites

In *Senna alata* leaf extract, total alkaloids, tannins, glycoside steroids, and terpenoids were found at  $8.5 \pm 2.0 \text{ mg/100g}$ ,  $12.5 \pm 1.0 \text{ mg/100g}$ ,  $17.0 \pm 2.0 \text{ mg/100g}$ ,  $11.5 \pm 3.0 \text{ mg/100g}$ , and  $16.0 \pm 3.1 \text{ mg/100g}$ , respectively (Table 2).

Table 2. Determination of total secondary metabolites in ethanol leaf extract

Analysis of variance and post-hoc mean comparisons with Turkey's multiple range tests were performed in SPSS version 20.0 (IBM Corp., Armonk, NY, USA). P-values below 0.05 indicated statistical significance.

### RESULTS

This phytochemical investigation found alkaloids, glycosides, tannins, steroids, and terpenoids in *Senna alata* leaves. The plant leaf lacked saponins and sesquiterpenes (Table 1).

Active principle	Quantity (mg/100g) $\pm$ SD
Alkaloids	$8.5 \pm 2.0$
Tannins	$12.5 \pm 1.0$
General glycoside	$17.0 \pm 2.0$
Saponins	ND
Steroids	$11.5 \pm 3.0$
Terpenoids	$16.0 \pm 3.1$
Sesquiterpenes	ND

ND = Not detected

### DPPH scavenging activity

Results showed that the concentration-dependent DPPH scavenging activity was found (Table 3). At concentrations of 50, 100, 150, 200, and  $250 \mu\text{g/ml}$ , ascorbic acid values were  $30.55 \pm 2.34\%$ ,  $50.00 \pm 2.33\%$ ,  $60.754 \pm 1.98\%$ ,  $85.35 \pm 2.50\%$ , and  $95.64 \pm 1.54\%$ . *Senna alata* had values of  $7.45 \pm 2.05\%$ ,  $13.74 \pm 3.50\%$ ,  $20.40 \pm 2.40\%$ ,  $32.51 \pm 3.17\%$ , and  $42.95 \pm 3.32\%$ .

**Table 3.** DPPH Scavenging activity of ascorbic acid and leaf extract of *Senna alata*

Concentration ( $\mu\text{g/ml}$ )	DPPH scavenged by ascorbic acid (%) $\pm$ SD	DPPH scavenged by leaf extract (%) $\pm$ SD
50	$30.55 \pm 2.34$	$7.45 \pm 2.05$
100	$50.00 \pm 2.33$	$13.74 \pm 3.50$
150	$60.75 \pm 1.98$	$20.40 \pm 2.40$
200	$85.35 \pm 2.50$	$32.51 \pm 3.17$
250	$95.64 \pm 1.54$	$42.95 \pm 3.32$

SD = Standard deviation

### Antimicrobial properties

The inhibition zones against test organisms ranged from  $14.0 \pm 0.1 \text{ mm}$  to  $19.5 \pm 3.1 \text{ mm}$  in diameter. Inhibition zones of  $12.5 \pm 0.2 \text{ mm}$ ,  $15.8 \pm 1.2 \text{ mm}$ , and  $13.5 \pm 0.4 \text{ mm}$  were observed for aqueous, methanol, and ethanol extracts against *E. coli* (Table 4). Extract

values of  $14.0 \pm 0.1 \text{ mm}$ ,  $19.5 \pm 3.1 \text{ mm}$ , and  $16.5 \pm 2.0 \text{ mm}$  were recorded against *E. faecalis*. Table 4 shows that aqueous, methanol, and ethanol extracts yielded  $12.4 \pm 1.6 \text{ mm}$ ,  $17.0 \pm 2.1 \text{ mm}$ , and  $15.5 \pm 1.0 \text{ mm}$ , respectively, when tested against *B. subtilis*. The MIC of *S. alata* methanol leaf extract

against *E. coli* was 25mg/ml, but against *S. aureus*, *E. faecalis*, and *B. subtilis* it was 40, 30, and 35mg/ml (Figure. 1).

Table 4. Antibacterial activity of leaf extracts of *Senna alata*

Bacteria strain	Leaf extract (50mg/ml)			Standard antibiotic
	Diameter of zone of inhibition (mm) ± SD			
	Aqueous	Methanol	Ethanol	Amoxicillin
<i>E. coli</i>	12.5±0.2	15.8±1.2	13.5±0.4	30.6±2.2
<i>S. aureus</i>	11.8±2.2	16.9±0.1	14.8±0.1	38.5±1.2
<i>E. faecalis</i>	14.0±0.1	19.5±3.1	16.5±2.0	40.5±0.1
<i>B. subtilis</i>	12.4±1.6	17.0±2.1	15.5±1.0	39.3±0.1

SD = Standard deviation

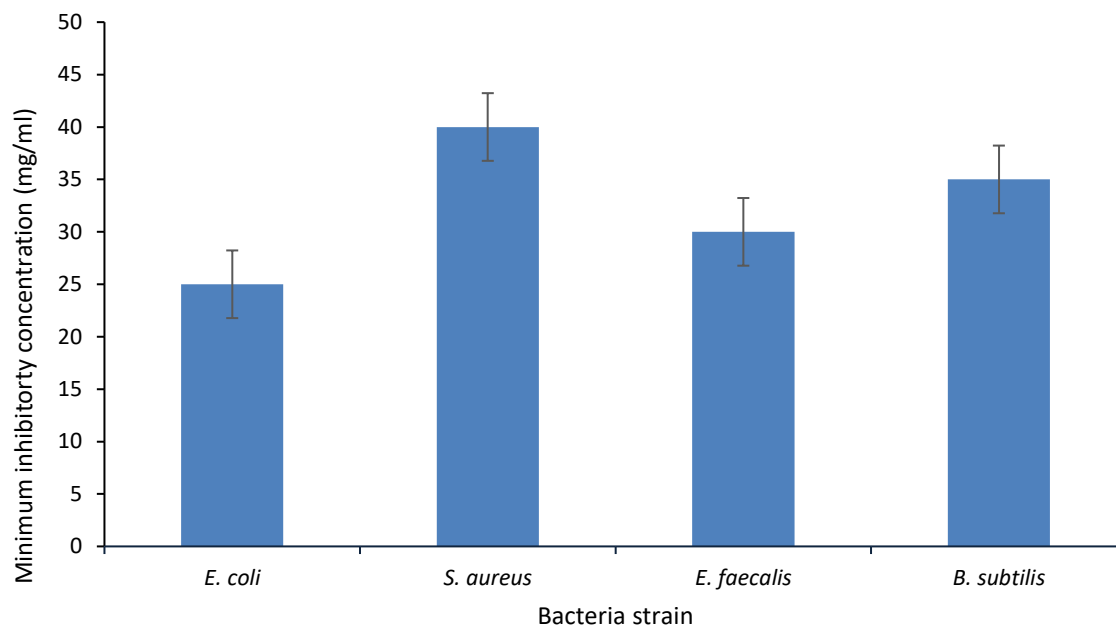


Figure 1. The MIC of ethanol extract of *Senna alata* against bacteria strains

## DISCUSSION

*S. alata* leaf extract has tannins, alkaloids, steroids, glycosides, and terpenoids but no saponins or sesquiterpenes. The results support Yang et al. (2018), who suggested that certain secondary metabolites may only be found in certain plant locations and that environmental stress may impact their abundance. Several agronomic parameters, including the developmental stage of the plant, the specific plant parts, fertilisation, and soil pH, can have an impact on both the quality and quantity of secondary metabolite synthesis. Furthermore, the quantity and makeup of phytochemicals can be

influenced by several factors such as environmental circumstances and genetic factors (Bjorkman et al., 2011). This investigation showed that the scavenging activity of the leaf extract of *S. alata* rose as the concentration of DPPH increased. However, the antioxidant capacity of the leaf extract was reduced compared to ascorbic acid. Antioxidants hinder the process of oxidation in molecules, hence preventing the occurrence of oxidative chain reactions. The oxidative capacity of *S. alata* leaf is likely attributed to the presence of flavonoids, phenolics, tannins, and glycosides inside the leaf. These chemicals, specifically phenolics, demonstrate redox characteristics that are essential for counteracting

free radicals, oxygen singlets, triplets, and degrading peroxides. Phenolic compounds, which have inherent antioxidant properties, can convert DPPH, a stable purple radical, into a colourless state. This indicates that they can donate hydrogen and reduce DPPH to DPPH-H. This phenomenon can be detected by the discolouration of a purple DPPH solution (Asmamaw and Yalemtehay, 2017).

The study discovered that extracts derived from the leaves of *S. alata* showed antibacterial effects against *E. coli* (ATCC28923), *S. aureus* (ATCC28923), *E. faecalis* (ATCC29212), and *B. subtilis* (ATCC6051). The magnitude of the inhibitory zones exhibited variation across the organisms under examination, indicating varying degrees of vulnerability to the plant extracts. Banso et al. (2020) and Banso et al. (2024d) observed that the efficacy of an agent can vary based on the specific species being targeted. Furthermore, Banso et al. (2021) highlighted that the location of the zone of inhibition might be influenced by parameters such as the starting population density, growth rate, and diffusion rate of the antimicrobial agent. The plant extract's various forms suggest its value in medicine, potentially due to phytochemicals, which are key sources of pharmacological compounds (Mann et al., 2008). *S. alata* ethanol leaf extract has the lowest MIC against *Escherichia coli* (ATCC28923) and the highest against *Staphylococcus aureus*. According to Banso et al. (2020), low-activity antimicrobials have higher MIC values than high-efficiency ones. This study suggests that *S. alata* leaf extract chemicals could be employed in chemotherapy.

## CONCLUSION

*S. alata* leaf extracts contain a high concentration of alkaloids, tannins, glycoside steroids, and terpenoids. The concentration of DPPH in the plant leaf extract rises as the extract's concentration increases. The presence of tannins and glycosides in *S. alata* leaf extract might be responsible for its antimicrobial activities. These chemical components found in the extract have the potential to be used as antibacterial agents in the development of novel medication formulations.

## CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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