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Phytochemical Screening, Microbicidal Potentialities and FT-IR Finger printing profile of selected *Cassia* species

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ABSTRACT

Secondary metabolites are natural plant constituents that exhibit various pharmacological activities. According to Ayurveda, the leaves and seeds of many *Cassia* species are acrid, laxative, antiperiodic, anthelmintic, ophthalmic, liver tonic, and cardiotonic. Additionally, some *Cassia* species are used as natural pesticides in organic farms. It is, in fact, the responsibility of the scientific community to validate the medicinal properties of these traditional claims through scientific research. In this context, the present study aimed to qualitatively screen the phytochemicals in five selected *Cassia* species and validate the compounds using Fourier transform infrared spectroscopy (FT-IR). Furthermore, the study sought to analyze the microbicidal potential of these compounds against selected bacterial strains. Five *Cassia* species (*C. tora*, *C. alata*, *C. fistula*, *C. occidentalis*, and *C. mimosoides*) from the family Leguminosae were selected for this purpose. Finely powdered leaves were subjected to hot continuous Soxhlet extraction using solvents ranging from non-polar to polar. Phytochemical screening of the different solvent extracts revealed the presence of various phytochemicals. FT-IR fingerprinting analysis confirmed the presence of alcohols, phenols, alkanes, carboxylic acids, aldehydes, alkenes, nitro compounds, esters, ethers, aliphatic amines, and alkyl halides, validating the presence of diverse phytochemicals in the plants. Additionally, the microbicidal potential of the extracts was observed as inhibition zones on agar plates via the disc diffusion assay. Gram-negative bacteria were found to be more sensitive than Gram-positive ones. From the results it could be concluded that the studied species of *Cassia* are potent sources of phytochemicals having significant biological activities.

Keywords: *Cassia*, FT-IR, Phytochemicals, Bactericidal, FT-IR Finger printing

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INTRODUCTION

Plants continue to serve as valuable sources for new drugs and chemicals derived from various parts, which can be highly useful as lead structures for synthetic modification and bioactivity optimization. Plant-derived substances have gained considerable interest due to their versatile applications in traditional medicine, modern pharmaceuticals, nutraceuticals, food supplements, and synthetic drug intermediates. Medicinal plants are considered one of the richest resources for drugs used in traditional systems of medicine, modern treatments, and even folk remedies. The chemicals that protect plants from environmental hazards like pollution, stress, drought, UV exposure, and pathogenic attacks are referred to as phytochemicals. These naturally occurring, biologically active secondary metabolites have the potential to prevent diseases and play an essential role in plant defense. Evaluation of phytochemical content is regarded as a crucial step in medicinal plant research (Banso & Adeyemo, 2007). Phytochemicals are known to accumulate in different parts of plants such as roots, stems, leaves, flowers, fruits, and seeds (Costa et al., 1999).

Phytochemicals are classically classified into primary and secondary metabolites based on their roles in plant metabolism. Secondary metabolites encompass a wide range of chemicals such as alkaloids, terpenes, flavonoids, lignans, plant steroids, curcuminoids, saponins, phenolics, and glucosides (Ramawat et al., 2009).

Infrared spectroscopy (FT-IR) is a valuable analytical technique, provides a unique fingerprint for organic compounds, making it possible to identify them unambiguously, even among isomers. FT-IR works by measuring the amount of infrared radiation absorbed or emitted by a sample as a function of its wavelength (Chalmers & Griffiths, 2001). FT-IR spectroscopy This technique has been widely adopted for the identification, quality control, and manufacturing supervision of herbal medicines, as the efficacy of these medicines depends on the concentration of active components, which can vary significantly.

Cassia is a genus belonging to the family Leguminosae (subfamily Caesalpiniaceae), with approximately 800 species. Historically, *Cassia* was considered a catch-all genus for plants that did not fit into other classifications. However, many species of *Senna* were previously included in *Cassia*. In India, around 45 species of *Cassia* have been introduced for medicinal purposes and as tanning materials. These species are included in the traditional system of medicine and have been documented in ancient Ayurvedic literature. *Cassia* species are reported for their therapeutic value in folk medicine, particularly for treating various skin diseases such as ringworm, eczema, and scabies (Manojlovic et al., 2006). The leaves and seeds of *Cassia* species are known to be acrid, laxative, antiperiodic, anti-helminthic, ophthalmic, liver tonic, cardiotonic, and expectorant. They have also been used for leprosy, ringworm, flatulence, colic, dyspepsia, constipation, cough, bronchitis, and cardiac disorders. In addition to their medicinal uses, *Cassia* species are employed as natural pesticides in organic farming. The extracts of these plants have shown therapeutic potential in treating skin ailments, rheumatic diseases, and as laxatives (Manojlovic et al., 2006). Studies have found that the leaves of *Cassia* possess significant hepatoprotective and anti-inflammatory activities. The whole plant has been utilized in treating impetigo, ulcers, helminthiasis, and as a purgative.

Given the importance of active components in determining the efficacy of herbal medicines, the present study aims to investigate the phytochemical composition of five selected *Cassia* species, assess their microbicidal potential, and explore their functional groups using FT-IR fingerprinting technology. This approach is increasingly used for the identification, quality control, and manufacturing process supervision of herbal medicines in recent years.

MATERIALS AND METHOD

Plant Material

Five species of *Cassia* (*Cassia tora, C. alata, C. fistula, C. occidentalis* and C. *mimosoides*), belonging to the family Leguminosae, were selected for this study. The leaves of these plants were collected, air-dried, and finely powdered. The powdered leaves were subjected to phytochemical extraction using hot continuous Soxhlet extraction with solvents of varying polarity: hexane, ethyl acetate, and water.

Microorganisms

For the analysis of the bactericidal potential of the different *Cassia* extracts, two Gram-positive bacteria, *Staphylococcus aureus* and *Bacillus subtilis*, and two Gram-negative bacteria, *Escherichia coli* and *Klebsiella pneumoniae*, were used.

Extraction of Phytochemicals

The hot continuous Soxhlet extraction method was employed to extract the phytochemicals from the fresh leaves of the selected Cassia species. Approximately 100 grams of chopped, air-dried leaves were sequentially extracted using 250 ml of non-polar to polar solvents, namely hexane, ethyl acetate, and water. The extraction process was carried out for six hours. The resulting extracts were concentrated and stored in a refrigerator for further analysis.

Preliminary Phytochemical Analysis

The presence of various phytochemicals in the solvent extracts was analyzed following the protocol of Khandelwal (2007). This preliminary phytochemical screening aimed to identify the different types of bioactive compounds present in the *Cassia* leaf extracts.

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

The dried leaves of the *Cassia* species were prepared for FTIR analysis. After being oven-dried for two days at 60°C, 2 mg of leaf powder was mixed with potassium bromide (KBr) in a 1:100 ratio by weight. Tablets were formed using agate mortars, and the absorbance spectra of the samples were measured between 300 and 4500 cm^{-1}. At least three spectra were obtained for each sample to ensure accuracy (Anilkumar et al., 2016).

Microbicidal Assay by Disc Diffusion Method

All selected bacterial stock cultures were maintained at 4°C on nutrient agar medium (Hi-Media). For experimental purposes, active bacterial cultures were prepared by transferring a loopful of the stock culture to 10 ml of nutrient broth (Mueller Hinton Agar, Hi-Media). The cultures were then incubated at 37°C for 24 hours to promote bacterial proliferation. The Kirby-Bauer disc diffusion method was used to assess the antibacterial activity of the Cassia extracts. The results were compared with the standard antibiotic Ampicillin, and dimethyl sulfoxide (DMSO) served as the positive control (Zakaria et al., 2011). All experiments were conducted in triplicate for accuracy.

RESULTS AND DISCUSSION

The table 1 presents the presence or absence of specific phytochemicals across five Cassia species using three different solvent extracts (hexane, ethyl acetate, and water). The phytochemicals tested include alkaloids, phenols, flavonoids, saponins, tannins, terpenoids, and coumarins. All five *Cassia* species exhibited the presence of almost all phytochemicals when extracted using water. Water appears to be the most effective solvent for extracting a broad range of phytochemicals. Species like *C. fistula* and *C. occidentalis* lack certain phytochemicals, such as coumarins, in some extracts, indicating species-specific variations in phytochemical composition.

All species exhibit significant phytochemical diversity, particularly in the water extracts, which consistently show the presence of nearly all phytochemicals tested. *Cassia tora* and *C. mimosoides* have the most diverse range of phytochemicals in the water extract, with all seven compounds present. *Cassia fistula* and *C. occidentalis* display a consistent presence of phenols, flavonoids, tannins, and terpenoids across all solvent types, indicating their broad phytochemical content.

According to Yueh et al. (2002), *Cassia fistula* is particularly rich in phenolic antioxidants such as anthraquinones, flavonoids, and flavan-3-ol derivatives. The results also indicated the presence of alkaloids, steroids, reducing sugars, saponins, tannins, carbonyl compounds, phlobatannins, and terpenoids in this species. The therapeutic value of plants resides in their bioactive components, which can have significant physiological effects on the human body. The structural diversity of phytochemicals contributes to a wide range of biological activities that may protect organisms from chronic diseases. For example, alkaloids are known for their role in combating pathogenicity and alleviating pain, while saponins exhibit properties that help reduce cholesterol levels and possess antibiotic effects. Similarly, triterpenoids function as analgesics, and phenols and flavonoids exhibit multiple roles, including antimicrobial, antioxidant, antimetastatic, and anti-inflammatory activities (Ghasemzadeh & Ghasemzadeh, 2011).

Table 1: Qualitative analysis of different phytochemicals

Species	Solvent type	Alkaloids	Phenols	Flavonoids	Saponins	Tannin	Terpenoids	Coumarins
Cassia	Hexane	-	+	-	-	+	-	+
tora	Ethyl acetate	+	+	+	-	+	-	-
	Water	+	+	+	+	+	+	+
Cassia	Hexane		+	-	-	-	+	-
alata	Ethyl acetate	+	-	+	-	+	+	-
	Water	+	+	+	+	+	+	+
Cassia	Hexane	-	+	-	-	+	-	-
occiden	Ethyl acetate	+	+	+	-	+	+	-
talis	Water	+	+	+	+	+	+	-
Cassia	Hexane	+	-	-	-	-	+	+
fistula	Ethyl acetate	+	+	+	-	+	+	-
	Water	+	+	+	+	+	+	-
Cassia	Hexane		+				+	+
mimoso	Ethyl acetate	+	-	+	-	+	+	-
ides	Water	+	+	+	+	+	+	+

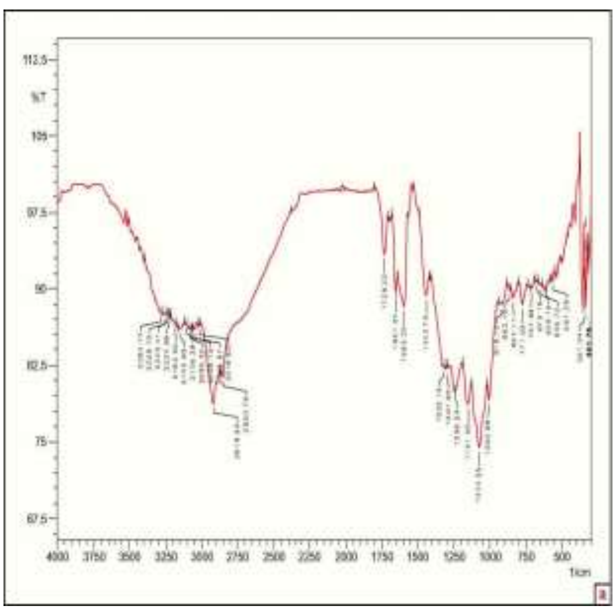
FTIR spectral analysis

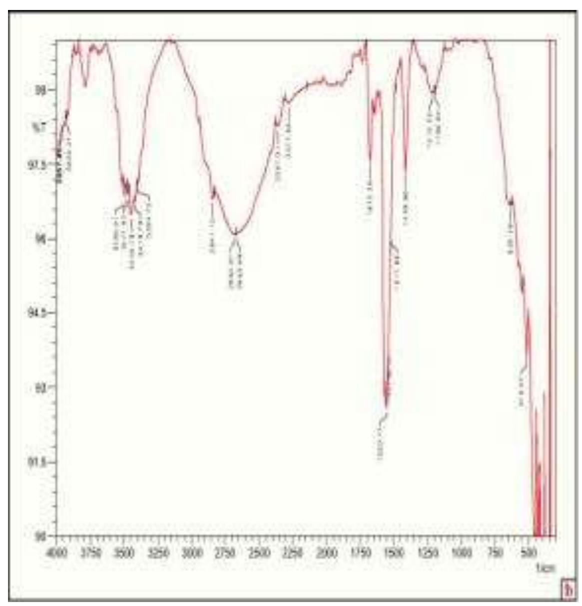
The FTIR analysis confirms the presence of a wide range of functional groups, validating the presence of various bioactive phytochemicals in the *Cassia* species. The variation in peaks between species suggests that each plant may have unique compounds contributing to its medicinal properties. IR spectral absorption bands are shown in Figure1 a-e.

Cassia tora exhibits unique peaks at 489.92 cm^{-1}, corresponding to metal-O or metal-N complexes, suggesting the possible presence of metal-chelating phytochemicals. *Cassia fistula* contains peaks in the 2000–2300 cm^{-1} range, indicating the presence of carbodiimide functional groups, which are uncommon in plant extracts. These could be indicative of unique secondary metabolites. *C. occidentalis* shows unknown peaks at 513.07, 628.79, and 3923.21 cm^{-1}. These unknown peaks may indicate unique compounds that have not yet been fully characterized but could offer distinct bioactive properties. *C. mimosoides* displays high-frequency peaks (3446.79–3957.93 cm^{-1}), some of which remain unidentified. This suggests the presence of highly polar or complex molecules that are less commonly observed in the other species.

Common functional groups across species- Alcohols, Phenols, and Hydroxyl Groups (-OH), present in all species, particularly in the high-frequency range (3000–3600 cm^{-1}). This indicates the presence of phenolic compounds and alcohols, which are known for their antioxidant properties. C-H Stretching found consistently across species in the 2800–3100 cm^{-1} range, showing the presence of alkanes and aldehydes, indicating fatty acids and other hydrocarbons. Nitro compounds commonly found in all species at ~1593 cm^{-1}, associated with nitrogen-containing phytochemicals. These compounds can be related to potential antimicrobial properties.

Alkenes are present in multiple species, especially in the 1600–1700 cm^{-1} region, indicating unsaturated compounds like flavonoids or terpenoids, which contribute to the plant's therapeutic potential. Aromatic rings detected in all species in the 1440–1500 cm^{-1} range. This is consistent with the presence of flavonoids and other aromatic phytochemicals, contributing to the medicinal properties of the plants.





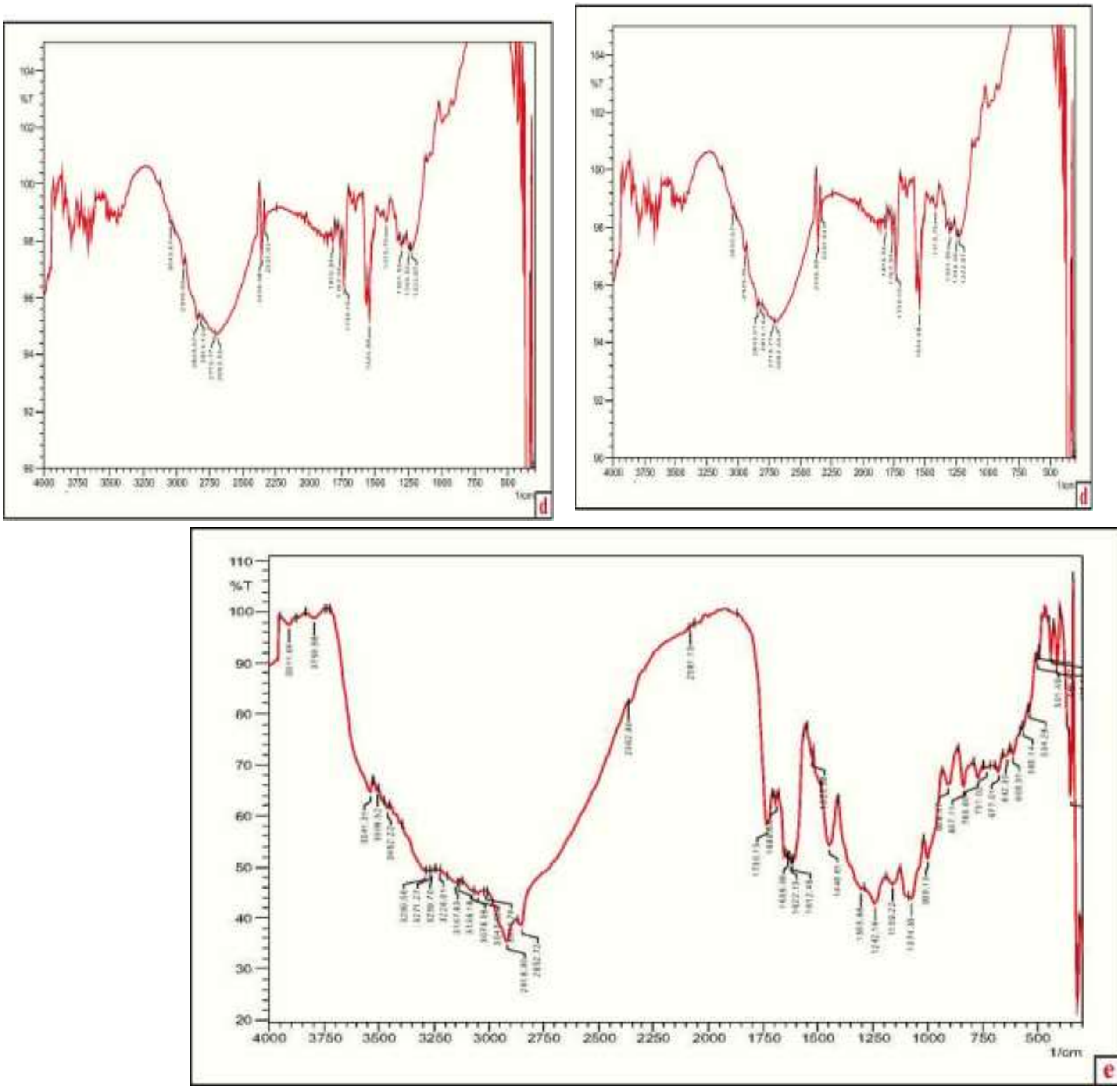


Figure 1 a-e. FTIR Finger printing profile of *Cassia species*; 1a. *Cassia alata,* 1b. *Cassia mimosoides,* 1c. *Cassia tora,* 1d. *Cassia fistula,* 1e. *Cassia occidentalis*.

Antimicrobial activity

The bactericidal activity of various solvent extracts from *Cassia* species exhibited varying degrees of effectiveness against the tested bacterial strains (Figure 2a-e). Microbicidal potential was assessed through inhibition zones formed during the disc diffusion assay, where treated pathogens were spread on agar plates. Significant inhibitory effects were observed across all extracts against the four tested strains, with Gram-negative bacteria showing greater sensitivity compared to Gram-positive bacteria.

Ampicillin demonstrated potent bactericidal activity across all bacterial strains tested, whereas DMSO showed minimal antibacterial effects. The diameter of the inhibition zones is presented in Table 2. Notably, the highest inhibitory activity was observed against *Klebsiella pneumoniae* and *E. coli*, with inhibition zone diameters ranging from 28 to 33 mm, while the lowest activity was recorded against *Bacillus subtilis* and *Staphylococcus aureus*, with inhibition zones measuring between 9 and 14 mm. Each plant extract exhibited comparable results across the tested strains.

These findings align with the reports of Seyyed et al. (2011), who found that *Cassia fistula* extracts were effective against *Bacillus cereus, Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli,* and *Klebsiella pneumoniae*. The most susceptible microorganisms to ethanolic and methanolic extracts were *E. coli* and *Klebsiella pneumoniae*, respectively, while B*acillus cereus* and *Staphylococcus aureus* exhibited the least sensitivity to ethanolic and methanolic extracts. The antimicrobial properties of *C. fistula* have been linked to its secondary metabolites, including phenolic compounds, saponins, triterpenoids, glycosides, anthraquinones, steroids, and flavonoids, which inhibit bacterial growth (Aneja et al., 2011; Draughon, 2004).

Rizvi et al. (2009) reported that *Cassia* species exhibited significant activity against Gram-positive microorganisms, attributing this effect to the flavonoids and polysaccharides present in the plant extracts. Additionally, Abo et al. (1999) observed considerable antimicrobial activity in the leaf extracts of *C. fistula.* Vasudevan et al. (2009) found that methanolic extracts inhibited Gram-positive bacteria more effectively than Gram-negative species.

Similarly, Gaurav et al. (2011) demonstrated the antibacterial potential of methanolic extracts of *Cassia auriculata* dry flowers using the agar disc diffusion method. They tested the extract against *Staphylococcus aureus, Bacillus subtilis, Salmonella typhi,* and *Escherichia coli*, with the highest activity observed against all tested microorganisms. The minimum inhibitory concentration (MIC) varied depending on the microorganism.

The *in vitro* antimicrobial potential of *Cassia occidentalis* leaf extracts also showed varying degrees of activity against isolates of *Salmonella typhi*, *Salmonella paratyphi* A, and *Salmonella paratyphi* B, with inhibition zones ranging from 7 mm to 23 mm, MIC values between 62.5 µg/ml and 125 µg/ml, and minimum bactericidal concentrations (MBC) between 125 µg/ml and 500 µg/ml (Adamu et al., 2018). The extracts of *Cassia* species demonstrate promising potential for treating infectious diseases and should be explored further as sources of potent antibacterial agents.

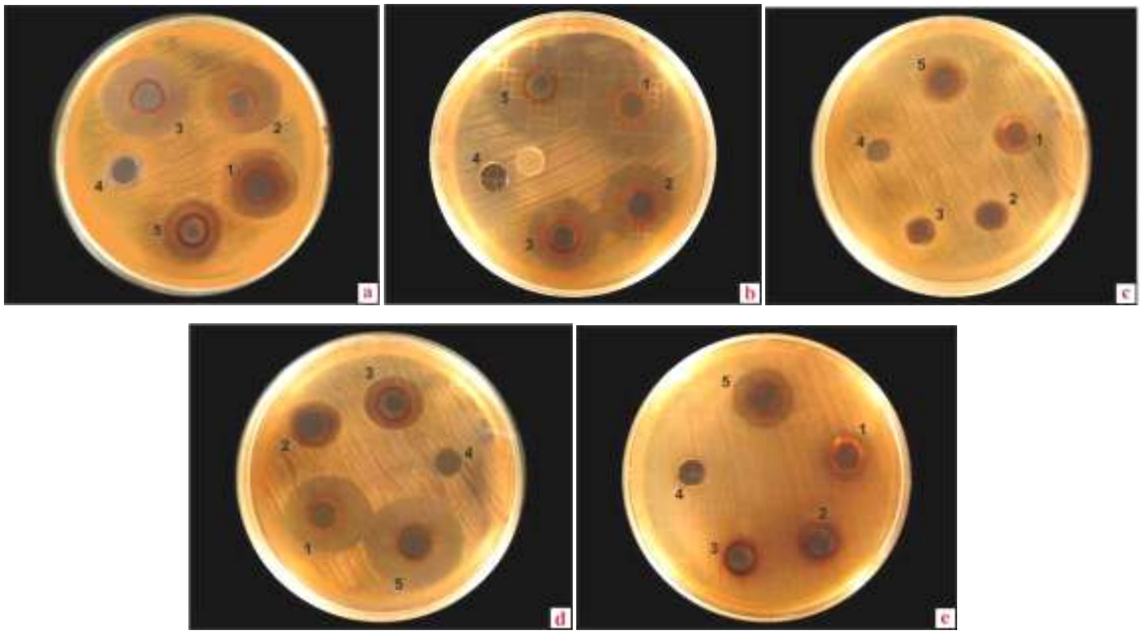


Figure 2a-e. **Bactericidal potentiality of *Cassia* species against bacterial strains**

a. *C. tora* against K. pneumoniae, b. *C. alata* against E. coli, c. *C. fistula* against *S. aureus*, d. *C. occidentalis* against E. coli,e. *C. mimosoides* against B. subtilis

1- Hexane, 2- Ethyl acetate, 3- Water, 4- DMSO, 5- Ampicilin

CONCLUSION

In conclusion, the present study successfully screened the phytochemical content and bactericidal potential of five selected *Cassia* species. The phytochemical analysis revealed the presence of a diverse range of bioactive compounds, including alkaloids, tannins, saponins, flavonoids, coumarins, terpenoids, glycosides, and phenols, across different solvent extracts. FTIR fingerprinting further confirmed the presence of various functional groups such as alcohols, phenols, alkanes, carboxylic acids, and more, validating the phytochemical composition of the plants. The bactericidal potential of these species demonstrated significant inhibitory effects, particularly against Gram-negative bacterial strains, highlighting their potential as potent antibacterial agents. The findings suggest that *C. tora, C. alata, C. fistula, C. occidentalis,* and *C. mimosoides* represent valuable sources of phytochemicals with promising therapeutic applications in combating bacterial infections. Further research could explore their potential in developing novel antibacterial agents.

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