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Screening of Primary and Secondary Metabolites Profile of Different Extracts of *Cassia fistula* Flowers and its Perspectives on the Antimicrobial and Antidiabetic Potential of Active Extract

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Abstract: This article provides an overview of the pharmacological activity and phytochemical constituents found in *Cassia fistula* flower extract. Some phytonutrients and antioxidants of plant extract may be compromised due to the relatively high concentration of organic solvents. The main goal of this study was to find out how well an aqueous extract from *Cassia fistula* flowers fights diabetes and germs in a lab setting. The maceration extract yielded the best results among others. The antimicrobial activity of the extract was evaluated using bacterial and fungal strains, while the anti-diabetic activity was assessed at different concentrations, ranging from 10 to 50 µg/mL. The maximum inhibition of 54.07% was observed at a concentration of 50 µg/mL, compared to the standard acarbose, which showed an inhibition of approximately 62.23%. The IC_{50} value of the flower extract was 49.3494 µg/mL, while that of the standard acarbose was 42.1726 µg/mL. The presence of phytochemicals in the extract was determined using UV and FTIR spectral studies, which are effective methods for extracting a broad range of chemical constituents. The results show that *Cassia fistula* flower extract has promising anti-diabetic and antimicrobial activity. It can be concluded that *Cassia fistula* has high alpha-amylase inhibitory activity, and these extracts have the potential to be effective antimicrobial agents against pathogenic microorganisms.

Keywords: Phytochemicals, *Cassia fistula*, Anti-microbial, Anti-diabetic, FTIR.

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1. INTRODUCTION

The present study aims to evaluate the antidiabetic effect of flowers of *Cassia fistula*. *Cassia fistula* is a small tree or shrub found in many western countries like Indonesia, Vietnam, Thailand, and India. Traditional folk medicine has a long history of utilizing all parts of the *Cassia fistula* plant. Studies have quantitatively evaluated the plant's antioxidant content, which has a direct impact on regulating blood sugar levels. Diabetes, a chronic and serious disease that affects people worldwide, poses a significant risk to the function of major organs such as the heart and kidneys. As a result, it is considered a dangerous illness (2,3). Currently, over 500 million people worldwide are affected by type 1 and type 2 diabetes mellitus. Medications, including insulin, are critical for people with diabetes to survive. The high cost of treatment and the serious nature of the disease have led to a growing awareness of the need for efficient, economical, and safe substitute medications (4).

Researchers have investigated the use of herbs and isolated compounds, such as glucosides, alkaloids, and steroids, to treat different stages of diabetes. Due to its greater accessibility, the focus of research has been on plant-based alternative medicines (5). In vitro and animal tests have been conducted to evaluate the effectiveness of plants and their extracts in regulating blood sugar levels. Given the rising number of people affected by diabetes in recent years, it is essential to explore alternative remedies. Investigating the effectiveness of

medicinal plants known to be useful in many traditional systems of medicine is a promising approach to discovering new antidiabetic drugs (6,7).

To address the critical issue of growing bacteria resistance against conventional antibiotics, it is essential to find novel antimicrobial chemicals or extracts. Biodiversity in plant chemicals is a rich potential resource. The flowers were studied with polar and non-polar solvents like hexane, chloroform, methanol, and ethanol, which exhibit good antibacterial activity, especially for gram-positive bacteria. Hence, the floral part of the *Cassia fistula* can be used for good antimicrobial drug formation (8). The pod of *Cassia fistula* was evaluated for its antidiabetic activity in rats with various concentrations of ethanolic extract, which exhibited a significant lowering of the blood sugar level. It is well recognized that the plant components of *Cassia fistula* constitute a significant source of secondary metabolites, particularly phenolic compounds (9,10). The current investigation examines the various techniques for extracting *Cassia fistula* flowers and assesses the antimicrobial and antidiabetic properties of the resulting active extract.

2. EXPERIMENTAL SECTION

2.1. Materials

Ferric chloride, glacial acetic acid, ammonia, Dragendroff's reagent, ethanol, hydrochloric acid, sulfuric acid, sodium hydroxide, and double distilled water were purchased from SRL chemicals and Merck. All chemicals utilized in the research were of superior quality and high purity, with a minimum purity level of ≥99.0%. The flowers of *Cassia fistula* were collected from Trichy district, India, in May.

2.2. Preparation of Flower Extracts

The fresh flowers of *Cassia fistula* were collected and dried at room temperature. To prepare the extract, the dried flower samples were ground into a fine powder. Each of the four portions of the 200g flower sample was extracted using maceration, digestion, infusion, and decoction methods with 1000 mL of double-distilled water. The methods chosen have been devised to achieve effectiveness in removing a wide range of chemical constituents. These techniques facilitate both heating and heatless extraction that enable the isolation of compounds with varying solubility. The maceration extraction method involved soaking the flower sample (200g) in 1000 mL of double distilled water for 72 hours, with periodic shaking and subsequent filtration for further use. For the digestion method, 200g of the flower sample was mixed with 1000 mL of double distilled water and heated in a water bath at 50C for 30 minutes, followed by filtration of the extract for further studies. The infusion method involved the same solvent-to-sample ratio as the maceration process, with a shorter soaking time of up to 4 hours, followed by filtration. Finally, the decoction method entailed heating 200 g of the flower sample with 1000 mL of double distilled water continuously for 30 minutes, with the concentrated extract then filtered for further analysis. (5,11).

2.3. Phytochemical Screening of Plant Extracts

The qualitative evaluation of phytoconstituents in the four different extracts was carried out using some standard procedures. The most crucial chemical analyses are those that examine the specific physiological advantages of the phytochemicals. Four extracts were examined qualitatively for the presence of such significant phytochemicals as flavonoids, alkaloids, phenolic compounds, tannin, terpenoids, saponin, carbohydrates, and steroids; outcomes were compared (7,12).

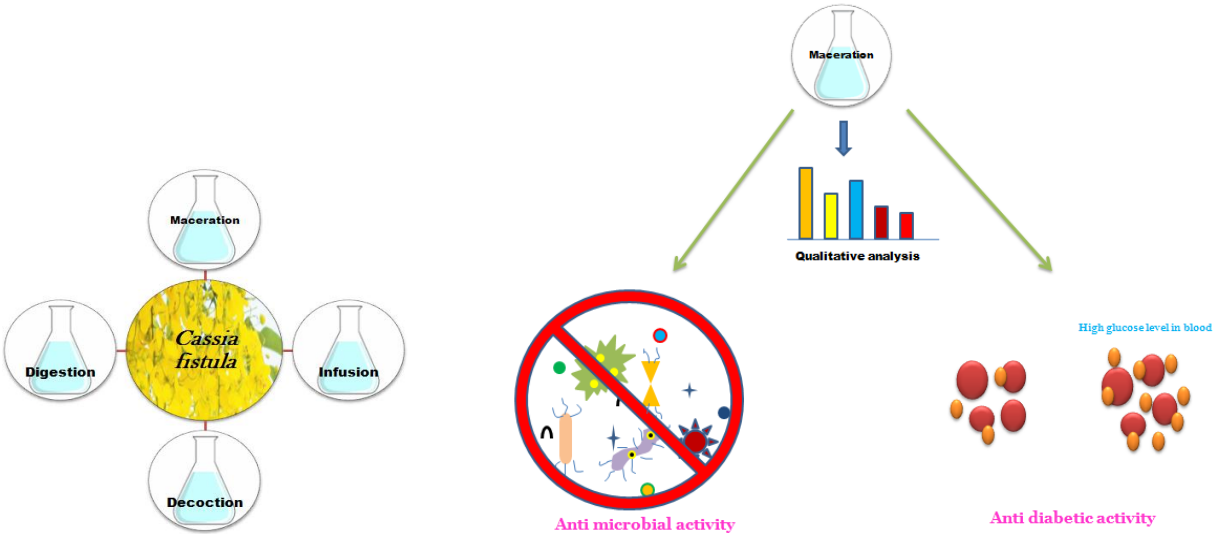

Figure 1: Schematic diagram of antimicrobial and antidiabetic activity of *Cassia fistula*.

2.4. Characterization of Flower Extract

The best outcomes of the phytochemical constituents were selected from the screening, and the extract obtained by the maceration technique was further analyzed. The macerated extract of *Cassia fistula* was subjected to spectral analysis, like UV-Vis and Fourier transform infrared spectroscopy. The aqueous extract of *Cassia fistula* was studied by a

UV-Vis spectrometer (Perkin Elmer, Lambda 35 model) with a wavelength range of 200–800 nm (13,14). The flower extract was subjected to the FTIR spectrometer (Perkin Elmer, range 4000-400 cm^{-1}) for the functional groups (15).

2.5. Antimicrobial Activity of Flower Extract

The agar well diffusion method was used to assess the flower extract's antibacterial and antifungal properties. The microbial test employed bacterial and fungal strains of *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Candida albicans, and Aspergillus niger*. Gentamicin was used as a control for these antimicrobial tests. The strains were subcultured in the nutrient broth, which was prepared just before the test. The nutritional agar medium was prepared, and the petri plates were prepared with wells (16,17). Each well was treated with the flower extract at a different concentration. The inhibition rate was calculated using the diameter of the zone of inhibition formed by the flower extract (18).

2.6. Antidiabetic Activity of Flower Extract

200µL of alpha-amylase solution was mixed with various concentrations of aqueous flower extract (10, 20, 30, 40, and 50 µL) and incubated for 10 mins (19). 200 mL dinitrosalicylic acid reagent was prepared using 12 g of sodium potassium tartrate tetrahydrate in 8.0 mL of 2 M NaOH and 20 mL of 96 mM of 3,5-dinitrosalicyclic acid solution, and the mixture was boiled, cooled and diluted for the absorbance of 540 nm. Acarbose was used as the standard reference. The inhibition rate and IC_{50} values were calculated (20).

3. RESULTS AND DISCUSSION

The occurrence of phytochemicals in the four different extracts was studied. The maceration method of extraction was found to be the most effective, as it is a low-tech technique that requires minimal equipment. This method does not involve heat, which may contribute to the preservation of bioactive compounds. In contrast, the decoction method, which involves continuous heating, is commonly used for extracting compounds from tough plant materials. This method is particularly useful for extracting less soluble compounds. The majority of the phytochemicals were present in all of the extracts since polar solvents were used. In comparison to other extracts, the maceration extraction process produced significantly better results. Some phytochemicals were absent or showed mild occurrence in the digestion and infusion processes, which may be due to the heating process of the plant extract.

Table 1: Phytochemical screening of aqueous flower extract of *Cassia fistula.*

S.No.	Phytochemicals	Extraction method			
		Maceration	Infusion	Digestion	Decoction
1	Flavonoid	+++	++	++	++
2	Alkaloid	+++	+	+	++
3	Phenols	++	-	+	+
4	Tannin	++	-	+	+
5	Terpenoid	++	+	+	+
6	Saponin	-	+	-	+
7	Carbohydrates	+++	++	++	++
8	Steroid	++	++	+	+

(+ : slightly present, ++ : moderately present, +++ : strongly present, - : absent)

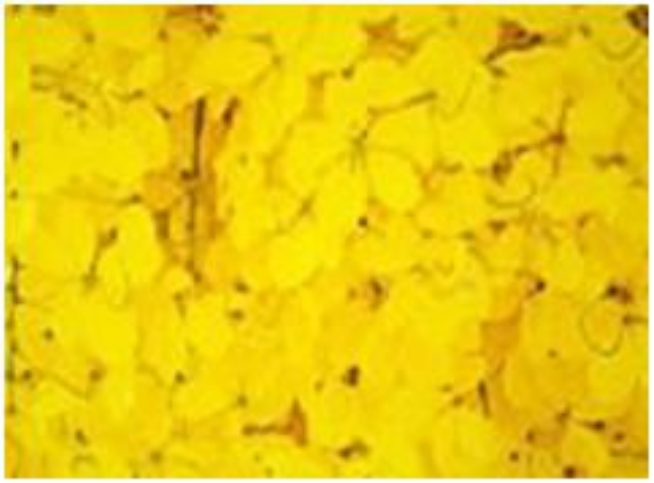

Figure 2: Flower of *Cassia fistula*.

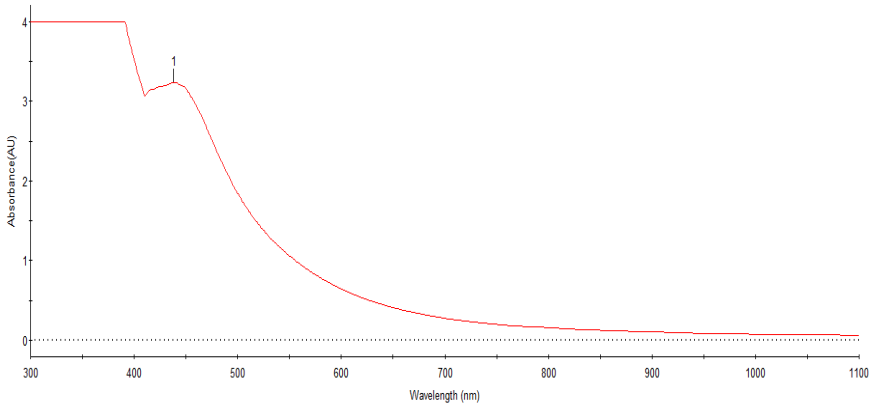

Figure 3: UV-Vis spectrum of flower extract of *Cassia fistula.*

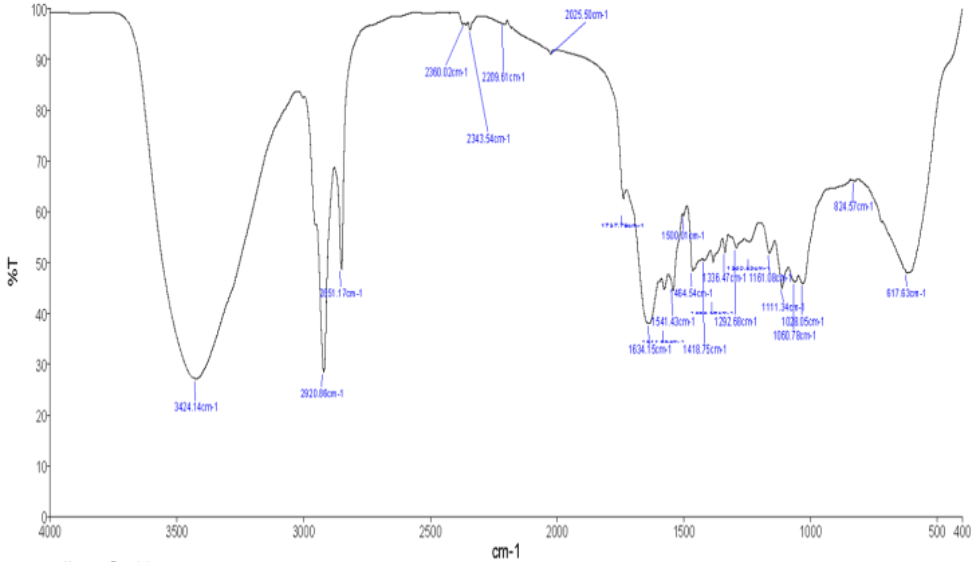

Figure 4: FTIR spectrum of flower extract of *Cassia fistula.*

The extract made using the maceration and decoction methods included significant amounts of flavonoids and phenolic substances. These phytochemicals have antioxidant properties, supporting several therapeutic qualities (7,39). Flavonoids are one of the major antioxidant agents present in most plant species. It is significantly present in maceration and decoction extracts. This plays a vital role in antimicrobial and antidiabetic activity. This secondary metabolite is widely studied for anticancer and anti-inflammatory activity (21,22). Many flavonoids were successfully isolated, and their structures were identified. The polyphenolic compounds and functional groups are responsible for the chelation of metals by scavenging free radicals and acting as the best antioxidant agents (12,36).

All different kinds of extracts contain substantial amounts of tannin. Typically, tannin is found in the tree's bark, protecting it from microbes. As a result, tannins have built-in antibacterial properties. These specific tannins, which were extracted from plant samples, have antiviral and antibacterial properties (7,21,35). Previous studies back up the claim that it slows tumor growth, which has a positive impact on numerous cancer types, like breast and lung cancer. Steroid content in *Cassia fistula* flower extracts is noticeable. These phytosterols are very beneficial for lowering cholesterol and blood sugar. Important phytosterols like stigmasterol and sitosterol have reduced the absorption of cholesterol (23,27,34). The maceration technique-adapted extract had the highest concentration of phytoconstituents of all the extracts. Hence, this extract was used for further antimicrobial and antidiabetic studies. The process of digestion necessitates high temperatures, which may cause the degradation of heat-sensitive compounds, resulting in the loss of biological activity. Furthermore, this extraction method exhibits reduced efficiency in extracting non-polar compounds. The infusion method displays limited extraction efficiency and possesses a brief shelf-life with aqueous extracts, allowing for microbial growth and oxidation. The decoction technique may cause the evaporation of volatile compounds in the extract, which are responsible for numerous medicinal properties. Each of these methods has its unique advantages and disadvantages, and the study assists in selecting the most appropriate extraction procedure for further research based on the specific requirements of biological studies.

The *Cassia fistula* flower extract was subjected to UV-Vis spectroscopy. The spectrum gives the details of σ and π bonds, chromophores, and other lone pairs of electrons (13,28). Hence, this information can help

identify the phytocompounds present in the plant extract. The aqueous extract shows absorption at a wavelength range of 438 nm, which indicates the presence of unsaturated groups (35,40). The peaks formed may be due to the presence of tannin, flavonoids, and carotenoids. This absorption range indicates the π – π* transition in the aromatic ring (24,29).

The FTIR spectrum of the flower extract of *Cassia fistula* is shown in figure 4. The well-defined absorbance peaks indicate the presence of many alkyl and alkane groups, as well as some hydroxyl groups. The absorption peaks at 3424 cm^{-1} and 2920 cm^{-1} indicated stretching of the hydroxyl group and symmetric stretching of saturated compounds in the extract. The peak that appears at 2209 cm^{-1} indicates the alkyne group. The peaks at 1541 and 1292 cm^{-1} may be due to the C=C stretching and C=O stretching (15,30). Hence, this FTIR spectrum proved the presence of phenolic compounds, aromatic compounds, amines, and alkanes in the aqueous extract of *Cassia fistula* flowers.

The aqueous extract of *Cassia fistula* flower was tested at various concentrations (25, 50, 75, and 100 µL) in gram-positive, gram-negative, and fungal strains. The microorganisms chosen for this study were selected based on their clinical relevance, specifically *Staphylococcus aureus* and *Escherichia coli*, which are the most common causes of skin infections and pneumonia, respectively, and are known to exhibit heavy antibiotic-resistant strains. Furthermore, these microorganisms represent a diverse range of organisms, including both gram-negative and gram-positive bacteria, as well as fungal strains, which allows for a broader understanding of the antimicrobial activity of *Cassia fistula* flowers. These microorganisms are ideal for studying antimicrobial resistance mechanisms and can be easily cultivated with well-defined growth requirements. Collectively, the pathogens responsible for a wide range of human diseases are these microorganisms and studying them can provide valuable insights into treatment strategies for various types of infections. The growth of *Staphylococcus aureus* bacteria and *Aspergillus niger* fungi was inhibited better than that of other microorganisms. On increasing the concentration of the flower extract, the zone of inhibition increased. The *Bacillus subtilis* and *Pseudomonas aeruginosa* have less inhibition at 100µl of the extract when compared to other microorganisms. Urinary tract infections caused by *Escherichia coli* can be treated with the *Cassia fistula* flower extract (25,31,33). Hence, the extract can be used as an alternative antimicrobial medicine and for further drug discovery studies. This kind of plant-based alternative medicine has been well appreciated in recent years for its less toxic and more effective antimicrobial behavior. Lethal diseases like pneumonia were caused by *Bacillus subtilis*, which can be treated by the *Cassia fistula* flower extract (17,32,41). Natural antioxidant agents like flavonoids and phenolic compounds may be responsible for the good antimicrobial activity of the *Cassia fistula* extract, which could be a suitable alternative to synthetic medicine.

Table 2: Antimicrobial activity of aqueous extract of *Cassia fistula* flower.

Microorganisms	Concentration of extract and zone of inhibition (mm/mL)				
	25 µL	**50 µL**	**75 µL**	**100 µL**	**Control**
Bacillus subtilis	14	16	19	22	20
Staphylococcus aureus	16	18	21	25	20
E. coli	15	18	20	23	20
Pseudomonas aeruginosa	15	18	20	22	25
Candida albicans	15	18	21	24	23
A. Niger	16	19	22	26	23

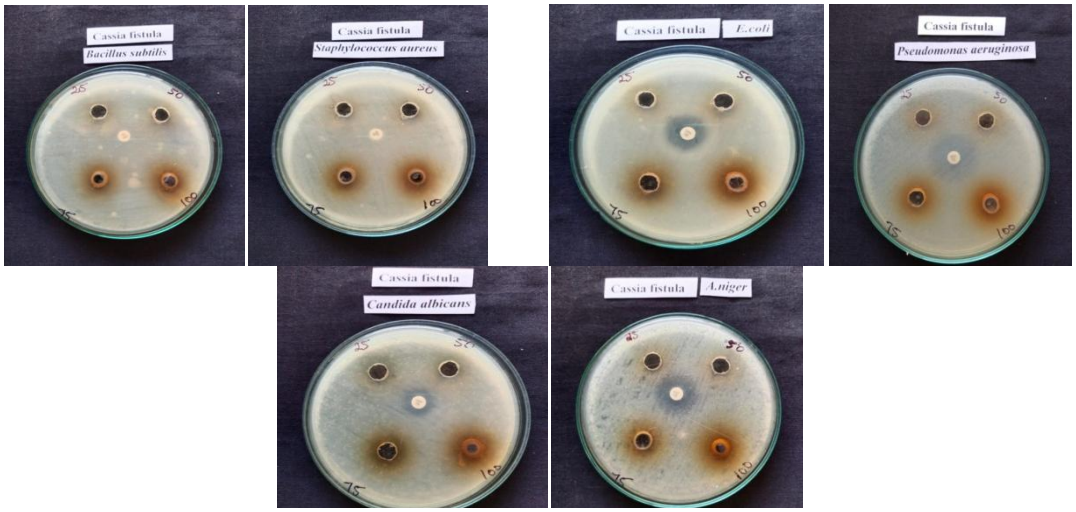

Figure 5: Antimicrobial activity of flower extract of *Cassia fistula.*

Table 3: Antidiabetic activity of aqueous extract of *Cassia fistula* flower.

Volume of the extract (µg/mL)	Absorbance at 540 nm		Percentage inhibition (%)	
	Standard	Extract	Standard	Extract
10	0.215	0.222	7.7253	4.7210
20	0.188	0.208	19.3133	10.7296
30	0.153	0.182	34.3347	21.8884
40	0.129	0.146	44.6351	37.3390
50	0.088	0.107	62.2317	54.0772

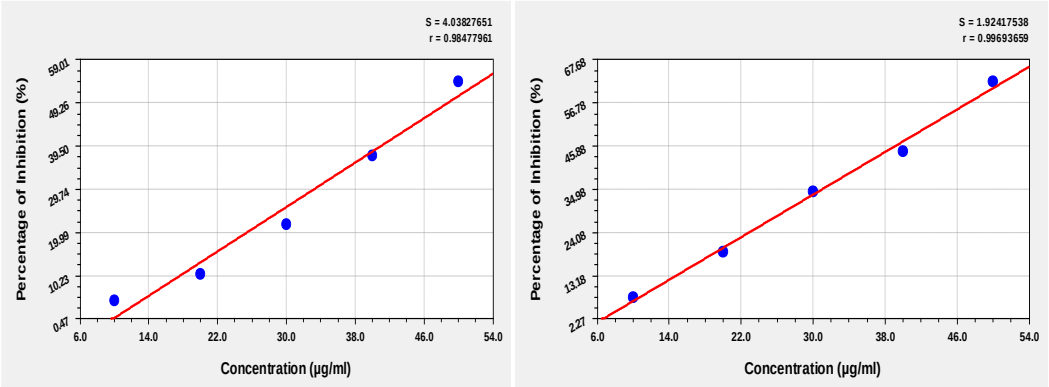

Figure 6: Graph of concentration of flower extract/standard and percentage of inhibition.

Various concentrations of *Cassia fistula* flower extract were evaluated for their antidiabetic activity using the α-amylase enzyme inhibition method. An *in vitro* α-amylase assay was carried out using 10, 20, 30, 40, and 50 µg/mL of *Cassia fistula* flowers and acarbose as a standard drug. The aqueous extract of *Cassia fistula* flower exhibits an inhibitory activity of α-amylase as IC_{50} 49.3494 µg/mL, whereas acarbose exhibits an IC_{50} 42.1726 µg/mL. Maximum inhibition of 54.07% was found at a concentration of 50 µg/mL of extract, which is compared with standard acarbose, which shows 62.23%. The IC_{50} value serves as a crucial parameter for assessing the efficacy of substances in biological functions. The IC_{50} value obtained for *Cassia fistula* extract is considered decent, indicating a high potency to inhibit 50% achievement at a lower concentration. While IC_{50} is a valuable metric, it is only one aspect of an extract's profile. It should be evaluated in conjunction with other factors to assess its therapeutic potential for antidiabetic treatment fully. Therefore, the obtained IC_{50} value for *Cassia fistula* flower is essential for understanding the extract's potency, effectiveness, and potential for development into therapeutic agents. Diabetes can be treated by reducing the production and absorption of glucose in the body. This can be done through the inhibition of enzymes like α-amylase, which helps digest carbohydrates. The results show that a significant amount of flower extract inhibits the a-amylase enzymes; hence, it can be used for the therapeutic approach to diabetes mellitus (37,38). The flowers of *Cassia fistula* could be used as an alternative natural antidiabetic agent as the disease is genetic, and a high number of cases have been recorded in recent years. Future research could focus on isolating and characterizing bioactive compounds from *Cassia fistula* extract and elucidating their mechanism of action. Additionally, optimizing the extraction method and formulating *Cassia fistula* for pharmacological uses could be explored.

4. CONCLUSION

Cassia fistula flower contains a significant source of bioactive compounds. The traditional medicinal system is becoming increasingly apparent as a global concern. Because of its low toxicity and widespread usage of its therapeutic properties, *Cassia fistula* could be one of them. UV and IR characterization of the aqueous extract of *Cassia fistula* flowers was used to predict the absorbance pattern and functional present groups. The antimicrobial activity of the *Cassia fistula* flower extract shows a maximum zone of inhibition against tested microbes, especially against fungi. The findings show that the anti-diabetic activity of the aqueous extract reveals high alpha-amylase inhibitory activity. It can be used as a green medicine for diabetes mellitus. It can be concluded that the aqueous extract of *Cassia fistula* flower can be used as a better alternative for treating diabetes mellitus and is also effective for pathogenic disorders.

5. CONFLICT OF INTEREST

The authors declare that they have no conflict of interest in this work.

6. ACKNOWLEDGMENTS

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