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Quantitative, qualitative phytochemical analysis and antibacterial activity of *Cassia fistula* L

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Abstract
In the present study medicinal plants of *Cassia fistula* L were collected from Thanjavur, Thanjavur (Dt), Tamilnadu. *In vitro* antibacterial activity were performed by agar well diffusion method against five human pathogenic bacterial cultures such as *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris* and *Staphylococcus aureus*. The methanolic leaves extract of *Cassia fistula* L. showed highest antibacterial activity against *Escherichia coli.* Phytochemical analysis of leaves extracts of *Cassia fistula* L. revealed the presence of alkaloids, phenols, proteins, saponins, sterols and tannins. Antibiotics are an essential part of medicine. However, bacterial resistance emerges and reduce efficacy of antibiotics in the human population.

Keywords: medicinal plants, solvents, bacterial cultures, phytochemical analysis, antibacterial activity

Introduction

Plants have been utilized to treat many diseases and ailments for thousands of years because they are a natural source of numerous therapeutic compounds. Because medicinal plants contain bioactive chemicals (secondary metabolites) that are primarily responsible for various healing effects, they have been intensively explored as a source of safe and effective medicine. Medicinal plants contain a variety of bioactive components and are a major source of novel medications and health-care products (Tiwari *et al*., 2008, Rahman *et al* 2020) [21, 8].

Non-nutritive plant compounds with disease-preventive or protective qualities are known as phytochemicals. These substances are produced by plants to protect themselves, but new research shows that many phytochemicals can also protect humans from disease. Plants and plant derivatives have long been utilized as sources of medicine. More than 80% of the world's population, especially in developing countries, relies on traditional plant-based medicines for their primary healthcare needs, according to the World Health Organization (WHO).

Medicinal plants play an important role in the pharmaceutical industry as manufacturers of chemicals that are used as drug development precursors. Plants that have been employed in the treatment of human ailments in various traditional, complementary, and alternative systems. Plants include a wide range of secondary metabolites, such as tannins, terpenoids, alkaloids, flavonoids, and other compounds that have been shown to have antibacterial activities in *in-vitro* (Dahanukar *et al*., 2000; Cowan, 1999; Tayyiba Afzal *et al*., 2021) [6, 20]. *Cassia fistula* L is an essential medicinal plant utilised in Ayurveda and Chinese Traditional Medicine, among other traditional medical systems. It's a medium-sized deciduous tree with pulpy elongated and rod-shaped fruits and bright yellow blossoms, earning it the name 'Yellow Shower.' (Mwangi *et al*., 2021; Aabideen *et al*., 2021) [17, 1].

Materials and Methods

Plant collection

The leaves of *Cassia fistula* L. were collected from Thanjavur, Thanjavur (Dt), Tamilnadu, India. Brought into the laboratory for further processes.

Sterilization of plant materials

For this experiment, disease-free and fresh plants were chosen. For each solvent extract, including aqueous, about 2gm of fresh and healthy leaves were obtained. The surface was then sanitized for a few seconds with 0.1 percent mercuric chloride or alcohol. The plant materials were thoroughly cleaned with distilled water once more (Three times).

Preparation of plant extracts

In 10ml organic solvents such as chloroform, methanol, and aqueous, two grams of sterilized plant leaves were preserved. The leaves were then ground with a mortar and pestle.

The material from the grind plant was centrifuged for 10-15 minutes at 10,000rpm. It was filtered once again with Whatmann No. 1 filter paper. The supernatant was taken and preserved for future research.

Selection of bacteria

Totally five pathogenic bacterial cultures such as *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris* and *Staphylococcus aureus* were selected for the present investigation.

Preparation of nutrient agar medium

1000mL of Nutrient agar medium was created, and the pH was adjusted to 6.8 with the addition of acid or alkali using a pH metre.

The medium was sterilized in an autoclave at 121°C for 15 minutes at 15 pounds of pressure and then allowed to cool.

Screening for antibacterial activity assay (Agar well diffusion method)

Antibiotic sensitivity test on bacteria

Bauer *et al.,* (1996) [3] used the approach to analyze antibiotic sensitivity tests using conventional antibiotics (gentamycin and streptomycin for bacteria). Each sterile petriplate was filled with sterilized nutrient agar media and allowed to harden. Fresh bacterial cultures with known population counts were distributed over the plates using sterile cotton swabs utilizing the spread plate technique. Then, on the bacterial plates, typical antibiotic discs such as gentamycin and streptomycin were inserted. The plates were then cultured for bacteria for 24 hours at 37°C. The results were examined after the incubation period, and the diameter of the inhibitory zone around the isolates was assessed.

Preliminary phytochemical screening of leaves extracts of *Cassia fistula* L.

To investigate the nature of phytochemical elements present in *Cassia fistula* leaf extracts such as chloroform, methanol, and aqueous extract, preliminary phytochemical analysis was performed. (Brindha *et al*., 1982; Harborne, 1998) [4, 9].

Alkaloids

Two ml of test solution are taken with 2N HCl. Aqueous layer formed was decanted and then added with one or a few drops of Mayer's reagent. Formation of white precipitate or turbidity formed indicates the presence of alkaloids.

Lignins

Phloroglucinol with HCl are added with the test solution. Formation of pink color indicates the presence of lignins.

Phenols

Two ml of test solution with alcohol were added with one drop of neutral ferric chloride (5%) solution. Formation of intense blue color indicates the presence of phenols.

Proteins

Picric acid is added with the test solution. Formation of yellow color indicates the presence of protein.

Saponins

Two ml of test solution were added with H_2O and shaked. Formation of foamy indicates the presence of saponins.

Sterols

Two ml of test solution and minimum quantity of chloroform were added with 3-4 drops of acetic anhydride and one drop of concentrated H_2SO_4. Formation of purple color changes into green color that indicates the presence of steroids.

Tannins

About 0.5 g of the dried powdered sample was boiled with 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added to the filtrate. Development of brownish-green or bluish black color indicates the presence of tannins.

Quantitative determination of the chemical constituents in *Cassia fistula* L.

Determination of alkaloids

Five grams of plant material (Leaves) were placed in a 250ml beaker, along with 200ml of 10% CH_3CO_2H in C_2H_5OH. Covered, the mixture was left to sit for 4 hours. It was then filtered and concentrated on a water bath until it was just a fourth of its original volume. NH_4OH was added in a concentrated form until the precipitation was complete. The precipitate was collected on weighted filter paper and rinsed with dilute NH_4OH once the mixture had settled. The alkaloid precipitate was dried and weighed. By subtracting the percentage of alkaloid, the percentage alkaloid was computed. (Krishnaiah *et al,* 2009) [13].

Determination of flavonoids

At room temperature, ten grams of plant sample (Leaves) were extracted several times using 100ml of 80 percent aqueous methanol. The mixture was then filtered through a filter paper into a 250ml beaker that had been pre-weighed. The filtrate was placed in a water bath and allowed to dry completely before being weighed. The percentage flavonoid was calculated by subtracting the percentage flavonoid from the total flavonoid. (Krishnaiah *et al,* 2009) [13].

Determination of total phenols

One gram of plant samples (Leaves) was cooked for 15 minutes at 70°C in 10 ml of 80% methanol. One milliliter of methanolic extract was mixed with five milliliters of distilled water and 250 milliliters of F.C. reagent and incubated for three minutes. The mixture was then incubated for 1 hour at 25ºC with 1 ml of saturated sodium carbonate (Na_2CO_3) added.

A spectrophotometer set to 725nm was used to test the absorbance of the generated blue color. The standard was phenol, and the amount of phenolics was measured in milligram's per 100 grams of dry weight. (Zieslin and Ben-Zaken, 1993) [23].

Determination of saponins

A 250ml conical flask was filled with 20 grams of plant sample (Leaves). A total of 100 mL of 20% C_2H_5OH was added. At roughly 55°C, the mixture was cooked for 4 hours over a hot water bath with continuous stirring. After that, it was filtered with a Whatman No.42 filter paper. The residue was extracted one more using 200ml of 20% C_2H_5OH.

Over a water bath at around 90°C, the combined extract was reduced to 40ml.

After that, the concentrated extract was transferred to a 250ml separator funnel, where 20ml of $(CH_3CH_2)2O$ was added and violently shaken. The aqueous layer was saved, while the (CH_3CH_2) O_2 layer was thrown away. This purification procedure was carried out once more. (Krishnaiah *et al,* 2009) [13].

The combined n-butanol extract was washed twice with 10ml of 5% NaCl after 60ml of n-butanol was added. The residual solution was then heated in a pre-weighed 250ml beaker over a water bath. After evaporation, the residue was dried to a consistent weight in a Gallenkamp moisture extraction oven (Size 1). The saponin percentage was obtained using the difference method.

Determination of tannins

In a mechanical shaker, one gram of the sample (Leaves) was weighed into 100 ml of water and agitated for one hour. This was then filtered into a 100 mL volumetric flask and brought up to the required concentration. 5 milliliters of filtrate were pipette into a test tube and mixed with 2 milliliters of 0.1 M $FeCl_3$ in 0.1 NaCl and 0.008 milliliters of potassium ferrocyanide. Within 10 minutes, the absorbance was measured at 120 nm. The amount of total tannic acid in the extract was measured in milligram's of tannic acid equivalent per gram of extract. (Van-Burden and Robinson, 1981) [22].

Results

Table: 1 Antibacterial activity of leaves extracts of *cassia fistula* l. against some bacterial species

Bacterial cultures	Solvent Extracts (Zone of inhibition in mm)		
	Chloroform	Methanol	Aqueous
Bacillus subtilis	10	12	11
Escherichia coli	8	14	8
Klebsiella pneumoniae	12	13	10
Proteus vulgaris	8	10	8
Staphylococcus aureus	9	12	8

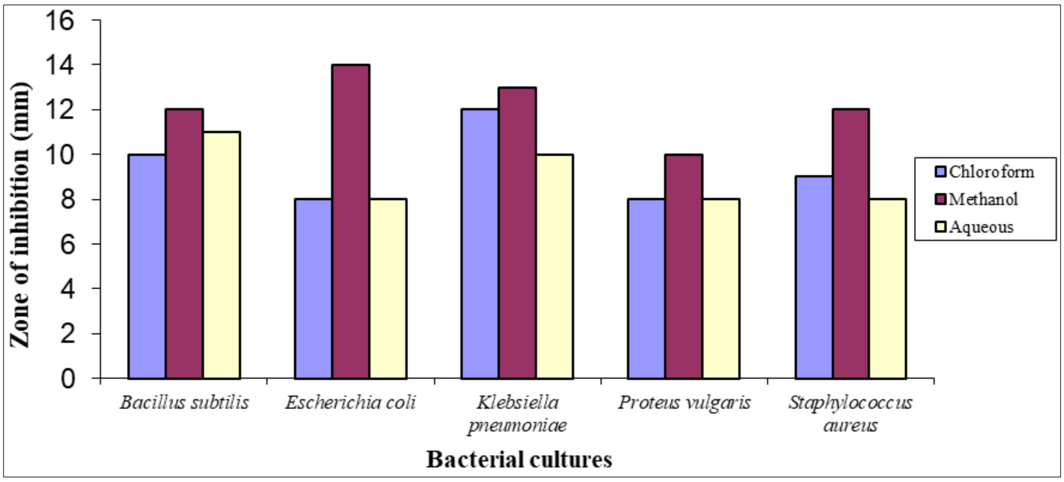

Fig 1: Antibacterial activity of leaves extracts of *Cassia fistula* L. against some bacterial species

Table 2: Quantitative phytochemical analysis of leaves extracts of *Cassia fistula* L

Quantitative Phytocompounds	Percentage (%)
Alkaloids	1.70
Flavonoids	6.58
Phenols	1.9
Saponins	1.4
Tannins	1.2

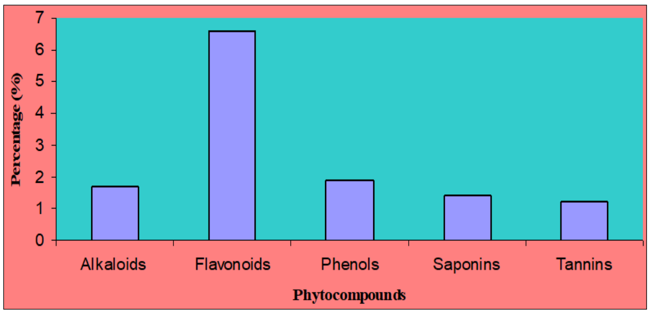

Fig 2: Quantitative phytochemical analysis of leaves extracts of *Cassia fistula* L.

Table 3: Qualitative phytochemical analysis of leaves extracts of *Cassia fistula* L.

Phytocompounds	Chloroform	Methanol	Aqueous
Alkaloids	+	+	-
Lignins	-	-	-
Phenols	+	-	-
Proteins	+	+	+
Saponins	+	+	+
Sterols	-	+	-
Tannins	+	+	+

+ Indicate presence of compounds
- Indicate absence of compounds

Discussion

Because the majority of plant extracts have antibacterial activity, the current study supports the long-term use of herbal medicine. Furthermore, the presence of a high number of phytochemicals in these two botanicals supports their use in the treatment of a variety of disorders. Natural goods and medicinal plants are now a hot topic for the discovery of novel antibacterial agents all around the world. (Kilonzo and Munisi 2021, Sashikala *et al.*, 2009) [12, 18]. This could be linked to antibiotics' recent failure to combat the rapid emergence of multidrug-resistant organisms, as well as the rapid spread of novel illnesses. (Abdallah, 2011) [2]. According to Chaerunisaa *et al.*, 2018 [5], The extract was tested for antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* using the diffusion agar method. The results revealed that the ethyl acetate fraction's Minimum Inhibitory Concentration against *S. aureus* was 0.625%, while the water fraction was more than 10%. The water fraction had a MIC of greater than 10% against *E. coli*, while the ethyl acetate fraction had a MIC of 1.25 percent. The antibacterial activity of the difusion approach was compared to that of amoxicillin, a commonly used oral antibiotic. The ethyl acetate fraction had the most effectiveness against both *S. aureus* and *E. coli*, according to the findings. The study indicated that the potential antimicrobial activities of *Cassia fistula* ethyl acetate fraction demonstrate promising activity in the search for novel antibacterial agents. The antibacterial activity of *Cassia fistula* L leaf extracts (chloroform, methanol, and aqueous) was tested *in-vitro* using the agar well diffusion method with gentamycin and streptomycin as positive controls against chosen bacterial strains (*Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris* and *Staphylococcus aureus*). The outcomes of *Cassia fistula* L. leaf extracts using solvents were shown in table 1, and fig 1 respectively.

Qualitative phytochemical analysis of *Cassia fistula* L.

Recent developments in phytochemical analysis, according to Ishak *et al.*, 2021 [15], have allowed crop researchers to accumulate data due to its ability to identify and discriminate compounds existing within organisms, tissues, or cells. Aside from genotypic qualities, small changes in metabolite abundances caused by biotic or abiotic stimuli will have a major impact on metabolite abundances, which will be visible through physicochemical properties. Stauth (2007) discovered tannins, saponins, flavonoids, phenolics, and alkaloids in the leaves of four medicinal plants under inquiry after conducting phytochemical analyses. Clearly, Mandloi *et al.*, (2018) [14] found the existence of some intriguing secondary metabolites in the stem bark of *Cassia fistula* during qualitative analysis, including tannins, flavonoid, carbohydrate, saponins, glycosides, phenol, and terpenoid. In the current study, qualitative phytochemical analysis of several solvent extracts of *Cassia fistula* L. leaves, such as chloroform, methanol, and aqueous, revealed the existence of secondary metabolites in a variety of ways. A phytochemical examination of *Cassia fistula* L. leaf extracts revealed the presence of alkaloids, phenols, proteins, saponins, sterols, and tannins, but no lignin.

Quantitative phytochemical analysis of leaves extract of *Cassia fistula* L.

According to Herlin Sheeba Gracelin *et al.*, (2013), the *Pteris confusa* extract included ten milligrams of alkaloids, twelve milligrams of flavonoids, nine milligrams of phenolic compounds, six milligrams of saponins, and three milligrams of tannins. There were 12 mg of alkaloids, 14 mg of flavonoids, 10 mg of phenolic compounds, 8 mg of saponins, and 5 mg of tannins discovered in *Pteris vittata* extract. There were 11 mg of alkaloids, 13 mg of flavonoids, 8 mg of phenolic compounds, 7 mg of saponins, and 4 mg of tannins found in *Pteris argyreae* extract. Table 4 shows the results of the quantitative analysis performed on a sample of *Cassia* fistula L. leaves in this study. Total alkaloids, saponins, and phenols were found to include 1.7, 1.4, and 1.9 percent of the total alkaloids, saponins, and phenols, respectively. When compared to tannins (1.2 percent), total flavonoids concentration was found to be greater (6.58 percent).

Antibacterial activity of *Cassia fistula* L. against some bacterial species

The botanical aqueous extract of *Hymenelobium petraeum* inhibited the colony formation of *Staphylococcus aureus*, *Enterococcus faecalis*, and *Salmonella typhi*, according to Oliveira *et al.*, (2013) [16], while the aqueous extracts of *Vatairea guianensis* and *Symphonia globulifera* were active against *Staphylococcus aureus*. *Klebsiella ozaenae* and *Acinetobacter baumannii* were also suppressed by the aqueous extracts of *Ptychopetalum olacoides* and *Pentaclethra macroloba*. Recently, Sharma and Seemarai (2019) [19] used isolated steam distillation and the Clevenger apparatus to evaluate the antibacterial and antifungal activities of two commonly available medicinal plants, Cassia fistula and Bauhinia variegate leaf oil, against commonly pathogenic bacteria (*Proteus vulgaris*, *Pseudomonas aeruginosa*, *Solmonella newport*, and *Salmonella Stanley*) and fungi (*Rhizopus stol)* The disc diffusion method was used to test the antibacterial activity. Throughout the experiment, nutrient agar medium was utilized to sustain the culture as well as for testing. The antibacterial activity of penicillin and tetracycline were compared.

Kamath and Kizhedash 2019 [10] previously observed that the methanolic extract had antibacterial action against *Staphylococcus aureus*. *E. coli*, *Proteus*, and *Pseudomonas aeruginosa* were all resistant to the extract. The extract was also found to be ineffective against *Candida albicans* and *Aspergillus niger*. Khatak *et al.*, (2019) [11] investigated antimicrobial analysis and phytochemical assay in relation to the amount of lovely resources that are squandered or blown away. These plants had moderate to strong antibacterial activity against the pathogens that were tested. Furthermore, the phytochemical study confirmed the presence of bioactive elements that may be used to build medication development procedures and investigate the potential of medical treatment to benefit society. The antibacterial activity of methanol leaves extract against *Escherichia coli* (14mm), *Klebsiella pneumoniae* (13mm), *Bacillus subtilis*, and *Staphylococcus aureus* was the greatest in this investigation (12mm). Significant efficacy against *Klebsiella pneumoniae* (12mm), *Bacillus subtilis* (10mm), and *Staphylococcus aureus* was found in chloroform extract (9mm). The largest zone of inhibition (11mm) was recorded against *Bacillus subtilis*, whereas the least zone of inhibition (8mm) was observed against *Escherichia coli*, *Proteus vulgaris*, and *Staphylococcus aureus*.

Conclusion

The results of this study show that the plant species tested had antibacterial capabilities. Because of the presence of many compounds that are essential for good health, the plant analysed for phytochemical elements appeared to have the potential to operate as a source of helpful pharmaceuticals as well as to improve the health status of customers.

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