WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
Coden USA: WJPRAP Impact Factor 8.63
Volume 15, Issue 17, 1030-1039. Research Article ISSN 2277–7105

PHYSICOCHEMICAL STANDARDIZATION, PHYTOCHEMICAL PROFILING, AND BROAD-SPECTRUM ANTIBACTERIAL ACTIVITY OF *CASSIA ANGUSTIFOLIA*

Md. Al Amin Pramanik[1], Md. Fazle Rabbi Lemon[2], Md. A. Razzak[3], Mohammad Abu Bin Nyeem[4]*, Md. Abdul Mannan[4]

[1]Department of Microbiology, Jagannath University, Dhaka-1100.
[2]Department of Unani Medicine, Hamdard University Bangladesh, Munshiganj, Bangladesh.
[3]Assistant Professor, Hamdard Unani Medical College & Hospital, Bogra, Bangladesh.
[4]Associate Professor, Department of Unani Medicine, Hamdard University Bangladesh, Munshiganj, Bangladesh.

Article Received on 04 July 2026,
Article Revised on 24 August 2026,
Article Published on 01 Sept. 2026,

https://doi.org/10.5281/zenodo.22160594

***Corresponding Author**
Mohammad Abu Bin Nyeem
Associate Professor, Department of Unani Medicine, Hamdard University Bangladesh, Munshiganj, Bangladesh.

How to cite this Article: Md. Al Amin Pramanik[1], Md. Fazle Rabbi Lemon[2], Md. A. Razzak[3], Mohammad Abu Bin Nyeem[4]*, Md. Abdul Mannan[4]. (2026). Physicochemical Standardization, Phytochemical Profiling, And Broad-Spectrum Antibacterial Activity of Cassia Angustifolia. World Journal of Pharmaceutical Research, 15(17), 1030-1039.

ABSTRACT

Cassia angustifolia (Fabaceae), commonly known as Senna is a medicinal plant extensively used in traditional systems of medicine for gastrointestinal and inflammatory disorders. The present study investigated the physicochemical characteristics, qualitative phytochemical composition, and antibacterial activity of the methanolic leaf extract of *C. angustifolia* against clinically important Gram-positive and Gram-negative bacteria. Physicochemical parameters including moisture content, total ash, acid-insoluble ash, water-soluble ash, alcohol-soluble extractive value, pH, swelling index, and bulk density were determined following standard pharmacognostic procedures. Preliminary phytochemical screening was carried out using established qualitative tests for major secondary metabolites. Antibacterial activity was evaluated using the Kirby-Bauer agar disc diffusion method against *Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus pyogenes*. The physicochemical analysis showed acceptable quality parameters, including moisture content (9.22% w/w), total ash (7.81% w/w), alcohol-soluble extractive value (13.63% w/w), and pH (6.35). Phytochemical analysis confirmed the presence of alkaloids, flavonoids, tannins, phenolic

compounds, saponins, cardiac glycosides, terpenoids, steroids, and carbohydrates. The methanolic extract exhibited concentration-dependent antibacterial activity, with inhibition zones of 20 mm against *E. coli*, 21 mm against *S. aureus*, and 16 mm against *S. pyogenes* at 1000 µg/disc. The findings suggest that *C. angustifolia* possesses significant phytochemical richness and broad-spectrum antibacterial potential. The integration of physicochemical and biological evaluations provides a scientific basis for the authentication, standardization, and therapeutic application of *C. angustifolia* as a promising source of natural antibacterial agents.

KEYWORDS: *Cassia angustifolia*; physicochemical analysis; phytochemical screening; antibacterial activity.

INTRODUCTION

Medicinal plants remain an indispensable source of bioactive compounds for the discovery of novel therapeutic agents and pharmaceutical products. According to the World Health Organization (WHO), a substantial proportion of the global population relies on plant-based medicines for primary healthcare, emphasizing the importance of scientifically validating traditional medicinal plants (World Health Organization [WHO], 2013). Among these medicinal plants, *Cassia angustifolia* (syn. *Senna alexandrina* Mill.), commonly known as Indian senna, belongs to the family Fabaceae (Thaker et al., 2023) and has long been used in Unani, Ayurvedic, and traditional systems of medicine for the treatment of constipation, hemorrhoids, intestinal disorders, and inflammatory conditions (Ahmad et al., 2023).

The pharmacological properties of *C. angustifolia* are primarily attributed to anthraquinone glycosides, particularly sennosides A and B, which stimulate colonic motility and facilitate bowel evacuation (Dhangar et al., 2024). In addition to anthraquinones, the plant contains flavonoids, tannins, alkaloids, phenolic compounds, saponins, terpenoids, and steroids, which have been associated with antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, and other pharmacological activities (Harborne, 1998; Kokate et al., 2010). Physicochemical and phytochemical standardization of medicinal plants is essential for quality control, authentication, and reproducibility of herbal formulations (Khare et al., 2017; Singanaboina & Chinna, 2018).

The increasing prevalence of antimicrobial resistance among pathogenic bacteria such as *Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus pyogenes* has created an urgent

need for alternative antibacterial agents derived from natural sources (WHO, 2014). Plant secondary metabolites, including flavonoids, phenolic acids, tannins, and saponins, exhibit antibacterial activity through mechanisms such as disruption of bacterial membranes, inhibition of protein synthesis, interference with nucleic acid replication, and suppression of quorum sensing pathways (Cushnie & Lamb, 2005; Patil et al., 2020).

Although previous studies have reported either the phytochemical composition or antibacterial activity of *C. angustifolia*, integrated investigations combining physicochemical standardization, phytochemical profiling, and antibacterial evaluation remain limited. Therefore, the present study was designed to comprehensively evaluate the physicochemical characteristics, qualitative phytochemical constituents, and broad-spectrum antibacterial activity of the methanolic leaf extract of *C. angustifolia* against clinically important Gram-positive and Gram-negative bacterial pathogens.

MATERIALS AND METHODS

Plant Material Collection and Authentication

Fresh leaves of *Cassia angustifolia* were collected from Dhaka, Bangladesh, during March 2023. The plant material was authenticated by a qualified taxonomist, and a voucher specimen was deposited in the departmental herbarium for future reference.

Preparation of Methanolic Extract

The collected leaves were washed thoroughly with distilled water, shade-dried at room temperature, and pulverized into coarse powder using a mechanical grinder. Approximately 100 g of powdered leaf material was macerated with 100 mL methanol for 48 h at room temperature with intermittent stirring (Marjhan et al., 2024).. The extract was filtered through Whatman No. 1 filter paper and concentrated at 40°C using a water bath. The crude methanolic extract was stored in airtight amber-colored containers at 4°C until further analysis.

Physicochemical Analysis

The powdered leaves of *C. angustifolia* were subjected to physicochemical evaluation according to WHO guidelines for medicinal plant materials (WHO, 2011) and established protocols (Haruna et al., 2023; AOAC, 2006; De & Sharma, 2023; Khandelwal, 2008; Kokate et al., 2010). Moisture content (loss on drying), total ash, acid-insoluble ash, water-soluble ash, alcohol-soluble extractive value, pH, swelling index, and bulk density were determined.

Qualitative Phytochemical Screening

Preliminary phytochemical analysis was performed using standard methods described by Harborne (1998), Trease and Evans (2009), and Kokate et al. (2018). The extract was screened for alkaloids (Mayer's, Wagner's, Dragendorff's, and Hager's tests), flavonoids (Shinoda test), tannins and phenolic compounds (ferric chloride test), saponins (froth test), cardiac glycosides (Keller-Killiani test), terpenoids (Salkowski test), steroids (Liebermann-Burchard test), carbohydrates (Molisch's test), and proteins (Biuret test).

Bacterial Strains

Clinical isolates of *E. coli*, *S. aureus*, and *S. pyogenes* were obtained from the Microbiology Laboratory of Dhaka Medical College, Bangladesh. The bacterial cultures were maintained on appropriate media and adjusted to 0.5 McFarland turbidity before antibacterial testing.

Antibacterial Assay

Antibacterial activity was evaluated by the Kirby-Bauer agar disc diffusion method following NCCLS (2006) guidelines. Methanolic extract discs containing 250, 500, and 1000 µg/disc were tested against each bacterial strain. Amikacin (30 µg/disc) served as the positive control. The inoculated plates were incubated at 37°C for 16-18 h, and the diameters of inhibition zones were measured in millimeters.

RESULTS

Physicochemical Analysis

The physicochemical characteristics of powdered *C. angustifolia* leaves are presented in Table 1.

Table 1: Physicochemical analysis of powdered leaves of *Cassia angustifolia*.

Parameter	Value
Moisture content (Loss on Drying)	9.22% w/w
Total Ash Value	7.81% w/w
Acid-Insoluble Ash	1.30 % w/w
Water-Soluble Ash	2.83 % w/w
Alcohol-Soluble Extractive Value	13.63 % w/w
pH	6.35
Swelling Index	3.57 mL/g
Bulk Density	0.70 g/mL

The low moisture content and ash values indicate good quality and minimal inorganic contamination, while the relatively high alcohol-soluble extractive value suggests efficient extraction of ethanol-soluble bioactive constituents.

Qualitative Phytochemical Screening

The methanolic leaf extract showed the presence of multiple classes of secondary metabolites (Table 2).

Table 2: Qualitative phytochemical screening of methanolic leaf extract of *Cassia angustifolia.*

Phytochemical Test		**Methanol**
Alkaloids	Mayer's test	+
	Wagner's test	+
	Dragendorff's test	+
	Hager's test	+
Flavonoids	Shinoda test	+
	Alkaline reagent test	+
	Ferric chloride test	+
	Lead acetate test	+
Tannins	Ferric chloride test	+
	Gelatin test	+
	Lead acetate test	+
	Potassium dichromate test	+
Saponins	Froth test	+
	Foam test	+
Glycosides	Keller–Killiani test	+
	Legal's test	+
	Bornträger's test	–
Phenolic compounds	Ferric chloride test	+
	Folin–Ciocalteu test	+
	Ellagic acid test	+
Terpenoids	Salkowski test	+
	Liebermann–Burchard test	+
	Copper acetate test	+
	Vanillin–sulfuric acid test	+
Steroids	Liebermann–Burchard test	+
	Salkowski test	+
Carbohydrates	Molisch's test	+
	Benedict's test	+
Proteins	Biuret test	-
	Ninhydrin test	-
	Xanthoproteic test	-

(+ = Present; - = Absent)

Antibacterial Activity

The methanolic extract exhibited concentration-dependent antibacterial activity against all tested bacterial strains (Table 3).

Table 3: Antibacterial activity of methanolic extract of *Cassia angustifolia.*

Sample	Concentration (µg/disc)	*E. coli* (mm)	*S. aureus* (mm)	*S. pyogenes* (mm)
MECA1	250	11	11	10
MECA2	500	16	15	12
MECA3	1000	20	21	16
Amikacin	30	12	16	10

The highest antibacterial activity was observed at 1000 µg/disc, producing inhibition zones of 20 mm against *E. coli*, 21 mm against *S. aureus*, and 16 mm against *S. pyogenes*.

DISCUSSION

The present study provides an integrated evaluation of the physicochemical properties, phytochemical composition, and antibacterial potential of *Cassia angustifolia* leaves. The physicochemical parameters obtained fall within acceptable limits for medicinal plant materials and may serve as reference standards for quality control and authentication of *C. angustifolia* leaf powder (WHO, 2011). The relatively low moisture content (9.22% w/w) suggests enhanced storage stability and reduced susceptibility to microbial deterioration, while the total ash value (7.81% w/w) reflects the overall mineral content of the plant material.

Qualitative phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, phenolic compounds, saponins, cardiac glycosides, terpenoids, steroids, and carbohydrates. These findings are consistent with previous reports on *Cassia* species, which have documented abundant anthraquinones, flavonoids, tannins, and glycosides as major bioactive constituents (Verma et al., 2021; Kokate et al., 2018). Flavonoids and phenolic compounds are known to possess potent antioxidant and antibacterial properties through inhibition of nucleic acid synthesis, disruption of membrane integrity, and interference with bacterial enzyme systems (Cushnie & Lamb, 2005). Tannins exert antimicrobial effects by precipitating microbial proteins and inhibiting bacterial adhesion, whereas saponins increase membrane permeability and enhance the antibacterial activity of other phytochemicals.

The methanolic extract exhibited significant concentration-dependent antibacterial activity against both Gram-positive and Gram-negative bacteria. *Staphylococcus aureus* showed the

greatest susceptibility (21 mm), followed by *E. coli* (20 mm) and *S. pyogenes* (16 mm) at the highest concentration tested. The slightly higher sensitivity of Gram-positive bacteria may be attributed to the absence of an outer membrane, which facilitates the penetration of plant-derived phytochemicals (Singh et al., 2019; Ali et al., 2020). The observed activity against *E. coli* indicates that the extract can also overcome, at least partially, the permeability barrier characteristic of Gram-negative bacteria.

Anthraquinone glycosides, characteristic constituents of *C. angustifolia*, together with flavonoids, phenolic acids, and saponins, may act synergistically to produce the observed antibacterial effects. These compounds have been reported to disrupt bacterial cell membranes, inhibit protein synthesis, interfere with DNA replication, and suppress quorum sensing mechanisms involved in bacterial virulence and biofilm formation (Patil et al., 2020). Additionally, quorum sensing inhibition, a process critical to bacterial virulence and biofilm formation, could contribute to the antibacterial effects (Chavan et al., 2020).

While the precise mechanisms of action require further elucidation, existing evidence suggests that flavonoids and phenolic acids in *C. angustifolia* disrupt bacterial cell membranes, causing leakage of cellular contents (Patil et al., 2020). Saponins may further compromise membrane integrity by interacting with sterols. Additionally, quorum sensing inhibition, a process critical to bacterial virulence and biofilm formation, could contribute to the antibacterial effects (Chavan et al., 2020).

CONCLUSION

The present investigation successfully integrated physicochemical standardization, qualitative phytochemical profiling, and antibacterial evaluation of *Cassia angustifolia*. The physicochemical parameters established in this study provide valuable reference standards for the authentication and quality control of the plant material. The methanolic leaf extract was found to contain a diverse range of bioactive secondary metabolites, including alkaloids, flavonoids, tannins, phenolic compounds, saponins, cardiac glycosides, terpenoids, steroids, and carbohydrates. Furthermore, the extract demonstrated significant broad-spectrum antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus pyogenes*, with the strongest activity observed against *S. aureus*. The antibacterial activity is likely associated with the synergistic action of flavonoids, phenolic compounds, tannins, saponins, and anthraquinone glycosides. These findings provide scientific support for the traditional therapeutic applications of *C. angustifolia* and justify further studies involving

chromatographic characterization, isolation of active compounds, mechanistic investigations, and in vivo pharmacological evaluation for the development of plant-derived antibacterial therapeutics.

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