مجلة الدراسات التربوية والعلمية - كلية التربية - الجامعة العراقية
العدد الخامس والعشرون - المجلد الثاني عشر - علوم الحياة - آب 2025 م
doi.org/10.52866/esj.2023.12.25.15

Antimicrobial study of *Senna alata* green and dried leaf extracts

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ABSTRACT:

This study aimed to investigate the chemical composition and antimicrobial activity for *Senna alata* leaf by using an alcohol solvent, in both the green and dry leaf states. **FTIR spectroscopy** was performed to identify the functional groups present in the extracts. The results revealed the presence of bioactive compounds such as phenols, carboxylic acids, and alkanes, suggesting the potential therapeutic properties of this plant. **Antimicrobial activity** was evaluated using the minimum inhibitory concentration **(MIC)** and minimum lethal concentration (**MBC**) assays. The results showed that both the green and dry extracts possessed significant antimicrobial activity, with efficacy varying depending on leaf condition and extract concentration. These findings suggest that Senna slant is a promising source of natural antimicrobial compounds and could be used in future therapeutic and pharmaceutical applications.

KEYWORDS: Candle bush**,** alcohol extraction**,** Antimicrobial activity**.**

دراسة مضادات الميكروبات لمستخلصات أوراق السنا المجففة والخضراء

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مستخلص:

هدفت هذه الدراسة إلى دراسة التركيب الكيميائي والنشاط المضاد للميكروبات لمستخلص أوراق السنا المائل باستخدام مذيب كحولي، في كل من حالتي الأوراق الخضراء والجافة. أُجريت تقنية التحليل الطيفي بالأشعة تحت الحمراء (FTIR) لتحديد المجموعات الوظيفية الموجودة في المستخلصات. كشفت النتائج عن وجود مركبات فعاله بيولوجيًا مثل الفينولات والأحماض الكربوكسيلية والألكانات، مما يشير إلى الخصائص العلاجية المحتملة لهذا النبات. كما تم تقييم النشاط المضاد للميكروبات باستخدام اختباري التركيز المثبط الأدنى (MIC) والتركيز القاتل الأدنى (MBC). أظهرت النتائج أن كلاً من المستخلصين الأخضر والجافة يمتلكان نشاطًا مضادًا للميكروبات ملحوظًا، مع اختلاف الفعالية تبعًا لحالة الورقة وتركيز المستخلص. تشير هذه النتائج إلى أن ممكن اعتباره مصدر واعد للمركبات الطبيعية المضادة للميكروبات، ويمكن استخدامه في التطبيقات العلاجية والصيدلانية المستقبلية.

الكلمات المفتاحية: نبات الشمعدان ، الاستخلاص الكحولي ، النشاط المضاد للميكروبات.

Introduction

Medicinal plants are have distinctive therapeutic properties which used to treat a variety of health conditions. These plants contain bioactive compounds that can promote healing and improve overall health, making them essential in traditional medicine and modern pharmacology. (Smith and Johnson, 2023). Iraq is known for its great diversity of medicinal plants, due to its unique geographical location and varied climatic conditions. It is located at the crossroadsof three continents: Asia, Africa, and Europe, resulting in a great diversity of plants growing within its borders (Hussein and Ali, 2022).

However, several studies have evaluated compounds within medicinal plants that act as antibiotics. The plant *senna alata*, also known as Candle bush is an ornamental shrub widely grown throughout different region of the globe especially during the dry season. Its leaves are traditionally used to treat several infectious diseases, including ringworm and parasitic skin diseases (Hameed, 2018 and Hussain, 2018). Also, fresh of Senna leaf juice is known worldwide by local healers as a remedy for parasitic and fungi skin diseases (Idu *et al*.,2006 and Makinde *et al*., 2007). Fourier transform infrared (FTIR) analysis was performed to by studying its infrared absorption. FTIR analysis is used in botanical studies to detect the active components for plant extracts, its helping to understand their biological and medicinal properties. The biological activity of the plant extracts was evaluated using different types of bacteria and fungi to determine their ability to inhibit or combat microbes, which contributes to determining their effectiveness as natural antibiotics.

Materials and Methods

Plant collection

The candle bush leaves were collected from the Mustansiriyah University campus in Baghdad during the period of September 2024 after the identification by plant taxonomist Dr. Hadeel Radawi Hussein, Curator of the Mustansiriyah University Herbarium (MUST). When the leaves were collected, they underwent thorough disinfection process using tap water, fol-

lowed by a second rinse with distilled water.

Then, the leaves were left to dry in a shaded area for a maximum of seven days. The dried leaves were ground into a fine by using a grinder and stored for later use. (Ayodele *et al*., 2015).

Plant extracts preparation (Cold alcoholic extract (Maceration)

To prepare the cold alcoholic extract, 100 g of dried and 125g of green leaves were placed in a beaker. Then 500 ml of ethanol (99%) was added to the beaker (separately) (Khoddami *et al*., 2013). The beaker was placed in a shaking incubator equipped with a shaker for 24 h at 37 °C. After that, the alcoholic extracts were filtered. The first filtration was done using a Buchner funnel with a piece of gauze, and the second filtration was done using Whatman No. 1 filter paper. The liquid passed through the filter at 2500 rpm for 15 min. The liquid was then kept at 40-45 °C for 48-72 h to remove moisture and obtain a dry extract. The dried extracts f both green and dried leaves were stored in the refrigerator at 4 °C until needed. Plant extracts used to compare the efficiency of the antimicrobial and biochemical properties

Fourier transform infrared spectroscopy FTIR to examine leaves extracts

Plant extracts were by using Fourier transform infrared spectroscopy at the Ibn Al-Baytar Center, Research and Development Authority, Ministry of Industry and Minerals. This technique was used to identify functional groups and active components in plants. The Folin-Ciocalteu method was used to determine UV/vis spectroscopy at 760 nm. Gallic acid was also present and used as a reference to construct a calibration curve that allowed for the determination of total phenols. The extract was spread in to the surface of an ATR (Attenuated Total Reflectance) crystal. Fourier transform infrared spectroscopy (Bruker Vertex) analysis of the dry extract surface was then performed. Infrared light was transmitted through the sample to obtain the corresponding spectrum, which was averaged across multiple data acquisitions. Infrared spectra were acquired in the wavenumber range of 400–4000 cm. The crystal surface was cleaned after each measurement. It was cleaned with

deionized water and dried with a soft cloth (Ni'matul Izza *et al*., 2018).

Microorganism isolates

Antimicrobial activity test was conducted by using four pathogenic bacterial strains: *Staphylococcus aureus*, *Staphylococcus epidermidis, Acinetobacter*, *Escherichia coli*, and one fungal species: *Candida albicans*. The pathogenic strains were obtained from the Advanced Bacteriological Studies Laboratory in the Department of Biology, College of Science, Mustansiriyah University.

Minimal inhibitory concentration (MIC), minimal bactericidal concentration (MBC)

Resazurin (7-hydroxy-3H-phenoxazin-3-one, 10-oxide) is a blue dye used in MIC testing. First, 0.015 g of the dye was dissolved in 10 ml of distilled water, and the solution was then filtered using 47 mm filter paper with a nominal pore size of 22 µm. Preparing the dye for laboratory use requires great care and precision to ensure accurate results. After filtration, the filtrates were transferred to clean glass bottles to prevent contamination. To ensure long-term viability, the solution was refrigerated in the dark at 4 °C for up to two weeks (Dai et al., 2020). Resazurin dye reduction is widely used to determine bacterial viability and metabolic activity (Sychev et al., 2023). Resazurin is added to a growth medium, and as the bacteria grow and metabolize, the dye is reduced, resulting in a color change from blue to pink. This change can be imaged and quantified to assess bacterial growth and metabolic activity under various conditions. Ten concentrations (100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39, and 0.19) of the substance were added to each tube, while the extracts were passed through a series of opaque tubes until they reached the minimum concentration that inhibits bacterial growth (MIC). The MBC test was initially performed by selecting tubes that showed no growth during the MIC determination. A complete inoculation loop from each tube was placed on extract free agar plates and incubated at 37°C for a further 24 hours. This minimum concentration, at which no growth was observed, was considered the MBC.

Results & Discussion:

Functional groups related to the bioactive constituents of the raw (fresh and dry) leaves of *Senna alata* were analyzed by FTIR spectroscopy. Tables 1–2 & Figures 1–2 summarize the specters and main functional groups. Fig. FTIR analysis of dry Leaves (Fig 1 and Table 1)

Broad O−H stretching band (3325, 3335 cm^{-1}), which are typically representative of hydroxyl groups in phenols or alcohols that are connected to antioxidant activity (Okafor et al., 2022).

C-H stretching at 2924–2935 cm^{-1} indicated the presence of aliphatic hydrocarbons, as confirmed by the presence of C=O stretching at 1648–1652 cm^{-1} belong to the carbonyl group of ketones or carboxylic acids, which is featured for their antimicrobial property (Gupta et al., 2021). The absorption peaks indicated flavonoids and polysaccharides at 1517–1525 cm^{-1} and 1030–1040 cm^{-1} corresponding to aromatic C=C stretching and C-O bonds (Rahman et al., 2022; Mazumder et al., 2020), respectively, which is related to the inhibition of inflammation and immune-modulatory effects. FTIR of Fresh Leaves (Fig. 2, Table 2) , FTIR analysis of fresh leaves represented a strong peak at 3367 cm−1, which is associated with secondary amines and N-H stretching. 2, Table 2). Functional groups were similar as well but significantly different peak intensities were also observed in fresh leaf extracts. Increased transmission in the C-O region (1030–1040 cm^{-1}) indicated a higher polysaccharide content of C.O-M than that in dry leaves, which may improve bioavailability (Tran et al., 2023). This sharper C=O peak (1648–1652 cm^{-1}), suggests a relatively high concentration of antimicrobial carbonyl compounds.

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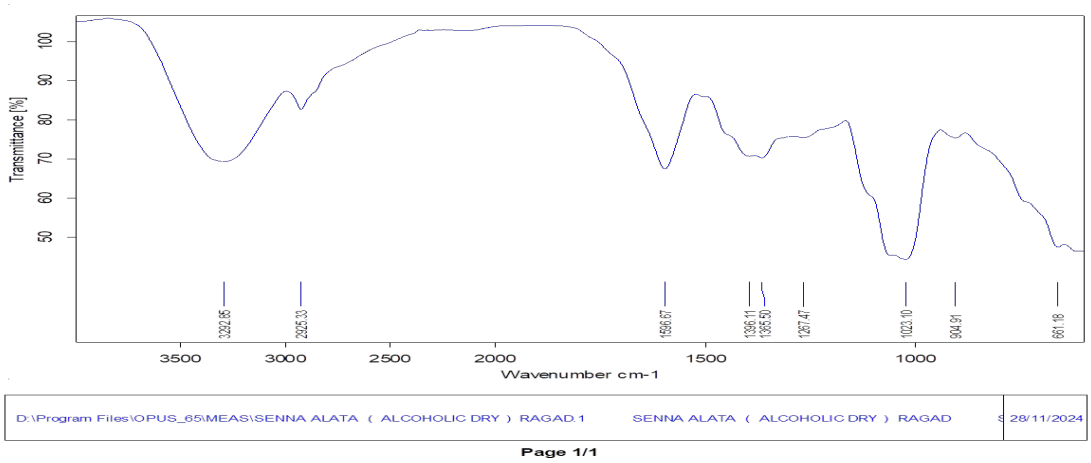

Fig. (1) FTIR spectroscopic analysis of alcoholic of *Senna alata* dry leaves

Table No. (1) Functional group and their quantitative frequencies for cold alcoholic extract of Senna alata dry leaves

Wavenumber (cm^{-1})	Type of Bond	Functional Group	Assignment/Indication
3325-3335	O-H Stretching	Alcohols, Phenols	Alcohols, Phenols
2924-2935	C-H Stretching	Alkanes	Aliphatic compounds (alkanes)
1648-1652	C=O Stretching	Ketones, Aldehydes, Acids	Carbonyl group (aldehydes, ketones, or carboxylic acids)
1517-1525	C=C Stretching	Aromatic Compounds	Aromatic compounds (flavonoids, phenols)
1400-1380~	CH_3	Methyl	Alkanes or aromatic compounds
1030-1040	C-O stretching	Alcohols, Ethers, Polysaccharides	Indicates carbohydrate derivatives or plant polysaccharides

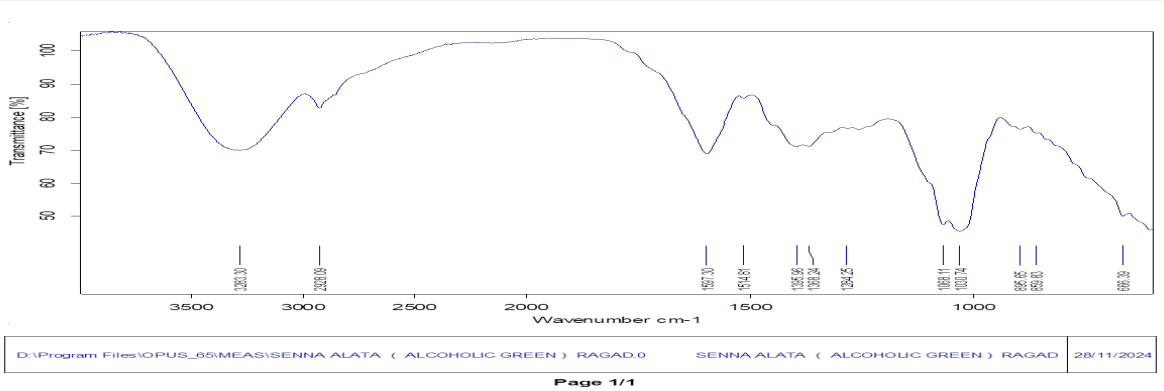

Fig. (2) FTIR spectroscopic analysis of alcoholic of *Senna alata* fresh leaves

Table No. (2) Functional group and their quantitative frequencies for cold alcoholic extract of Senna alata fresh leaves

Wavenumber (cm^{-1})	Type of Bond	Functional Group	Assignment/Indication
3325-3335	O-H Stretching	Hydroxyl (Alcohols, Phenols	Indicates hydrogen bonding, com-.mon in phenolic or water content
2924-2935	C-H Stretching	Alkanes	(Aliphatic compounds (alkanes
1648-1652	C=O Stretching	Ketones, Aldehydes, Carboxylic Acids	Carbonyl group
1517-1525	C=C Stretching	Aromatic Rings	Aromatic ring vibrations
1030-1040	C-O Stretching	Alcohols, Ethers, Esters	Presence of C-O bonds in esters or polysaccharides

The peaks indicated to enhanced ransmission for specific wavenumbers. Absorption of those wavelengths is indicated by dips. These spectra exhibit several well-defined absorption peaks in the low- to mid-wavenumber region. Generally observed bands are attributed to the functional groups which suggests the presence of hydroxyl (O-H), carbonyl (C=O), or aromatic nature. It indicates the identification of the alcoholic extract functional groups of Senna alata. Infrared spectroscopy usually identifies phytochemicals, including flavonoids, phenols, and alkaloids. The bands observed between3200–3600 cm^-1 could be attributed to the stretching of hydroxyl (O H) groups caused by alcohols and phenols, which are common in plant extracts. In aromatic compounds, the C=C stretching can be seen around 1600 cm^1. At 1000–1300 cm^-1 we expect the C–O stretch, which means alcohols or ethers (Coates.2000: Pavia *et al*.,2014). This spectral analysis is effective for: Phytochemical screening of *Senna alata* confirming its health benefits (e.g., antioxidant or antimicrobial activity) which are often associated with its chemical composition.

Minimal inhibitory concentration (MIC), minimal bactericidal concentration(MBC) The medicinal potential of alcoholic

extracts for Senna alata fresh leaves exquisite antimicrobial activity against Gram-positive and Gram-negative bacteria, proved by Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values (Table3&4). *Acinetobacter* spp. was the most susceptible strain. The most susceptible bacteria to the extracts was *Acinetobacter pneumonia* (MIC = 3 mg/mL; MBC = 2 mg/mL), followed by *Escherichia coli* (MIC = 4 mg/mL; MBC = 3 mg/mL), while *Staphylococcus aureus* (MIC = 8 mg/mL; MBC = 6 mg/mL) and *Staphylococcus epidermidis* (MIC = 7 mg/mL; MBC = 6 mg/mL) relatively less susceptible. The ratio of minimum bactericidal concentration (MBC) to minimum inhibitory concentration (MIC) (all strains were identified with the ratio <2) confirms that the extract led to such a strong bactericidal effect that pathogens were killed, not only inhibited from growing. Significantly, the enhanced activity observed against Gram-negative bacteria (*E. coli and Acinetobacter*) can be explained potential for disrupting Gram-negative outer membranes, a mechanism that has recently been evidenced in studies using terpenoids and flavonoids from plants (Okafor et al., 2022; Rahman et al., 2022). This is especially critical for treatment of multidrug-resistant *Acinetobacter*, which is encountered most commonly in hospital-acquired pneumonia and sepsis in increasingly rising numbers (Vijayakumar et al., 2020; Gupta *et al*., 2021).

Staphylococcus spp., the most common pathogens in skin infections, have higher MIC values, indicating that the extract would need to be used at higher concentrations to inhibit these Gram-positive bacteria, although the extract's bactericidal activity (MBC = 6 mg/mL) is also promising. This activity is probably due to the bioactive phytochemicals present in *Senna alata*, such as anthraquinones and phenolic acids that damage bacterial membrane or inhibit some important enzymes (El-Mahmood, 2009). These findings are consistent with previous studies that reported MIC values between 3–8 mg/mL for extracts of *Senna* against similar pathogens (Awal *et al*., 2004), although some variations may exist due to the different extraction methods

or origins of the plants. Recent work by Tran *et al*. therapeutics (2023) strengthen the observation that *S. alata* extracts used as adjunct therapy in combination with antibiotics can aid in fighting methicillin-resistant Staphylococcus aureus (MRSA), subverting antibiotic resistance.

Overall, these results indicate the potential of Senna alata as a source of antimicrobial compounds with activity against drug resistant Gram-negative strains. In addition, in future should be work isolate the active compounds, evaluate the in vivo efficacy and delves into synergy with conventional antibiotics before therapeutic applications (Adetunji *et al*., 2020 Alzomor *et al*., 2023)..

Senna alata dry leaves (alcoholic extracts)	MIC	MBC
*Staphylococcus aureu*s	4	3
Staphylococcus epidermidis	8	6
Escherichia coli	7	6
*Acinetobacte*r	5	3
Candida albicans	9	8

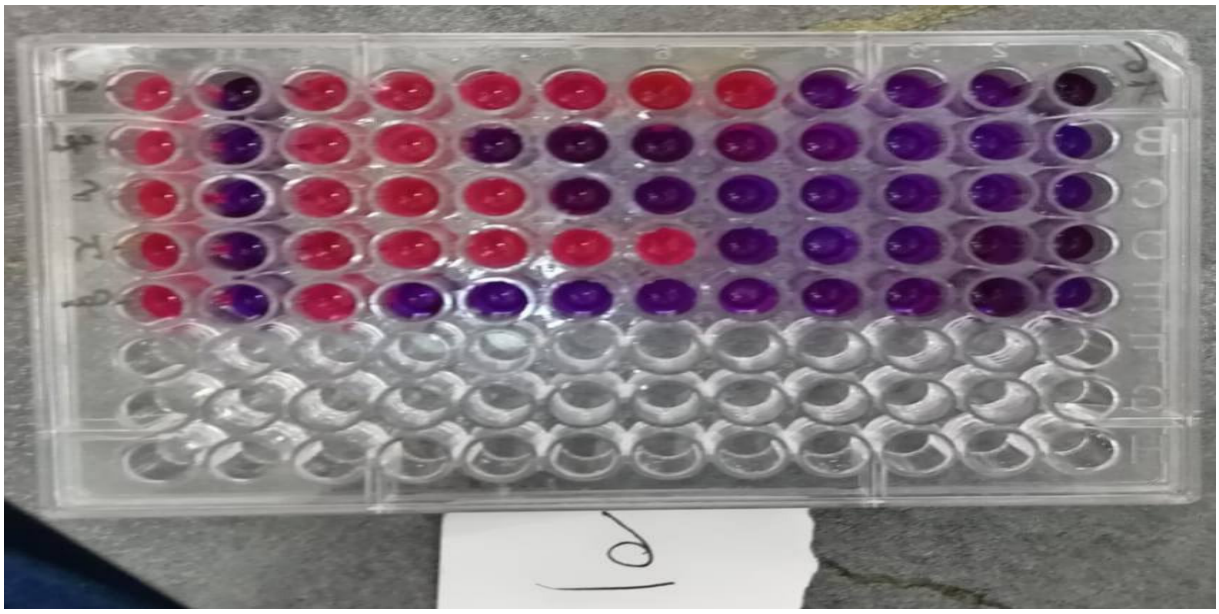

Fig. (3) (MIC) and (MBC) of *Senna alata* dry leaves

Table No. (4) (MIC) and (MBC) of *Senna alata* fresh leaves

Senna alata fresh leaves (alcoholic extracts)	MIC	MBC
Staphylococcus aureus	8	6
Staphylococcus epidermidis	7	6
Escherichia coli	4	3
Acinetobacter	3	2
Candida albicans	7	5

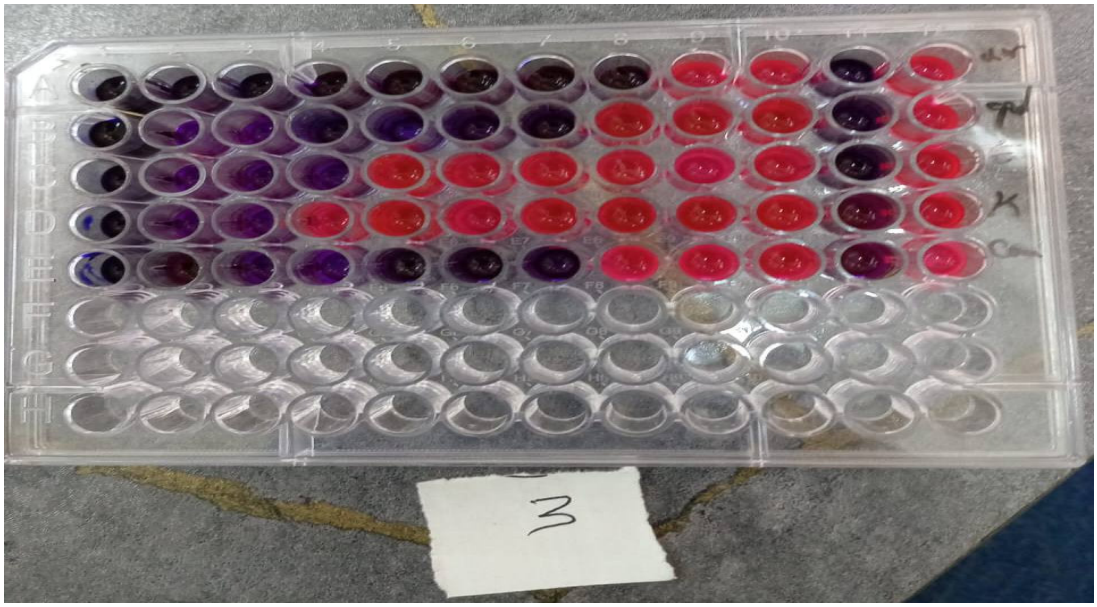

Fig. (4) (MIC) and (MBC) of *Senna alata* fresh leaves

Conclusion

Senna alata bioactive compounds (phenols, carboxylic acids) from leaf extracts (green/dry) showed antimicrobial activity, as revealed in this study. Green extract was more effective against Gram-negative bacteria (*E. coli*, *Acinetobacter spp.*), while dry extract was more active against Gram-positive bacteria (*Staphylococcus spp.*), emphases its merit as a natural antimicrobial source for pharmaceuticals.

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