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STUDY OF PHYTOCHEMICALS, *IN VITRO* ANTIMICROBIAL, ANTI INFLAMMATORY AND ANTI DIABETIC ACTIVITY OF *COCHLOSPERMUM GOSSYPIUM* EXTRACT

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ABSTRACT

The present study evaluated the phytochemical composition and in vitro antimicrobial, anti-inflammatory, and antidiabetic activities of the hydroalcoholic extract of *Cochlospermum gossypium*. The percentage yield of the extract was found to be 8.52%, indicating moderate extraction efficiency using a hydroalcoholic solvent system suitable for extracting both polar and semi-polar phytoconstituents. Phytochemical screening revealed the presence of alkaloids, flavonoids, phenols, proteins, carbohydrates, and diterpenes. The total phenolic and flavonoid contents were found to be 0.58 mg/100 mg and 0.96 mg/100 mg, respectively, suggesting a higher flavonoid concentration. The antimicrobial study demonstrated moderate activity against *Bacillus subtilis* and *Klebsiella pneumoniae*, with maximum zones of inhibition observed at higher concentrations. The anti-inflammatory activity showed a concentration-dependent increase, with 69.58% inhibition at 500 µg/ml, while the antidiabetic activity exhibited 62.58% inhibition at 150 µg/ml. Although the activities were lower than standard drugs, the extract showed significant pharmacological potential. These biological effects may be attributed to the presence of flavonoids and phenolic compounds. The study supports the traditional use of *Cochlospermum gossypium* and highlights its potential as a source of natural therapeutic agents. Further studies are required to isolate active constituents and confirm efficacy through in vivo investigations.

Keywords: *Cochlospermum gossypium*, Phytochemical screening, antimicrobial activity, anti-inflammatory activity, antidiabetic activity, flavonoids, phenolic compounds.

INTRODUCTION

Medicinal plants have long been recognized as a valuable source of bioactive compounds with diverse therapeutic applications. The growing interest in natural products is largely driven by their safety, affordability, and minimal side effects compared to synthetic drugs (Dar *et al.*, 2023). In recent years, there has been increasing scientific focus on exploring plant-derived compounds for the management of chronic diseases such as diabetes, inflammation, and microbial infections (Amin *et al.*, 2026).

Cochlospermum gossypium (family: Cochlospermaceae), commonly known as Yellow Silk Cotton Tree, is an important medicinal plant widely distributed in tropical and subtropical regions of India. Traditionally, various parts of the plant, particularly the roots and bark, have been used in the treatment of inflammation, wounds, infections, diabetes, and liver

disorders. The plant is known to contain a variety of phytoconstituents which are responsible for its pharmacological activities (Anumula *et al.,* 2019).

Phytochemicals play a crucial role in exhibiting biological effects such as antioxidant, antimicrobial, anti-inflammatory, and antidiabetic activities. The presence of phenolic and flavonoid compounds, in particular, contributes significantly to free radical scavenging and inhibition of oxidative stress, which is a major underlying factor in inflammation and diabetes. Moreover, the emergence of antimicrobial resistance has necessitated the search for new, effective, and safer antimicrobial agents from natural sources (Ahangarpour *et al.,* 2019).

Inflammation is a protective response of the body against injury or infection but, when chronic, it can lead to various pathological conditions. Similarly, diabetes mellitus is a metabolic disorder characterized by elevated blood glucose levels, leading to serious complications if not managed properly (Libby; 2007). Natural plant extracts have shown promising potential in managing both inflammation and diabetes by modulating biochemical pathways and improving physiological functions. The present study aims to investigate the phytochemical profile and evaluate the *in-vitro* antimicrobial, anti-inflammatory, and antidiabetic activities of *Cochlospermum gossypium* extract. The study is intended to provide scientific validation for its traditional uses and to explore its potential as a source of novel therapeutic agents.

MATERIALS AND METHODS

Materials

The plant material of *Cochlospermum gossypium* was collected, authenticated, dried, and powdered for extraction. A hydroalcoholic solvent system was used for the preparation of the extract. Various analytical-grade chemicals and reagents were employed for phytochemical screening, including Wagner's and Hager's reagents for alkaloids, concentrated sulphuric acid for glycosides, alkaline reagent and lead acetate for flavonoids, ferric chloride and Folin–Ciocalteu reagent for phenols, Fehling's and Benedict's solutions for carbohydrates, and copper acetate for diterpenes. For pharmacological evaluations, standard drugs such as ciprofloxacin (antimicrobial), diclofenac sodium (anti-inflammatory), and acarbose (antidiabetic) were used. Microbial strains including *Bacillus subtilis* and *Klebsiella pneumoniae* were utilized for antimicrobial studies. All experiments were carried out using standard laboratory glassware and instruments under controlled conditions.

Methods

Extraction by maceration method

Defatting of plant materials

50 gram shade dried leaves was coarsely powdered and subjected to extraction with hexane by Maceration process. The extraction was continued till the defatting of the materials had taken place.

Extraction by maceration method

Defatted powdered of *Withania coagulans* has been extracted with hydroalcoholic solvent (ethanol: water; 70:30v/v) using Maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40°C (Mukherjee, 2007).

Determination of percentage yield

The extraction yield is evaluate of the solvent's efficiency to extracts bioactive

components from the selected natural plant samples and it was defined as quantity of plant extracts recovered in mass after solvent extraction compared with the initial quantity of plant samples. After extraction, yield of the plant extracts obtained were calculated in grams and then converted it into percentage. The percentage yield of each extract was calculated by using following formula:

Percentage Yield

$$= \frac{Weight\ of\ Extract}{Weight\ of\ Powder\ drug\ taken} x\ 100$$

Phytochemical screening

Medicinal plants are resources of traditional medicines and many of the modern medicines are produced indirectly from plants. Phytochemical constituents are of two type primary bioactive constituents (chlorophyll, proteins, amino acids, sugar etc.) and secondary bioactive constituents include (alkaloids, terpenoids, phenols, flavonoids etc.). Phytochemical examinations were carried out for all the extracts as per the standard methods (Kokate, 1994).

Quantitative estimation of bioactive compounds

Estimation of total phenol content

The total phenol content of the extract was determined by the modified folin-ciocalteu method (Mishra *et al.,* 2017). 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 5-25µg/ml was prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol. 2 ml of extract and each standard was mixed with 1 ml of folin-ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for color development. The absorbance was measured at 765 nm using a spectrophotometer.

Estimation of total flavonoids content

Determination of total flavonoids content was based on aluminum chloride method (Mishra *et al.,* 2017). 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5-25µg/ml were prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids. 1 ml of 2% $AlCl_3$ solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm.

In vitro* antimicrobial activity of hydroalcoholic extract of *Cochlospermum gossypium

The well diffusion method was used to determine the antimicrobial activity of the hydroalcoholic extract prepared from of *Cochlospermum gossypium* using standard procedure (Bauer *et al.,* 1966).There were 3 concentration used which are 25, 50 and 100 mg/ml for extracted phytochemicals in studies. It's essential feature is the placing of wells with the antibiotics on the surfaces of agar immediately after inoculation with the organism tested. Undiluted over night broth cultures should never be used as an inoculums. The plates were incubated at 37°C for 24 hr. and then examined for clear zones of inhibition around the wells impregnated with particular concentration of drug.

In vitro* anti-inflammatory activity of hydroalcoholic extract of *Cochlospermum gossypium

Protein denaturation assay was done according to the method described by

Gambhire *et al*. (2009), with some modifications as described in Gunathilake *et al*. (2018). Diclofenac sodium, a powerful non steroidal anti-inflammatory drug was used as a standard drug. The reaction mixture consisted of 0.2 mL of 1% bovine albumin, 4.8 mL of phosphate buffered saline (PBS, pH 6.4), and 0.2 mL of extract (10-50 µg/mL), and the mixture was mixed, and was incubated in a water bath (37◦C) for 15 min, and then the reaction mixture was heated at 70°C for 5 min. After cooling, the turbidity was measured at 660 nm using a UV/VIS spectrometer. Phosphate buffer solution was used as the control. The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\% \text{ inhibition of denaturation} = (A1 - A2/A1) \times 100$$

Where A1 = absorption of the control sample, and A2 = absorption of the test sample

In vitro* anti-diabetic activity of hydroalcoholic extract of *Cochlospermum gossypium

Inhibition of alpha amylase enzyme

10 mg acarbose was dissolved in 10 ml methanol, and various aliquots of 100-500µg/ml were prepared in methanol. 10 mg of hydroalcoholic extract was dissolved with 10 ml methanol. 500 µl of this solution was used for the estimation of enzyme inhibition. A total of 500 µl of test samples and standard drug (25-150µg/ml) were added to 500 µl of 0.20 mM phosphate buffer (pH 6.9) containing α-amylase (0.5mg/ml) solution and were incubated at 25°C for 10 min. After these, 500 µl of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube (Kidane *et al.*, 2018). The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 1.0 ml of 3, 5 dinitrosalicylic acid colour reagent. The test tubes were then incubated in a boiling water bath for 5 min, cooled to room temperature. The reaction mixture was then diluted after adding 10 ml distilled water and absorbance was measured at 540 nm. Control represent 100% enzyme activity and were conducted in similar way by replacing extract with vehicle.

RESULTS AND DISCUSSION

The present study evaluated the phytochemical composition and in-vitro antimicrobial, anti-inflammatory, and antidiabetic activities of the hydroalcoholic extract of *Cochlospermum gossypium*. The percentage yield of the extract was found to be 8.52% (Table 1), indicating moderate extraction efficiency using a hydroalcoholic solvent system, which is suitable for extracting both polar and semi-polar phytoconstituents.

Phytochemical screening (Table 2) revealed the presence of important bioactive compounds such as alkaloids, flavonoids, phenols, proteins, carbohydrates, and diterpenes, while glycosides, saponins, tannins, and sterols were absent. The presence of flavonoids and phenolic compounds is particularly significant, as these are known to possess strong antioxidant, anti-inflammatory, and antimicrobial properties. The total phenolic and flavonoid contents were found to be 0.58 mg/100 mg and 0.96 mg/100 mg respectively (Table 3), indicating a relatively higher flavonoid content, which may play a major role in the observed biological activities.

The antimicrobial activity (Table 4) demonstrated that the extract exhibited

moderate inhibitory effects against both *Bacillus subtilis* and *Klebsiella pneumoniae*. The zone of inhibition increased with concentration, with maximum activity observed at 100 mg/ml (12±0.3 mm and 14±0.57 mm, respectively). Although the activity was lower compared to the standard drug ciprofloxacin (Table 5), the extract still showed appreciable antibacterial potential, which may be attributed to the presence of flavonoids and phenolic compounds that disrupt microbial cell membranes and metabolic pathways.

The anti-inflammatory activity was evaluated using the inhibition method with diclofenac sodium as a standard (Table 5). The extract showed a concentration-dependent increase in percentage inhibition, reaching 69.58% at 500 µg/ml. The IC_{50} value of the extract (244.24 µg/ml) was higher than that of diclofenac sodium (73.47 µg/ml), indicating comparatively lower but significant anti-inflammatory activity. This effect may be due to the ability of phytoconstituents to inhibit inflammatory mediators and enzymes.

Similarly, the antidiabetic activity assessed using acarbose as a standard (Table 6) demonstrated that the extract exhibited notable inhibitory activity against carbohydrate-metabolizing enzymes. The percentage inhibition increased with concentration, reaching 62.58% at 150 µg/ml. The IC_{50} value of the extract (57.16 µg/ml) was higher than that of acarbose (13.02 µg/ml), indicating moderate antidiabetic potential. This activity can be correlated with the presence of flavonoids and phenolic compounds, which are known to inhibit enzymes like α-amylase and α-glucosidase, thereby reducing glucose absorption.

The study indicates that *Cochlospermum gossypium* possesses significant pharmacological potential due to its rich phytochemical composition. The extract demonstrated moderate antimicrobial, anti-inflammatory, and antidiabetic activities in a dose-dependent manner. These findings support the traditional medicinal use of the plant and suggest its potential for development into natural therapeutic agents. However, further studies involving isolation of active constituents and in-vivo evaluations are necessary to validate and standardize its efficacy

Table 1: % Yield of *Cochlospermum gossypium*

S. No.	Extract	Colour	Odor	% Yield (w/w)
1.	Hydroalcoholic	Dark black	Characteristic	8.52

Table 2: Result of phytochemical screening of Hydroalcoholic extract of *Cochlospermum gossypium*

S. No.	Constituents	Hydroalcoholic extract
1.	**Alkaloids** Wagner's test: Hager's test:	 +Ve -Ve
2.	**Glycosides**	

	Conc. H_2SO_4 test	-Ve
3.	**Flavonoids** Alkaline reagent test: Lead acetate test:	 +Ve +Ve
4.	**Saponins** Froth test:	 -Ve
5.	**Phenol** Ferric chloride test: Folin ciocalteu test	 +Ve +Ve
6.	**Proteins** Xanthoproteic test:	 +Ve
7.	**Carbohydrate** Fehling's test: Benedict's test:	 +Ve -Ve
8.	**Diterpenes** Copper acetate test:	 +Ve
9.	**Tanins** Gelatin test	 -Ve
10.	**Sterols** Salkowski test	 -Ve

Table 3: Total phenol and flavonoid content of *Cochlospermum gossypium*

S. No.	Extract	Total phenol content	Total flavonoid content
		mg/ 100mg	
1.	Hydroalcoholic extract	0.58	0.96

Table 4: Antimicrobial activity of standard drug against selected microbes

S. No.	Name of drug	Microbes	Zone of Inhibition (mm)		
			10 µg/ml	20 µg/ml	30 µg/ml
1	Ciprofloxacin	*Bacillus subtilis*	15±0.3	17±0.57	20±0.86
		Klebsiella pneumoniae	14±0.6	22±0.74	25±0.94

Table 5: Antimicrobial activity of hydroalcoholic extract of *Cochlospermum gossypium* against selected microbes

S. No.	Microbes	Zone of Inhibition (nm)		
		25mg/ml	50 mg/ml	100 mg/ml
1	*Bacillus subtilis*	8±0.86	10±0.94	12±0.3
2	*Klebsiella pneumoniae*	10±0.74	12±0.5	14±0.57

***Average of three determination, Mean ± SD**

Table 6: % Inhibition of Diclofenac sodium and hydroalcoholic extract of *Cochlospermum gossypium*

Concentration (µg/ml)	% Inhibition	
	Diclofenac sodium	***Cochlospermum gossypium* extract**
100	50.91	40.51
200	62.85	46.39
300	72.14	52.72
400	80.39	61.41
500	88.24	69.58
IC 50 value	**73.47**	**244.24**

Table 7: % Inhibition of acarbose and hydroalcoholic extract of *Cochlospermum gossypium*

S. No.	Concentration (µg/ml)	% Inhibition	
		Acarbose	***Cochlospermum gossypium* extract**
1	25	42.54	27.95
2	50	56.9	42.03
3	75	67.15	47.72
4	100	75.98	51.87
5	125	81.02	57.19
6	150	92.56	62.58
IC_{50} value		**13.02**	**57.16**

CONCLUSION

The present study demonstrated that the hydroalcoholic extract of *Cochlospermum gossypium* contains important bioactive phytoconstituents such as flavonoids and phenolic compounds. The extract exhibited moderate antimicrobial activity against *Bacillus subtilis* and *Klebsiella pneumoniae*, along with significant anti-inflammatory and antidiabetic activities in a concentration-dependent manner. Although the activity was comparatively lower than standard drugs, the results indicate promising therapeutic potential. Overall, the findings support the traditional use of *Cochlospermum gossypium* and suggest its potential as a natural source for developing antimicrobial, anti-inflammatory, and antidiabetic agents. Further *in-vivo* and isolation studies are recommended for validation.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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