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PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF EXTRACTS OF *COCHLOSPERMUM TINCTORIUM* AND *CASSIA SINGUEANA*

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ABSTRACT
Typhoid fever is a common disease reported in Africa and remains a major public health concern in recent times. While several factors contribute to this health challenge, it is primarily associated with pathogens such as Salmonella typhi and other microorganisms present in the environment. In this study, four clinical isolates (Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa and Salmonella typhi) were used to test for the antibacterial activity of Cochlospermum tinctorium and Cassia singueana. Four solvents—ethyl acetate, n-hexane, methanol and chloroform—were used for the extraction of active components from air-dried pulverized plant materials using cold maceration. Antibacterial activity was evaluated using the agar well diffusion method at a concentration of 2000 µg/mL. Phytochemical screening and FTIR analysis of chromatographically separated fractions were also conducted. Methanol extracts showed the highest antibacterial activity, with inhibition zones of 27 mm and 25 mm against E. coli and Staphylococcus aureus respectively for Cochlospermum tinctorium, and up to 23 mm against Salmonella typhi for Cassia singueana. Other solvent extracts showed moderate activity ranging from 10–17 mm. Phytochemical screening revealed the presence of tannins, saponins, glycosides, alkaloids and related secondary metabolites in both plant materials. FTIR analysis of purified fractions revealed functional groups such as alkanes, alkenes, aldehydes and hydroxyl-containing compounds. The findings from this study indicate that Cochlospermum tinctorium and Cassia singueana possess bioactive compounds with notable antibacterial activity, supporting their traditional medicinal use. However, the results are preliminary, and further studies involving compound isolation, toxicity evaluation, and in vivo analysis are required to establish their full therapeutic potential.

Keywords: Cochlospermum tinctorium, Cassia singueana, Phytochemical screening, Antimicrobial activity

Introduction

Infections results when pathogenic microorganisms like bacteria, fungi, and viruses find their way into human. These microorganisms are causative factors for many diseases (Omowumi et al., 2026). It then becomes a challenge termed as antimicrobial resistance. Antimicrobial Resistance threatens human and animal survival on earth affecring the entire world. Most of these microrganisms thrive through poor water and sanitation, vaccination, and other public health interventions, an improvement in this has reduced children death by 50% within 2000 and 2013 (Liu et al., 2015).

The ancient history of medicine rests on the potent possibilities and important provision of several medicinal plants. Plants have always provides advantages of cheap plant-based medicine with milder side effects. The medicinal value of plant parts lies in chemical substances that cause physiological changes in humans and animals (Predeep et al., 2014). Important bioactive compounds including alkaloids, flavonoids, tannins, tarpenoids, saponins and phenolic compounds constitute nearly 50% of drugs used as medicine. Analyzing plant extract procures valuable discovery of their chemical composition and antimicrobial activity to detect bioactive components (Sasidharan et al., 2011). These components called phytochemicals are naturally-occurring compounds found in all plant parts, providing antioxidant activity, antimicrobial effect, detoxifying enzyme modulation, immune stimulation and hormonal modulation (Abu, 2018). They are secondary metabolites synthesized in all parts of the plant body (Tiwari et al., 2011).

C. tinctorium is the most commonly used species of the family Cochlospermaceae. Several local applications have been reported on its roots or rhizomes, including treatment of ulcer, liver diseases, syphilis, hemorrhoids, intestinal worms, measles, rheumatism, yellow fever, gonorrhea, jaundice, snake bites, indigestion, convulsion, pneumonia, and bronchial infections. It is also used for women in labour and to alleviate menstrual pain, as well as in decoction or infusion with other herbs for malaria, urethral discharges, orchitis, and fever. Its extracts are used in traditional medicine for infectious diseases, diabetes mellitus, epilepsy, pain and inflammation, conjunctivitis, leprosy and testicular inflammation (Ahmad, 2021).

Cassia singueana is a plant of Cassia class, Fabaccae family and Caesalpinioideae sub family, common to East and West Africa. It is locally called Runfu in Hausa. Extracts of the plant have been used to treat a wide range of diseases such as skin cancer, liver diseases, malaria, body pain. Convulsions, Gonorrhea, constipation, heartburn, and many others in traditional herbal use (Kani Nura et al., 2024).

A review of medicinal plants used in southwestern Nigeria indicates rich flora diversity, and in Bauchi, located in the North Eastern zone, about 85% of dwellers use traditional medicine (Adamu et al., 2005). Herbal drugs are found in open spaces, shops, and markets, sold by traditional practitioners, with others moving through streets and villages. Despite interest in plant chemical components, approaches remain primitive with tendencies of unexpected reactions. Plants produce chemicals for self-protection, and research shows they also protect humans; an estimated 10,000 phytochemicals have potential effects on diseases such as cancer, stroke, or metabolic syndromes, though benefits may best derive from whole plant food consumption (Gimba et al., 2019).

This study provides a clear description of the antimicrobial potentials of Bauchi medicinal plants by showing the presence or absence of certain secondary metabolites. It proves the pharmaceutical implication of plant extracts used in local medicine. The aim of this study is to screen plant extract made using available solvent for their phytochemical components, ascertain the antimicrobial and biological activity, and isolate certain important compounds present. The

objectives include extraction using different solvents; testing for flavonoids, alkaloids, terpenes, tannins, saponins, steroids, glycosides, polyphenols and essential oils; testing antimicrobial (antibacterial) activity; purification; and isolating certain compounds. The study covers medicinal plant extraction, phytochemical screening and purification, using plants sourced only from Bauchi State, and reviewing works done on Bauchi and Nigerian medicinal plants for more than two decades. This study focuses on extraction using solvents of varying polarity, qualitative phytochemical screening of the extracts, evaluation of antibacterial activity against selected bacterial strains, chromatographic separation and purification of extract components, and characterization of functional groups using FTIR analysis.

Methods

1 Plant Collection and Identification

The plant materials used in this study consisted of the leaves, stems, barks and roots of selected medicinal plants collected from various locations within Bauchi metropolis. The samples were identified, proper labelling was carried out, and all samples were stored under conditions suitable for preserving their natural phytochemical composition. The leaves were washed carefully, dried thoroughly, ground into fine powder and stored in airtight polyethylene bags to prevent exposure to air. All collected samples were thoroughly washed with distilled water to remove dirt and contaminants. The plant materials were air-dried at room temperature (25–28°C) under shade to prevent degradation of heat-sensitive phytochemicals. The dried samples were then pulverized into fine powder using a mechanical grinder and sieved to obtain uniform particle sizes (30–40 mesh). The powdered samples were stored in airtight polyethylene containers at room temperature until extraction.

2 Physicochemical Analysis

Prior to extraction, dried plant powders were subjected to size reduction to enhance solvent penetration and mass transfer. The powdered materials (30–40 mesh size) ensured uniform extraction efficiency. Preliminary physicochemical observations of the crude extracts including colour, texture, and percentage yield were recorded after extraction. Extracts were stored in sterile, airtight containers at 4°C to preserve bioactive constituents prior to further analysis.

3 Preparation of Reagents

All reagents used were of analytical grade and prepared according to standard procedures:

- **Mayer's reagent:** Prepared by dissolving 1.36 g mercury (II) chloride in 40 mL distilled water and 5.0 g potassium iodide in 20 mL distilled water; both solutions were mixed and diluted to 100 mL.
- **Wagner's reagent:** Prepared by dissolving 30 g potassium iodide in 40 mL distilled water, followed by addition of 20 g iodine crystals and dilution to 100 mL.
- **Ferric chloride solution (1%):** Prepared by dissolving 1.0 g $FeCl_3$ in distilled water and making up to 100 mL.
- **Ammonia solution (10%):** Prepared by diluting 10 mL concentrated ammonia to 100 mL with distilled water.
- **Lead acetate solution (10%):** Prepared by dissolving 10 g lead acetate in distilled water and making up to 100 mL.
- **Hydrochloric acid (1%):** Prepared by diluting 1 mL concentrated HCl to 100 mL.
- **Barium chloride solution (1%):** Prepared by dissolving 1 g $BaCl_2$ in distilled water and making up to 100 mL.
- **Sulfuric acid (1%):** Prepared by carefully diluting 1 mL concentrated H_2SO_4 to 100 mL with distilled water.

4 Extraction of Plant Materials

Extraction was carried out using **separate solvent systems** to ensure methodological consistency.

Aqueous Extraction (Cold Maceration Method)

Approximately 100 g of powdered plant material was soaked in 1000 mL of distilled water (1:10 w/v ratio) and allowed to stand for 72 hours with intermittent stirring. The mixture was filtered using Whatman No. 1 filter paper. The filtrate was concentrated using a water bath at 40–50°C to obtain the crude aqueous extract.

Organic Solvent Extraction (Ethanol/Methanol Extraction)

For organic extraction, 100 g of powdered plant material was soaked in 1000 mL of ethanol (or methanol) and allowed to macerate for 72 hours with occasional agitation. The extract was filtered and concentrated using a rotary evaporator under reduced pressure at 40°C. The concentrated extract was further dried in a desiccator.

Percentage Yield Calculation

The percentage yield of each extract was calculated using:

$$\text{Percentage Yield (\%)} = \frac{\text{Weight of Extract Obtained}}{\text{Initial Weight of Plant Material}} \times 100$$

5 Phytochemical Screening

Qualitative phytochemical screening was conducted to determine the presence or absence of major classes of secondary metabolites. For alkaloid detection, extracts were dissolved in dilute hydrochloric acid and filtered. The filtrates were subjected to Mayer's, Wagner's, Dragendorff's and Hager's tests, with characteristic yellow, brownish-red and red precipitates indicating the presence of alkaloids. Flavonoids were detected using the alkaline reagent test, where an intense yellow colour appeared and later became colourless upon the addition of dilute acid, as well as the lead acetate test, which produced a yellow precipitate. Tannins were identified using ferric chloride, potassium cyanide and potassium dichromate, producing dark blue, greenish-black or yellow precipitates. Terpenoids were detected by adding chloroform and concentrated sulfuric acid to the extract, resulting in reddish-brown coloration at the interface. Steroids were detected by adding chloroform and sulfuric acid, which produced a reddish-brown ring at the interface. Glycosides were identified using Legal's test, Baljet's test and Borntrager's test, each producing specific colour changes such as pink-red, yellow-orange or pink-violet. Saponins were detected using the frothing test, foam test and mercuric chloride test. Anthraquinones were detected by mixing extracts with chloroform and ammonia, producing pink or red coloration in the ammonia layer. The total polyphenol content was determined using the Folin-Ciocalteu method, and coumarins were identified by heating extracts and exposing treated filter paper to UV light to observe greenish-yellow fluorescence.

6 Microbial Strains and Inoculum Standardization

Test microorganisms included clinically relevant bacterial strains obtained from a certified microbiology laboratory or culture collection. Reference strains with standard identification numbers were used (*Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa*, *Salmonella* spp., and *Klebsiella* spp.).

Bacterial inocula were standardized using the 0.5 McFarland standard, corresponding to approximately 1.5×10^8CFU/mL. This was prepared by adjusting the turbidity of bacterial suspensions in sterile saline solution and verified spectrophotometrically to ensure uniform inoculum density.

7 Antibacterial Activity Assay (Agar Well Diffusion Method)

The antimicrobial activity of the plant extracts was assessed against selected bacterial strains. Extracts of *Cochlospermum tinctorium* rhizome and *Cassia singueana* leaves in methanol, ethyl acetate, n-hexane and chloroform were tested against one gram-positive organism (*Staphylococcus aureus*) and four gram-negative bacterial strains (*Escherichia coli*, *Salmonella*, *Klebsiella* and *Pseudomonas aeruginosa*). Standard antibiotics including clindamycin, tetracycline, nalidixic acid and gentamicin served as reference drugs. Sterile Mueller–Hinton agar medium was prepared according to standard procedures by dissolving the appropriate quantity of agar and sodium chloride in 1000 mL of distilled water, followed by sterilization at 121 °C for 15–20 minutes. The medium was allowed to cool and poured into sterile Petri dishes to solidify. Standardized bacterial inoculum was uniformly spread on the agar surface using a sterile swab. Wells of 6 mm diameter were aseptically bored into the agar using a sterile cork borer, and measured volumes of plant extracts at defined concentrations were introduced into the wells. Dimethyl sulfoxide (DMSO) served as the negative control, while standard antibiotics served as positive controls. The plates were incubated at 37 °C for 18–24 hours, after which antibacterial activity was evaluated by measuring the diameter of the zones of inhibition in millimetres (mm).

8 Purification and Isolation Procedures

Purification of active extracts involved separating the components of crude plant extracts to obtain purified phytochemical fractions. Column chromatography was used extensively for this purpose. A cylindrical glass column was packed with silica slurry supported by a layer of cotton wool at the base. The extract sample, dissolved in a minimal amount of solvent to ensure even distribution, was carefully loaded onto the column. A series of solvents with varying polarities was allowed to pass through the column at a uniform rate under gravity. As the solvent flowed down, components of the extract migrated at different rates depending on their interactions with the stationary and mobile phases. Fractions were collected sequentially in labelled test tubes and stored for further analysis.

9 Chromatographic Analysis

Chromatographic analysis began with thin layer chromatography (TLC), which provided preliminary separation and identification of phytochemical components. TLC plates coated with silica gel G at a thickness of 0.2 mm were used. The solvent system consisted of butanol, acetic acid and water in the ratio 2:1:1. After development, the plates were dried and sprayed with 0.2% ninhydrin solution to visualize separated compounds. Retention factor (Rf) values were calculated by dividing the distance travelled by the solute by the distance travelled by the solvent front.

Column chromatography was then used to further isolate and purify compounds based on differential migration. Each collected fraction was analyzed and recorded for subsequent structural evaluation.

10 Spectroscopic Analysis

Spectroscopic analysis of the purified plant fractions was carried out to determine the functional groups present and to support the identification of the bioactive components. The study employed Fourier Transform Infrared (FTIR) spectroscopy to characterize the chemical bonds and structural features of the isolated fractions. FTIR analysis was performed on purified fractions obtained after column chromatographic separation of *Cochlospermum tinctorium* and *Cassia singueana* extracts. Each purified fraction was prepared according to laboratory requirements for infrared measurements. Absorption peaks corresponding to vibrational frequencies of chemical bonds were recorded and interpreted based on characteristic wave numbers. The method allowed the identification of major functional groups such as alkane (C–H stretch), aldehydic C–H,

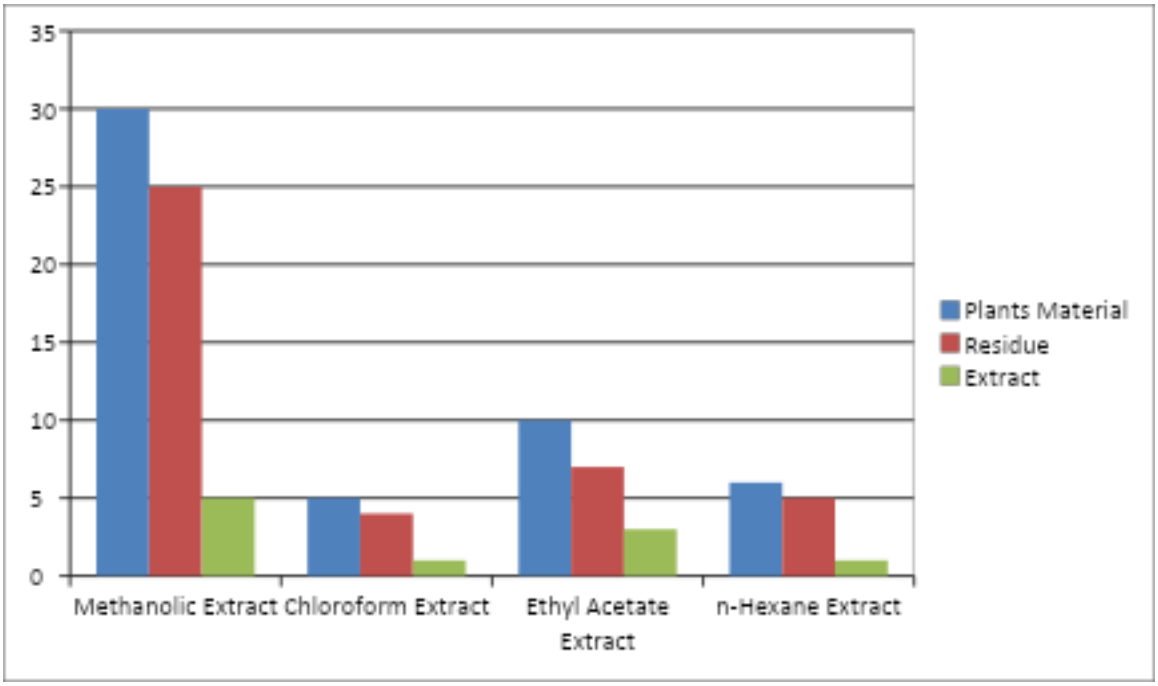
anhydride C=O, alkene C=C, hydroxyl (O–H) groups, CH_2 and CH_3 bending vibrations and other bond types. The FTIR analysis provided diagnostic information on structural features of phytoconstituents including alkanes, aldehydes, alcohols, carboxylic acids and unsaturated chains. Spectra for all purified fractions were recorded and compared with standard reference charts, and corresponding spectra are provided in the appendices. In addition to FTIR, spectroscopic tools listed under the materials included the Ultraviolet–Visible (UV–Vis) spectrophotometer and mass spectrophotometer. The UV–Vis spectrophotometer was used particularly in the quantification of total polyphenols using the Folin–Ciocalteu method, with absorbance readings taken at 765 nm.

Results

Extraction of the rhizome of *Cochlospermum tinctorium* and the leaves of *Cassia singueana* was carried out using four solvents of varying polarity: methanol, ethyl acetate, chloroform, and n-hexane. The cold extraction method employed involved soaking the pulverized samples for five days to ensure adequate solvent penetration and equilibrium. The crude extract yields obtained from each solvent reflected notable differences that are consistent with solvent polarity and phytochemical solubility. Methanol produced the highest extract yield for both plant samples, indicating the presence of predominantly polar constituents capable of dissolving efficiently in methanol. In contrast, chloroform extraction produced the lowest yield, particularly for *Cassia singueana*, suggesting that non-polar constituents were present in comparatively lower concentrations.

Table 1: Results of Crude recovery

Sample/ Extract	Weight of Plant A	Weight of Plant B
Powdered sample before extraction	300g	300g
Methanol	29g	25g
Chloroform	5g	4g
Methyl Acetate extract	10g	7g
n-Hexane	6g	5g



The percentage yield calculations (Table 2) further confirmed that methanol extracts exhibited the highest extraction efficiency, with *Cochlospermum tinctorium* showing approximately 9.7% yield and *Cassia singueana* approximately 8.0%. Chloroform extracts showed the lowest yields in both plants, which supports the observation that compounds present in the samples are largely hydrophilic or moderately polar.

Table 2 : Percentage yield of extract

SAMPLE/EXTRACT	**Weight of plant**		**% yield**	
	C. tinctorium	***C. Serguena***	***C. tinctorium***	***C. Serguena***
Methanol	29g	24g	9.7	8.0
Chloroform	5g	4g	1.7	1.3
Methyl Acetate extract	10g	7g	3.3	2.3
n-Hexane	6g	7g	2.0	2.3

Phytochemical screening results revealed that both *Cochlospermum tinctorium* and *Cassia singueana* possessed a rich array of secondary metabolites. The results presented in Table 3 show the presence of alkaloids, flavonoids, saponins, tannins, and glycosides in both plants. Slight differences were noted between the two samples: *C. tinctorium* displayed positive reactions for carboxylic acids and coumarins, whereas *C. singueana* tested positive for anthocyanins. These differences reflect the natural variation in chemical composition between plant species and may account for variations in their biological activity.

Table 3: Phytochemical content of *Cochlospermum tinctorium* and *Cassia Singueana*

S/N	Phytochemicals				
	Secondary Metabolites	**Test Method**	**Observation**	***C.. Tinctorium***	***C. Singueana***
1	Alkaloid	Dragendroff's	A reddish brown precipitate was observed		
		Kraut's Test			
		Wagner's Test	Brown/Reddish precipitate	+	+
2	Flavonoid	Lead Acetate Test	Yellow precipitate		
		Ferric Chloride Test	Green Precipitate	+	+
3	Saponins	Foam Test	Formation of foam which persist for more than 10minutes.	+	+
4	Tanins	10% NaOH Test	Formation of emulsion	+	+
5	Glycoside	Aqueous NaOH Test	Yellow Color	+	+
6	Cardiac Glycoside	Keller-Killani test	A blue coloured solution (in acetic acid layer)	+	+
7	Proteins and Amino acids	Xanthroproteic test	A yellow coloured solution	+	+
8	Carboxylic acids	Efferverscence Test	Appearance of Efferverscence	+	-
9	Phenolic Compound	Ferric Chloride	Dark green/bluish black color		
10	Quinone	Concentrated HCl Test	Green color		
11	Coumarins	NaOH test	A yellow Color	+	-
12	Anthocyanins	HCl Test	Pink-red solution, which turns blue-violet after addition of ammonia	-	+

Key: Present = + Absent = - ppt = Precipitate

Extracts obtained after the chromatographic process

Antimicrobial Activity Results

The antimicrobial activity of the crude extracts of both plants revealed notable variations across solvents and test organisms. For *Cochlospermum tinctorium*, the methanolic extract displayed the highest antibacterial activity, producing inhibition zones of 27 mm against *E. coli* and 25 mm against *Staphylococcus aureus*. These results indicate that methanol extracted a broader spectrum of bioactive compounds with antibacterial properties. Other solvents such as n-hexane, chloroform, and ethyl acetate demonstrated moderate activity, with inhibition zones ranging from 10–13 mm.

Table 4: Antimicrobial activity of *Cochlospermum tinctorium* on four isolates

TEST ORGANISM	Conc .(ug/ml)	Methanolic Extract (mm)	Ethylacetate Extract (mm)	n-Hexane Extract (mm)	Chloroform Extract (mm)	+ control	- Control
E- Coli	2000	27.00	11.00	10.00	11.00		0.00
S. aureus	2000	25.00	13.00	12.00	11.00		0.00
S. typhi	2000	16.00	13.00	12.00	10.00		0.00
Ps. auriginosa	2000	17.00	13.00	10.00	12.00		0.00

Likewise, *Cassia singueana* showed strong antimicrobial activity, with methanol and n-hexane extracts exhibiting inhibition zones as high as 23 mm against *Salmonella typhi* and 18 mm against *Staphylococcus aureus*. These values indicate that *C. singueana* also contains potent antibacterial compounds extractable using solvents of varying polarity, especially methanol.

Table 5: Antimicrobial activity of *Cassia Singueana* on four isolates

TEST ORGANISM	Conc. (ug/ml)	Methanolic Extract (mm)	Ethylacetate Extract (mm)	n-Hexane Extract (mm)	Chloroform Extract + Control (mm)	
E- Coli	2000	11.00	11.00	13.00	0.90	0.00
Staphyllococus Aureus	2000	18.00	11.00	10.00	13.00	0.00
Salmonella Typhi	2000	23.00	12.00	17.00	10.00	0.00
Pseudomonas auriginosa	2000	16.00	11.00	11.00	11.00	0.00

Chromatographic Results

Thin layer chromatography (TLC) analysis enabled the preliminary separation of compounds within the extracts. Spots were detected using a 0.2% ninhydrin spray, and retention factor (Rf) values calculated. Both plants produced two visible spots, showing the presence of at least two major components in each extract.

Table 6: TLC result

Plant	Number of spots	Rf Values
Cassia Singueana	2	1.9, 0.8
Cochlospermum tinctorium	2	3.5, 2.5

Column chromatography was performed to further isolate the constituents. *Cochlospermum tinctorium* yielded ten fractions, which after TLC purification were reduced to four distinct purified fractions. *Cassia singueana* yielded six fractions, with three remaining after purification. These findings are consistent with the number of distinct chemical species resolved on TLC plates.

Table 7: Column chromatography results

Plant	Number of spots	Number of Spots (After TLC)
Cassia Singueana	6	3
Cochlospermum tinctorium	10	4

FTIR Analysis Results

FTIR analysis was conducted on purified fractions to identify functional groups present in the plant constituents. For *Cochlospermum tinctorium*, Fraction 1 showed vibrational peaks at 2952 cm^{-1} and 2853 cm^{-1} corresponding to alkanes, while strong aldehydic C–H stretching was evident at 2920 cm^{-1}. An anhydride C=O stretch at 1746 cm^{-1} and alkene C=C at 1697 cm^{-1} were also present. Fraction 2 displayed similar functional groups with additional -COH alcohol stretching at 1015 cm^{-1}. Fraction 4 exhibited a broad O–H stretch at 3334 cm^{-1} and significant alkenic and alkane features, confirming the presence of alcohols, phenolics, and other oxygenated compounds.

Table 8: *Cochlospermum tinctorium* -Fraction 1

Wave number (cm-1)	Type of Bond	Remark
2952	-C-H stretch	Alkanes
2920	-C-H aldehydic	Aldehyde group
2853	-C-H stretch	Alkanes
1746	C=O anhydride	anhydride
1697	C=C alkene	Alkene group
1457	CH2 bend	Alkane
1377	-CH3 bend	R group
767 – 691	C-X	

For *Cassia singueana*, Fraction 1 displayed characteristic alkane and aldehydic peaks at 2952 cm^{-1} and 2920 cm^{-1}, respectively. Fraction 2 showed a broad O–H peak at 3390 cm^{-1}, alkene C=C stretches at 1653 cm^{-1} and 1615 cm^{-1}, and -CH_3 bending vibrations at 1448 and 1399 cm^{-1}, indicating the presence of alcohols and unsaturated compounds.

Table 9: *Cochlospermum tinctorium* - Fraction 2

Wave number (cm-1)	Type of Bond	Remark
2950	-C-H stretch	Alkanes
2920	-C-H aldehydic	Aldehyde
2853	-C-H stretch	Alkanes
1455	-CH2 bend	Branching present
1377	-CH3 bend	Branching present
1015	-C-OH Stretch	Alcohol
726 – 674	R-CH2	Alkane

Discussion

The extraction yields obtained in this study clearly demonstrate the influence of solvent polarity on the recovery of phytochemicals from plant materials. Methanol, being highly polar, extracted the highest quantity of constituents from both *Cochlospermum tinctorium* and *Cassia singueana*, with yields of approximately 9.7% and 8.0% respectively, as presented in the results. This agrees with common extraction trends in medicinal plant studies, where polar solvents efficiently dissolve phenolics, flavonoids, glycosides and related polar compounds, reflecting the phytochemical richness of these plant species. Similar observations have been reported in related studies, where methanol consistently produced higher yields compared to chloroform and n-hexane, confirming that the dominant phytochemicals present are largely polar or moderately polar in nature.

The qualitative phytochemical screening confirms that both plants contain multiple classes of bioactive compounds capable of producing therapeutic effects. The presence of alkaloids, flavonoids, tannins, saponins and glycosides has been associated with antimicrobial, anti-inflammatory, antioxidant and cytotoxic activities. The detection of coumarins in *C. tinctorium* and anthocyanins in *C. singueana* further highlights the unique phytochemical profiles of these species. These slight differences in composition may account for the variations observed in antimicrobial activity between the two plants, particularly in their response to different bacterial strains.

Antimicrobial activity results demonstrate that methanolic extracts of both plants possessed the strongest inhibitory effects on the test organisms. For *Cochlospermum tinctorium*, inhibition zones of 27 mm against *E. coli* and 25 mm against *Staphylococcus aureus* were

recorded, while *Cassia singueana* showed inhibition up to 23 mm against *Salmonella typhi*. These values indicate strong antibacterial activity and are comparable to inhibition ranges reported for plant-derived antimicrobials in similar studies. This can be attributed to methanol's ability to extract a broader range of active compounds, including phenolics, flavonoids, and triterpenoids known to possess antimicrobial potency. The significant inhibition of *E. coli*, *S. aureus*, and *Salmonella typhi* suggests that the plants have broad-spectrum antibacterial potential, supporting their traditional use in treating gastrointestinal and infectious diseases.

The moderate inhibition shown by non-polar extracts such as n-hexane (10–17 mm) indicates the presence of lipophilic antibacterial compounds as well. However, their relatively lower activity compared to methanol extracts suggests that the most potent antimicrobial constituents are predominantly polar. Differences observed across solvents cannot be attributed to polarity alone but may also be influenced by compound stability, solubility limits, and synergistic interactions among phytochemicals. The antimicrobial results, obtained at a concentration of 2000 µg/mL, reflect consistent trends across organisms, although further statistical evaluation (mean ± SD) would strengthen the reliability of these observations.

The chromatographic analyses (TLC and column chromatography) demonstrated successful separation of the extract components into distinct fractions. The number of spots recorded on TLC plates and the distribution of fractions after column chromatography indicate chemical diversity within the extracts. However, the recorded Rf values (greater than 1 in some cases) are unusually high and may be attributed to measurement or calculation inconsistencies, solvent front misidentification, or solvent system effects beyond simple polarity. This suggests that while separation was achieved, refinement of the TLC procedure may improve accuracy. The observed diversity justified the need for further structural elucidation using FTIR.

FTIR analysis revealed the presence of functional groups such as alkanes, aldehydes, alcohols, carboxylic acids and alkenes. These functional groups correspond to known phytochemical classes identified during screening, including phenolics (O–H stretch), saponins (multiple C–H and O–H functionalities), tannins (phenolic O–H), and terpenoids (C–H and C=C stretches). The FTIR results correlate strongly with the phytochemical screening and provide molecular evidence supporting the presence of bioactive compounds responsible for the antimicrobial activity observed. Mechanistically, these functional groups are known to contribute to antimicrobial action through membrane disruption, protein denaturation, and interference with microbial enzymatic systems, thereby explaining the inhibitory effects recorded in this study.

Overall, the results of this study confirm that *Cochlospermum tinctorium* and *Cassia singueana* possess significant phytochemical and antimicrobial properties. The strong activity exhibited by methanolic extracts is consistent with the high solubility of antimicrobial phytochemicals in polar solvents. The combined outcomes of extraction, phytochemical screening, antimicrobial tests, chromatographic separation and FTIR analysis validate the traditional medicinal use of these plants and highlight their potential for future phytopharmaceutical applications.

Conclusion

The study confirms that *Cochlospermum tinctorium* and *Cassia singueana* contain important phytochemicals with significant antimicrobial activity. Their extracts justify traditional medicinal uses and demonstrate potential for further pharmaceutical development. However, these findings are preliminary and based on in vitro analyses only. The absence of toxicity evaluation, dosage determination, and isolation of specific bioactive compounds limits the extent to which definitive pharmaceutical applications can be established.

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