

Phytochemical Investigation and Antibacterial Activity of *Cassia fistula* Leaf Extract Against RTI Pathogens

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Abstract

Cassia fistula is a medicinal plant possesses various phytochemical components those were responsible for the pharmacological activities of the plant. In the present study, plant leaves were collected and extracted by using hot and cold aqueous followed by phytochemical analysis were determined by following standard protocols which revealed the presence of alkaloids, carbohydrates, flavonoids, phenols, tannins and saponins. Antibacterial activities of leaf extract were determined against RTI pathogens such as *Staphylococcus aureus* and *Klebsiella pneumonia* by well diffusion method. Results revealed that hot aqueous leaf extract showed significant activity against pathogens. Hence, the study provides alternative natural solution to the current problems associated with respiratory infection causing pathogen. Separation and identification of bioactive components by chromatographic and FTIR analysis will be a further research process.

Keywords: *Cassia fistula*, phytochemical analysis, Antibacterial activity, RTI pathogens.

I. INTRODUCTION

Respiratory tract infection (RTI) is considered as one of the major public health problems. In recent years, the infectious diseases were leading in morbidity and mortality in many developing countries due to antibiotic resistant ability of some microorganisms [6]. Antimicrobial products of plant origin have been extensively studied nowadays as a therapeutic potential to subside the side effects and owing to resistance of pathogenic microorganisms against synthetic antibiotics. In order to combat the disadvantages in discriminate use of antibiotics, there is several other treatment options have been used now a days. The use of plants for therapeutic purposes was one of the oldest practices of humankind. Plants naturally possess various chemical in nature those compounds used to treat various microbial infections. One such plant was *Cassia fistula* commonly known as golden shower tree of the family *Caesalpinaceae* [8]. *Cassia fistula* is known to have various bioactive components such as alkaloids, carbohydrates, flavonoids, glycosides, phenols, saponins, steroids and tannins. This component was

found to be a factor responsible for antimicrobial properties of *Cassia fistula* [7].

Other components *Cassia fistula* include anthraquinones, methyl inositol, luteolin, pentacosane, fistulic acid, rhein, sennosides A and B, emodin, phlobaphenes, chrysophanic acid, babaloin, lupeol, beta-citosterol, 2-methyl, pibthiophane, fistuacacidin, isopropyl acetate, luteolin pentacosane and galactomannan. *Cassia fistula* plant is widely reported to use against skin diseases, liver problems, rheumatism, malaria, ulcers and diabetes etc. In addition *Cassia fistula* has various pharmacological activities including anti-bacterial, anti-fungal, anti-inflammatory, anti-oxidant and anti-tumor [1], [9].

II. MATERIALS AND METHODS

2.1 Collection of Plant Materials

Leaves of *Cassia fistula* were collected in a sterile polyethylene bags in and around Tirupur region, Tamilnadu. The collected samples were thoroughly washed with distilled water and shade dried. The shade dried leaves were grounded with sterile

blender until turn into fine powder and stored in an air tight container.

2.2 Extraction of Leaf Materials

Leaf powder was extracted by using hot and cold aqueous at 1:20 ratio. About 25 gms of *Cassia fistula* leaf powder were added in 500 ml hot aqueous at 80°C and cold aqueous respectively and placed in a rotary shaker for 48 hours. After 48 hours, the solution was filtered with filter paper and subjected into soxhlet apparatus for extraction purpose. After extraction, the residue of the extract was collected and stored for future purposes.

2.3 Qualitative Phytochemical Analysis

The leaf extract of *Cassia fistula* were used to analyse the presence of following phytochemical components: Alkaloids, Carbohydrates, Flavonoids, Glycosides, Phenols, Saponins, Steroids and Tannins.

2.3.1 Assay for alkaloids (Dragendroff's test)

About 1 ml of leaf extract was mixed with 1 ml of potassium bismuth iodide solution (Dragendroff's reagent) and shaken well. Formation of orange red precipitation would indicate positive [4].

2.3.2 Assay for carbohydrates (Molisch's test)

About 2 ml of leaf extract were mixed with 3 drops of Molisch's reagent and 2 ml of concentrated sulfuric acid. Formation of red color ring at the interphase of the two layers would indicate positive [2].

2.3.3 Assay for flavonoids (Alkaline reagent test)

About 0.4 ml of leaf extract was mixed with 2 ml of 2% sodium hydroxide. To this, 3 drops of diluted hydrochloric acid were added. Formation of yellow color became colorless after the addition of 3 drops of diluted hydrochloric acid which indicate positive [3].

2.3.4 Assay for glycosides (Keller-Kiliani test)

About 1 ml of leaf extract was mixed with 1 ml glacial acetic acid and 1 drop of ferric chloride. To this, 1 ml of concentrated sulfuric acid was added, formation of brown color ring would indicate positive [2].

2.3.5 Assay for phenols (Ferric chloridetest)

About 2 ml of leaf extract was mixed with 1 ml of 1% ferric chloride. Formation of blue green color would indicate positive [5].

2.3.6 Assay for saponins (Foam test)

About 0.5 ml of leaf extract was mixed 5 ml of distilled water and vigorously mixed. Formation of stable foam would indicate positive [5].

2.3.7 Assay for steroids (Liebermann-Burchard test)

About 1 ml leaf extract was mixed with 0.4 ml chloroform and 2 drops of sulfuric acid. Formation of red color would indicate positive [3].

2.3.8 Assay of tannins (Ferric chloridetest)

About 1 ml of leaf extract was mixed with 2 ml of 10% ferric chloride. Formation of blue black color would indicate positive [5].

2.4 Collection of RTI Pathogens

Cassia fistula leaf extracts were used against respiratory tract infection causing human pathogens such as methicillin resistant *Staphylococcus aureus* (MRSA) (MTCC 15029) and *Klebsiella pneumoniae* (MTCC 8911). They were collected from the Microbial type culture collection and genebank (MTCC), Chandigarh, India and stored at - 4°C for further research purposes.

2.5 Antibacterial Activity of Leaf Extract by Well Diffusion Method

Antibacterial activities of hot and cold aqueous extract of *Cassia fistula* were performed by well diffusion method against *Staphylococcus aureus* and *Klebsiella pneumoniae*. Mueller hinton agar medium were prepared and poured in sterile petri plates and allowed for solidification. 0.1 ml of 1 hour each bacterial culture were swabbed over the agar surface. 4 well with 6 mm in diameter were punched with the help of cork borer in all petriplates. About 25, 50, 75, 100 µl of leaf extract were added to each wells. Methicillin and kanamycin were act as positive control. Plates were incubated at 37°C for 24 hours. After incubation, different levels of zones were measured.

III. RESULT AND DISCUSSION

3.2 Extraction of Plant Materials

Cassia fistula leaf crude extract was obtained which is shown in figure 8 and stored for future purposes. Hot aqueous extract of *Cassia fistula* showed high zone of inhibition among all other solvents [2]. High level of bioactive components extracted by using hot aqueous as a solvent system [1].

3.3 Phytochemical Analysis

Phytochemical components were determined by preliminary qualitative phytochemical analysis. Results were shown in Table. *Cassia fistula* hot

aqueous leaf extract revealed presence of alkaloids, carbohydrates, flavonoids, phenols, saponins and tannins whereas absence for glycosides and steroids. *Cassia fistula* cold aqueous extract showed presence for the same but absence for carbohydrates, glycosides and steroids. Results were revealed that *Cassia fistula* leaf possessed various bioactive components those were responsible for the antimicrobial activity of plant leaf. *Cassia fistula* hot aqueous extract showed presence of various phytochemical components compare to other non polar solvents [3]. Hot aqueous leaf extract of *Cassia fistula* absence for glycosides and steroids [2].

Table 1: Qualitative phytochemical analysis *Cassia fistula* leaf extract
3.5 Antibacterial activity *fistula* showed highest zone of inhibition

S. No	Phytochemical components	Hot aqueous extract	Cold aqueous extract
1	Alkaloids	+	+
2	Carbohydrates	+	-
3	Flavonoids	+	+
4	Glycosides	-	-
5	Phenols	+	+
6	Saponins	+	+
7	Steroids	-	-
8	Tannins	+	+

Antibacterial activity of *Cassia fistula* leaf extract was determined by well diffusion method against RTI pathogens such as *Staphylococcus aureus* and *Klebsiella pneumoniae*. Hot aqueous extract showed 7 and 9 mm zone of inhibition at 100 µl concentrations against *Staphylococcus aureus* and *Klebsiella pneumoniae*. About 4 mm zone of inhibition were observed in cold extract at 100 µl concentrations. Organisms showed completely resistant against Methicillin and kanamycin. When compare to cold extract, hot aqueous extract of *Cassia*

against RTI pathogens. Hot aqueous extract were found to be effective against *Staphylococcus aureus* and *Klebsiella pneumoniae*. Phytochemical components were responsible for the antibacterial activity of the leaf extract. Hot aqueous extract of *Cassia fistula* showed highest inhibition activity over cold aqueous extract [1]. *Cassia fistula* leaf extract possess various phytochemical components which is responsible for the antimicrobial activity of leaf extract [2].

Antibacterial Activity of Leaf Extract –Well Diffusion Method

Table 2: Zone of inhibition by leaf extract of *Cassia fistula* leaf extract

RTI pathogen	Extract type	Leaf extract concentration in μl				Control
		25	50	75	100	
Zone of inhibition in mm						
<i>Staphylococcus aureus</i>	Hot	3	5	6	7	R
	Cold	2	3	3	4	R
<i>Klebsiella pneumoniae</i>	Hot	0	4	7	9	R
	Cold	0	2	3	4	R



A. 25 μl leaf extract B. 50 μl leaf extract C. 75 μl of leaf extract D. 100 μl of leaf extract E. Control

Figure 1: Antibacterial activity of hot aqueous extract against *Staphylococcus aureus* and *Klebsiella pneumoniae*



CONCLUSION

Based on the above research work, hot aqueous leaf extract of *Cassia fistula* showed potential activity against RTI causing MDR pathogens. Our findings provide a promising platform for the development of effective and eco friendly antimicrobial agents to inhibit infection by RTI causing MDR pathogens. When compare to other commercial antimicrobial drugs, plant materials showed better inhibition activity with no side effects. The findings in this study support the traditional therapeutic way of treatment against respiratory tract infections. Separation and identification of bioactive components by chromatographic and FTIR analysis will be our future research process.

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