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Antimicrobial and Antioxidant Activities and Phytochemical Profiling of Crude Extracts from *Senna alexandrina*

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Abstract

This study aimed to evaluate the biological activities and chemical characterization of crude extracts from *Senna alexandrina*. The plant extracts were prepared by sequential maceration of dried leaf powder using solvents of increasing polarity. Their antimicrobial activity was tested against two Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*), two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), and two fungi (*Candida albicans* and *Aspergillus niger*) utilizing the disc diffusion method. Antioxidant activity was measured by evaluating the scavenging of the stable 2,2-diphenyl-1-picrylhydrazyl free radical. Chemical characteristics such as total polyphenolic, flavonoid, and tannin contents were determined through spectrophotometric assays. Overall, the extracts showed stronger antifungal than antibacterial properties. Methanol and n-hexane extracts demonstrated significant antifungal activity with zones of inhibition measuring 25mm for *C. albicans* and 18mm for *A. niger*, respectively. The methanolic extract showed the highest antibacterial activity against *E. coli* with a 13mm zone of inhibition. It also exhibited the highest scavenging radical activity at 56%. Total polyphenolics were predominantly found in the ethyl acetate extract, reaching 136.8 ± 0.03 mg gallic acid equivalent per gram. Flavonoids were most abundant in the ethyl acetate extract with 499.33 mg quercetin equivalent per gram. Except for the methanolic extract of *S. alexandrina*, all extracts lacked tannins. In conclusion, this plant has potential as a valuable source of natural bioactive compounds.

Keywords: *Senna Alexandrina.*, Antimicrobial Activity, Antioxidant Activity; Total Phenolics.

INTRODUCTION

Natural products derived from plants have long been the mainstay for antibiotic development. With the growing acceptance of herbal medicines, the exploration of medicinal plants for novel active compounds has become a crucial avenue for discovering new antibiotic leads (Roy & Dutta, 2021). Senna species, part of the Fabaceae family, are found across the globe (NPGS, 2008). Notably, the tinnevelly senna (*Senna alexandrina* Mill.) is widely used in various laxatives. However, the agronomic characteristics of Senna species are not well-documented, as they have often been regarded as weeds. These species contain several phytochemicals with the potential for use in human medicine (Morris, 2009).

Senna alexandrina, commonly referred to as Senna, is naturally found across a range extending from Mali to Somalia and Kenya in Africa, as well as parts of Asia, including the Arabian Peninsu-

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la, India, and Sri Lanka. This plant is also widely cultivated for commercial purposes in countries such as India, Sudan, Egypt, Pakistan, China, and Korea. Renowned for its high-quality and valuable varieties, this small shrub reaches a height of approximately 2 feet. It features an upright, smooth, pale green stem with long, spreading branches that support leaflets arranged in clusters of four or five pairs. These leaflets are typically around an inch long, lanceolate or obovate in shape, and mucilaginous with a sweetish taste. The distinguishing feature of Senna leaves is their form at the base and the lack of bitterness, setting them apart from angel leaves, which tend to be thicker and stiffer. The plant produces small yellow flowers, with broadly oblong pods measuring approximately 2 inches in length and 7/8 inch in width, each holding around six seeds (Viswanathan & Nallamuthu, 2012).

Senna alexandrina, known for its pods and leaves, has been used as a laxative for centuries due to its strong cleansing properties. Beyond its laxative effect, the leaves are traditionally applied in the treatment of a wide range of ailments including anemia, anorexia, bile disorders, bronchial issues, burns, cancer, cholera, constipation, cramps, skin disorders, dysentery, indigestion, intestinal problems, fever, fungal infections, stomach issues, gonorrhea, gout, bad breath, hemorrhoids, liver problems, herpes, hiccups, infections, jaundice, leprosy, leukemia, fungal diseases, nausea, neural disorders, acne, ringworm, spleen disorders, syphilis, typhoid fever, sexually transmitted infections, viral diseases, as an anti-parasitic agent, and for healing wounds (El-Morsy, 2013). This herb has been used in traditional medicine to treat cholera, liver diseases, constipation, typhoid, and a variety of other ailments (Ahmed et al., 2016). This study aimed to evaluate the biological activities and chemical makeup of crude extracts from *Senna alexandrina.*

MATERIALS AND METHODS

Plant Material

Plant material

Fresh leaves of *Senna alexandrina* were sourced from Khartoum, Khartoum State, in October 2022. The plant underwent authentication by a taxonomist at the Department of Botany, Faculty of Science, University of Khartoum in Sudan. The leaves were carefully washed and dried in the shade to prevent any adverse effects on the phytochemical properties of the desired components. They were then stored in airtight containers at room temperature, ready for future use.

Preparation of extracts

In a separate procedure, 20 grams of dried powdered leaves of *Senna alexandrina* were extracted consecutively through maceration using hexane, chloroform, ethyl acetate, and methanol, with 400 mL of each solvent. This process involved a shaker apparatus for approximately 24 hours at room temperature. Following extraction, the mixture was filtered, and the solvents were evaporated under vacuum with a rotary evaporator. The dry extracts obtained from each sample were weighed and stored at 4°C until needed.

Biological activity

Antimicrobial activity

The bacterial cultures utilized in the study included *Bacillus subtilis* NCTC 8236, *Staphylococcus aureus.,* ATCC 25923, *Escherichia coli.,* ATCC 25922, and *Pseudomonas aeruginosa.,* ATCC 10145 were tested alongside fungal strains *Aspergillus niger*., ATCC 9763 and *Candida albicans.,* ATCC 7596. Each extract, loaded at 10 mg per disc, was assessed using the disc diffusion method as described (Ahmed et al., 2024b).

Antioxidant activity

The antioxidant activity of the extracts was assessed through the in vitro DPPH radical scavenging method, as described (Omer et al., 2024). Concentrations (1, 5, 10, 20, 40, 60, 80, and 100 µg/ml) were prepared by diluting the stock solution with methanol. The assay was conducted using 96-well microtiter plates. Each well received 70 µl of the sample solution, followed by the addition of 140 µl of 0.6×10^6 mol/l DPPH. The mixture was gently shaken and left to incubate for 30 minutes in the dark at room temperature. Absorbance was then measured spectrophotometrically at 517 nm using a microtiter plate reader. Propyl gallate served as the positive control. The DPPH radical-scavenging activity was calculated using the formula: DPPH radical scavenging (%) = [1 - (Ablank – Asample) / Ablank] × 100 Here, Ablank represents the absorbance of the control reaction (with all components except the test sample), and Asample refers to the absorbance of the extract or reference sample.

Quantitative Analysis of Total Polyphenol, Flavonoid, and Tannin Contents

Analysis of Total Polyphenol Content

The total polyphenolic content was measured using the method outlined by (Wolfe et al. 2003).

Analysis of Total Flavonoid Content

The total flavonoid content was assessed by following the procedure described by (Ordonez et al. 2006).

Analysis of Total Tannin Content

The total tannin content was evaluated according to the procedure provided by (Sun et al. 1998)

Statistical analysis

All the procedures for extraction, antimicrobial analysis, and antioxidant studies were repeated in triplicate. The descriptive analysis (mean and standard deviation) was used to discuss the results, assuming the normal distribution of the studied variables.

RESULTS AND DISCUSSION

Yields of crude extracts

The yield of n-hexane, chloroform, ethyl acetate, and methanolic extracts from the leaves of *Senna alexandrina* was assessed. The study involved sequentially macerating dried leaf powder with hexane, chloroform, ethyl acetate, and methanol. In general, the methanol extract showed the highest yield at 6.39%, whereas the ethyl acetate extract had a notably lower yield of 0.49%, as shown in Table 1. According to (Feudjio et al., 2020), methanol's ability to readily penetrate plant cells and dissolve a wide range of bioactive compounds, both polar and many nonpolar, may explain the superior extraction yields observed for the plant. Additionally, (Stalikas, 2007), highlighted that factors such as the type of plant parts used, storage duration, and temperature can also influence yield percentages.

Biological activities

Antimicrobial activities

The plant demonstrated stronger antifungal activity than antibacterial activity. The methanol and chloroform extracts showed the highest antifungal effects, inhibiting *C. albicans* with a 25mm zone of inhibition and *Aspergillus niger* with 18mm. In terms of antibacterial action, the n-hexane and methanolic extracts were most effective, both influencing *Staphylococcus aureus* and *Escherichia coli* with a 13mm zone of inhibition. Comparing these antimicrobial findings for *S. alexandrina* to earlier studies, it is consistent with (Vijaya Sekhar et al. 2016), which also reported that the methanolic extract exhibited strong antifungal properties.

Antioxidant activity

The antioxidant activity of extracts from the plant was assessed by examining their ability to scavenge DPPH free radicals, with results detailed in Table 3. The findings indicated that the methanolic extract exhibited the highest antioxidant activity at 56%, while ethyl acetate and hexane extracts showed lower activities at 8% and 3%, respectively, and the chloroform extract was inactive. Antioxidant activity is associated with phenolic (Thouri et al., 2017) and flavonoid (Kobus-Cisowska et al., 2019) compounds. Prior studies, including those by Ahmed et al. (2016), have also emphasized differences in antioxidant activity levels. The findings from this experiment align with and are supported by earlier research, such as the work of Akloul et al. (2014). Typically, a substance dissolves more easily in a solvent that shares a similar polarity (Wibisono et al., 2020).

Assessment of Total Polyphenolic, Flavonoid, and Tannin Content

The total polyphenolic content in the hexane, chloroform, ethyl acetate, and methanol extracts of *Senna alexandrina* was evaluated. The findings indicated that ethyl acetate extract had the highest concentration of polyphenolics, with 136.8 mg gallic acid equivalent (GAE) per gram. In contrast, most extracts showed a higher concentration of flavonoids, measured at 499.33 mg quercetin equivalent per gram. Notably, all extracts, except for the methanol extract from *Senna alexandrina*, were free of tannins.

Variations in the polyphenolic and flavonoid content of this studied species compared to values reported in previous literature could be linked to factors such as geographical locations and the climate conditions under which the plant grows (Khurm et al., 2020). Several researchers have noted a significant correlation between phenolic content and the antioxidant activity of extracts (Roy & Dutta, 2021). Despite their high phenolic content, these extracts exhibited little to no significant antiradical activity, indicating that the phytoconstituents present may lack potent antiradical properties. The study's calculated results for total flavonoid content in the solvents ethanol 96%, methanol, and ethyl acetate were 3.741%, 5.629%, and 7.492%, respectively. Similarly, the total phenolic content in ethanol 96%, methanol, and ethyl acetate solvents was recorded at 14.084%, 13.257%, and 12.007%, respectively (Ahmad et al., 2024a).

Table (1). Yields of crude extracts:

Plant	Yield (%)			
	N-Hexane	Chloroform	Ethyl acetate	Methanol
S. alexandrina	1.77	3.43	0.49	6.39

Table (2). Antimicrobial activity of extracts of *Senna alexandrina*

Botanical name	Extract	Inhibition zones diameter(IZD) in (mm)					
		B. subtitles.	*S.aureus*	*E.coli*	*P.aeruginosa*	*A.niger*	*C.albicans.*
S. alexandrina	N-hexane	NA	13 ± 0.1	11 ± 0.1	11 ± 0.4	17 ± 0.6	12 ± 0.8
	Chloroform	11 ± 0.3	NA	NA	NA	18 ± 0.6	NA
	Ethyl acetate	NA	NA	NA	NA	NA	NA
	Methanol	NA	11 ± 0.1	13 ± 0.8	11 ± 0.4	17 ± 0.8	25 ± 1.4
Gentamicin*	10µg/disc	23±0.01	28±0.01	23±0.02	23±0.0	-	-
Nystatin*	10µg/disc	-	-	-	-	22±03	20±.00

NA: not active,*positive control (10µg/disc). IZD (mm): >18mm:Sensitive: 14- 18mm: intermediate: <14mm: Resistant.

Table (3). Antioxidant Activities of *Senna alexandrina:*

Plant	Extract	RSA±SD(DPPH)
Senna alexandrina	N-h	3±0.04
	C-h	IA
	E-a	8±0.04
	MetH	56±0.01
Stander	PG	96±0.01

Key: IA= inactive, N-h: N- hexane, C-h: Chloroform, E-a: Ethyl-acetate, MetH: Methanol, PG: Propyl gallate.
SD: Standard Division,

Table (4). Total polyphenolic, flavonoids, and tannins contents in extracts of *Senna alexandrina.,*

Plant	Phenols (Y=0.005X+0.001) (mg GAE/g), R_2= 0.998	Flavonoids (Y=0.0012X+0.0958) (mg QE/g), R_2=0.0992	Tannin (Y=0.002X+0.590) (mg TAE/g), R_2=0.998
Hexane	0.00	262.33 ± 0.01	0.00
Chloroform	0.00	152.66 ± 0.11	0.00
Ethyl acetate	136.8 ± 0.03	499.33 ± 0.34	0.00
Methanol	78.0 ± 0.03	431.33 ± 0.06	14 ± 0.03

GAE: Gallic acid equivalent; QE: Quercitin equivalent; TAE: Tannic acid equivalent.

CONCLUSIONS

The extracts from *Senna alexandrina.,* displaying varying polarities, demonstrated diverse antimicrobial and antioxidant capabilities. The inhibitory zones varied depending on the type of microorganism being tested, with the extracts generally showing stronger antifungal activity than antibacterial. It is recommended to pursue further research to identify the specific phytochemicals responsible for these antimicrobial and antioxidant effects, alongside understanding their pharmacological mechanisms. Additionally, the potential for other beneficial biological activities such as anticancer, antimalarial, antiviral, and anti-inflammatory properties should be explored.

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