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Microbial Interventions for Nutrient Enrichment and Bioactive Compound Production in *Senna alexandrina* (*Cassia aungustifolia.* Vhal)

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ABSTRACT: *Cassia angustifolia* belongs to the Fabaceae family. The genus Cassia has historic therapeutic efficacy in treating a variety of illnesses caused by various infections. The current study aims to reveal the phytochemical evaluation and in vitro antimicrobial activity of *Cassia angustifolia* extracts, ranging from non-polar to polar. The findings of these studies demonstrated that *Cassia angustifolia* extracts contain a high amount of secondary metabolites. Ethanol is the most effective solvent for extracting metabolites from *Cassia angustifolia.* This also said that all of the extracts had measurable levels of phenols and Flavonoids. The ethanol extract has a high concentration of phenols and Flavonoids, followed by water. The extracts also demonstrate antibacterial action against a variety of harmful microorganisms. The results of the agar plates revealed that the plant aqueous extracts had no inhibitory zones. Acetone extracts had greater inhibitory zones than ethyl acetate or methanol extracts. *Aeromonas hydrophila* proved to be the most susceptible of the microorganisms examined in this investigation. More effort is needed to improve plant-based medications derived from *Cassia angustifolia*.

Keywords: Senna alexandrina, PGPMs, Microbial inoculants, Bioactive compounds, Antibacterial activity.

INTRODUCTION

Plants have been used as a source of medicine and wellness throughout history. Today, a large percentage Plants are commonly used in underdeveloped nations to meet basic medical needs (Karim *et al.,* 2011; Sultana *et al*., 2009). The principle "let food be thy medicine and medicine be thy food," introduced by Hippocrates 2500 years ago, highlights the health benefits of foods (Singh and Singh 2000). This idea gained modern traction in Japan in the 1980s with the creation of Foods for Specified Health Use (FOSHU) to address health concerns in an aging population (Karim *et al*., 2011; Chang *et al.,* 2002; Al-Naqqash *et al*., 2022).

The "Rig-Veda," written between 4500-1600 B.C., is considered the oldest reservoir of human knowledge and includes the first reference of medicinal plant use in Hindu culture (Jimoh *et al*., 2019 ; Wiart *et al.,* 2004). Ayurveda is the cornerstone of medicinal science in Hindu culture. Its eight divisions focus on medication characteristics, life science, and healing (Balasankar *et al.,* 2013; El-Morsy, 2013). Plant extracts inhibit specific species depending on extrinsic and intrinsic characteristics (Kaneria *et al*., 2009; Vijaya Sekhar *et al*., 2016).

Clinical microbiologists are interested in plant extracts that have antibacterial properties. These phytochemicals are likely to be included in antimicrobial medications recommended by physicians, with several already being evaluated in human trials (McDonald *et al.,* 2001 ; Mohanty and Das 2006). Annually, two to three antibiotics produced from microbes are launched. *Cassia angustifolia* is a plant that contains various medicines. Many medications utilized in modern medicine have their origins in traditional medicinal techniques, indicating their potential biological benefits (Jimoh *et al.,* 2019 ; Natarajan *et al.,* 2005). Plant-derived medicines are safer and more cost-effective than synthetic counterparts, providing significant therapeutic benefits (Kumar and Pandey 2013; Thayalini *et al.*, 2020).

Plants and plant-derived products have been utilized in traditional medicine to treat a variety of ailments. Plant-derived chemicals may combat pathogens by a different mechanism than recognized antimicrobials and may potentially have clinical benefit in treating disorders caused by pathogen-resistant strains (Natarajan *et al*., 2003 ; Shai *et al.,* 2008). The current study evaluates the phytochemical and in vitro antibacterial properties of Cassia angustifolia polar and non-polar solvent extracts. Cassia is the largest genus in the Caesalpinioideae subfamily, which belongs to the Leguminasae family. It contains over 600 species4-5 (Nkomo and Kambizi 2009; Viswanathan and Nallamuthu 2012). Members of the genus Cassia consist of annual or perennial herbs, shrubs and trees that have been classified by the number of leaflets, fertile and sterile stamens in a single flower and glands found on the leaves (Rosmalena *et al.,* 2022 ; Umedan *et al.,* 2020).

MATERIALS AND METHODS

Plant Material Collection and Preparation. Cassia angustifolia leaves were collected from SV University Campus near Vedic College, Tirupati. The collected leaves were washed, air-dried in the shade for 7-10 days, then powdered using an electric blender. The powdered material was stored in an airtight container at room temperature until further investigation.

Preparation of Plant Extract. The powdered material (50 g) was extracted using increasing polarity solvents (petroleum ether, Benzene, chloroform, ethanol and aqueous) to obtain a series of extracts. The extracts were concentrated under reduced pressure using a rotary evaporator and dried in a vacuum desiccators.

Standardized procedures for phytochemical screening

Phytochemical	Test Name	Procedure	Positive Result
Alkaloids	Dragendorff's Test	Add a few drops of Dragendorff's reagent to 1 mL of extract.	Orange or reddish-brown precipitate
	Mayer's Test	Add a few drops of Mayer's reagent to 1 mL of extract.	White or cream-colored precipitate
Flavonoids	Shinoda Test	Add magnesium ribbon and concentrated HCl to 1 mL of extract.	Pink or red color
	Alkaline Reagent Test	Add a few drops of NaOH to 1 mL of extract; add dilute acid if color appears.	Intense yellow color that turns colorless with acid
Phenols and Tannins	Ferric Chloride Test	Add a few drops of 1% ferric chloride solution to 1 mL of extract.	Dark blue or greenish-black color
	Lead Acetate Test	Add a few drops of 10% lead acetate solution to 1 mL of extract.	White precipitate
Saponins	Foam Test	Shake 2 mL of extract vigorously with distilled water.	Persistent foam
Steroids	Liebermann–Burchard Test	Add 2 mL of acetic anhydride and a few drops of concentrated H_2SO_4 to 1 mL of extract.	Blue-green color
Glycosides	Keller–Killiani Test	Add glacial acetic acid, 5% ferric chloride, and concentrated H_2SO_4 to the extract.	Reddish-brown ring at the interface
Carbohydrates	Benedict's Test	Mix extract with Benedict's reagent and heat.	Brick-red precipitate
	Molisch's Test	Add a few drops of Molisch reagent and concentrated H_2SO_4 to the extract.	Purple or violet ring

Documentation. The findings of the phytochemical tests were recorded based on color changes or precipitate formation and interpreted for the presence or absence of the various phytochemicals.

Antibacterial activity:

Microorganisms Used. The pathogenic bacterial strains Bacillus cereus, Escherichia coli, Klebsiella pneumonia, and Aeromonas hydrophila were employed in the present investigation. The microbial cultures were collected from the Microbiology Lab, Indira Gandhi Center for Advanced Research on Live stock (IGCARL), Genomix. Pvt. Ltd. Pulivendula A.P.

Preparation of Plant Extract. Fresh plant taken from SV University campus near Vedic college. The plant material was cleaned, peeled where necessary, and washed with sterile distilled water to remove contaminants. The extract was obtained by crushing approximately 100 g of the cleansed material with a mortar and pestle. The extract was filtered through a fine mesh cloth and then sterilized with a 0.45-micron membrane filter. For the sake of following experiments, this extract was assumed to be 100% concentrated.

Preparation of Filter Paper Discs. Filter paper discs (Whatman No. 1) with a diameter of 6 mm were prepared and autoclaved in a sterile Petri dish. After soaking for 6 hours in the prepared plant extract, the discs were shade-dried and used as the test material. The discs had an extract concentration of 0.1 g/mL. Filter paper discs soaked in butanol were prepared and used as the control.

Preparation of Nutrient Agar Medium (NAM). A nutrient agar medium was developed to cultivate the specified bacterial strains. The medium was steamed for 30 minutes, chilled to 37°C, and then steamed for another 30 minutes before filtration. Autoclaving at 15 pressure (121°C) for 20 minutes resulted in sterilization.

Antimicrobial Activity Assay

The antimicrobial activity assay was carried out on LB agar medium, which was prepared and sterilized at 121°C for 30 minutes. The sterile medium was put into 10 cm Petri dishes (about 15 mL per plate) and left to harden under aseptic conditions for 2 hours. A 1 mL suspension of each bacterial strain (**Bacillus cereus, Escherichia coli, Klebsiella pneumoniae and Aeromonas hydrophila**) was evenly distributed on the solidified agar plates using a sterile spreader. Sterile filter paper discs impregnated with plant extracts were carefully placed on the inoculation plates using sterile forceps, with each plate comprising four discs. Six replicates were made for each extract, and control discs soaked in butanol were included as a negative control. The plates were incubated at 37°C in a BOD incubator for 24 hours to allow inhibitory zones to form around the discs.

Measurement of Inhibition Zones. After incubation, the diameter of the zones of inhibition around the filter paper discs was measured in millimetres using a plastic ruler, and the average diameters of the inhibition zones were calculated based on two independent readings for

each extract to ensure the results' accuracy and reliability.

RESULTS AND DISCUSSION

Preliminary phytochemical screening was performed as per standardized procedure the various phyto constituents in petroleum ether, benzene, chloroform, ethanol and water leaf extracts of *Cassia angustifolia* Vahl presented in the Table 1.

Table 1: Preliminary Phytochemical analysis of *Cassia angustifolia* Vahl. With different solvents

Sr. No.	Name of the compound	Petroleum Ether (PE)	Benzene (Ben)	Chloroform ($CHCl_3$)	Ethanol (Eth)	Water (H_2O)
1.	Terpenoids	+	+	+	-	-
2.	Steroids, Sterols	+	+	+	-	+
3.	Alkaloids	+	+	+	+	-
4.	Anthraquinone (Glycosides)	-	-	-	+	+
5.	Flavonoids	-	-	-	+	+
6.	Cardiac Glycosides	-	-	-	+	+
7.	Tannins	-	-	-	+	+
8.	Phenolics	-	-	+	+	+
9.	Carbohydrates	-	-	-	+	+
10.	Saponins	-	-	-	-	+

(+) = Present, (-) = Absent

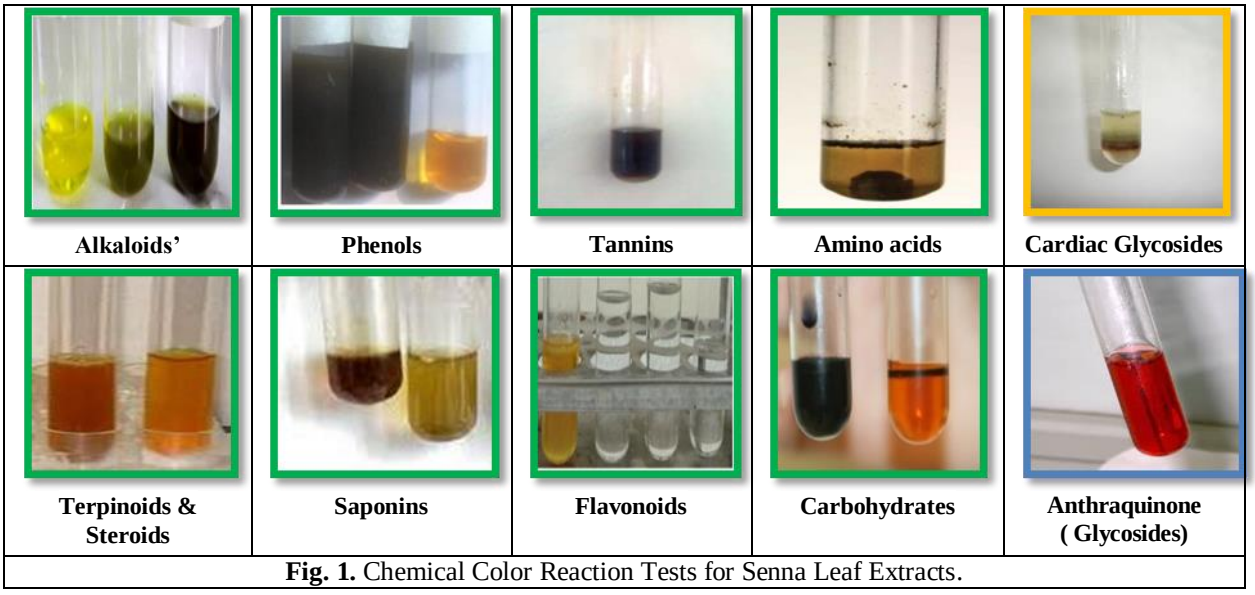

Fig. 1. Chemical Color Reaction Tests for Senna Leaf Extracts.

The qualitative study of phytochemicals across five solvent extracts—Petroleum Ether (PE), Benzene (Ben), Chloroform ($CHCl_3$), Ethanol (Eth), and Water (H_2O)—found different solvent-specific patterns. Non-polar solvents (PE, Ben, $CHCl_3$) were used to extract terpenoids, steroids, and sterols. Steroids also appeared in H_2O but not Eth. Except for H_2O, alkaloids had a broad solubility range. Polar substances, such as anthraquinone glycosides, flavonoids, cardiac glycosides, tannins, and carbohydrates, were limited to ethanol and water. $CHCl_3$, Eth, and H_2O were used to extract phenols, while H_2O was the only solvent for saponins. These findings highlight the importance of solvent polarity in phytochemical extraction, hence aiding targeted isolation techniques.

Antimicrobial activity of *Cassia Angustifolia* Vahl. The results of the agar plates demonstrated that the plant aqueous extracts had no inhibitory zones. Acetone extracts had better inhibitory zones than ethyl acetate and methanol extracts. The inhibitory zones indicate the sensitivity of bacteria in culture to plant extracts. Aeromonas hydrophila proved to be the most susceptible of the microorganisms studied in this investigation.

Fig. 2. Inhibition of *Klebsiella pneumoniae*.

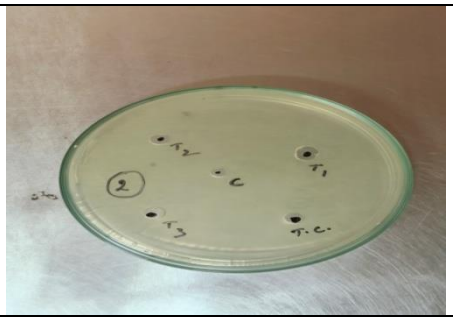

Fig. 3. Inhibition of *Bacillus cereus.*

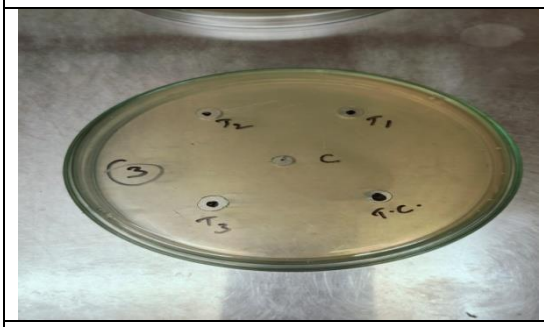

Fig. 4. Inhibition of *Aeromonas hydrophila.*

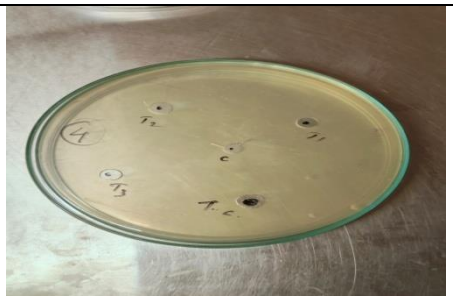

Fig. 5. Inhibition of *Escherichia coli.*

C: control; T1=Azosprillum; T2=PSB; T3=Azosprillum+PSB; T.C= Tetracyline

Bacterial strain	Control	T1 (mm)	T2 (mm)	T3 (mm)	Tetracyline(mm)
Klebsiella pneumoniae	1.24±0.68	5.46±1.02	11.4±0.68	15.7±1.98	26.0±2.04
Bacillus cereus	1.3±0.59	6.2±1.21	7.8±0.71	11.0±1.74	23.5±2.95
Aeromonas hydrophila	2.9±0.46	4.9±1.34	9.6±0.84	18.3±0.96	21.8±4.87
Escherichia coli	1.9±0.72	6.8±1.70	8.7±0.92	13.1±1.02	24.2±2.36

Values are mean of triplicates; ± SE

CONCLUSIONS

The study shows that phytochemical extraction in *Cassia angustifolia* Vahl. is extremely solvent-dependent, with non-polar solvents extracting terpenoids, Alkaloids, steroids and sterols and polar solvents such as ethanol and water targeting anthraquinone glycosides, Flavonoids, tannins, phenolics and carbohydrates. Antimicrobial studies revealed negligible action in aqueous extracts, whereas acetone extracts demonstrated the highest antibacterial activities, towards Aeromonas hydrophila. These findings highlight the relevance of solvent selection in optimizing phytochemical extraction and antibacterial activity.

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Conflict of Interest. None.

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