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Article

Phytochemical Screening and Antioxidant Activities of *Cassia fistula*

A. Uma Ravi Sankar[1]*, A. B. V. Kiran Kumar[2], M. Ravi[1], C. Raghavendra Reddy[3], K. S. V. Krshna Rao[4]

[1]Department of Humanities and Science, G. Pullaiah College of Engineering and Technology, Kurnool, Andhra Pradesh, India, [2]Vijaya Lab, Buchireddy Palem, Nellore, Andhra Pradesh, India, [3]School of Liberal Arts and Sciences, Mohan Babu University, (Erstwhile Sree Vidyani-Kethan Engineering College), Tirupati, Andhra Pradesh, India, [4]Department of Chemistry, Yogi Vemana University, Vemana Puram, Ganganapalle, Andhra Pradesh, India

ABSTRACT

The plant is rich in flavonoids such as rhein, phenolics (fistulic acid), lignans, glycosides, anthracene derivatives, and catechins, obtained from various parts. These phytochemicals possess pharmacological effects such as hepatoprotective, anti-inflammatory, antioxidant, antipyretic, antitussive activity, antiulcer, central nervous system activity, hypo-lipidaemia, larvicidal/ovicidal, laxative, leukotriene inhibition, anticancer, antidiabetic, antiepileptic, antifeedant, antifertility, urease inhibition, wound healing, antimicrobial, antifungal antibacterial, and antiviral activities. The present review has focused on the discussion of bioactives of the *Cassia fistula* (CF) and the bioeffects on the medicinal claims. Our findings provided evidence that CF aqueous and organic solvent extracts of these tested plants contain medicinally important bioactive compounds and it justifies their use in the traditional medicines for the treatment of different diseases.

Key words: Golden shower extracts, Phytochemicals, TPC, TFC, Antioxidant, DPPH.

1. INTRODUCTION

The plant kingdom is a treasure house of potential drugs, and in recent years, there has been an increasing awareness about the importance of medicinal plants. Drugs from the plants are easily available, less expensive, safe, and efficient and rarely have side effects. The plants that have been selected for medicinal use over thousands of years constitute the most obvious choice in examining the current search for therapeutically effective new drugs such as anticancer drugs [1], antimicrobial drugs [2], and antihepatotoxic compounds. According to the World Health Organization (WHO), medicinal plants are the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicines, which have compounds derived from medicinal plants. However, such plants should be investigated to better understand their properties, safety, and efficiency [3]. Medicinal plants contain some organic compounds that provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids, and flavonoids [4,5]. These compounds are synthesized by the primary or rather secondary metabolism of living organisms. Secondary metabolites are chemically and taxonomically extremely diverse compounds with obscure functions. They are widely used in human therapy, veterinary, agriculture, scientific research, and countless other areas [6]. A large number of phytochemicals belonging to several chemical classes have been shown to have inhibitory effects on all types of microorganisms *in vitro* [7]. Plant products have been part of phytomedicines since time immemorial. This can be derived from barks, leaves, flowers, roots, fruits, and seeds [8]. Knowledge of the chemical constituents of plants is desirable because such information will be valuable for the synthesis of complex chemical substances [9-11]. In the present work, qualitative and quantitative phytochemical analyses and antioxidant studies were carried out with *Cassia fistula* (CF) flower extract.

2. MATERIALS AND METHODS

2.1. Collection of Plant Materials

Fresh parts of the medicinal plant, *Cassia fistula* L. (Flowers), were collected from different regions of Chittoor districts of Andhra Pradesh (AP). The plant materials were taxonomically identified and authenticated by The Department of Life Science, S.V. University, Tirupathi, AP. The plant materials were shade-dried until constant weight achieved which indicates plants were dried. After drying, the plant materials were ground well using a mechanical blender into a fine powder and transferred into airtight containers with proper labeling for future use.

2.2. Solvent Extraction

CF flower extract was prepared by Soxhlet extraction method. About 25 g of powdered Flower material was uniformly packed into a thimble and extracted with 250 mL of different solvents separately [Figure 1]. The solvents used were mixture in equal ratio of methanol, ethanol, and acetone. The process of extraction continues till the solvent in the siphon tube of an extractor becomes colorless. The solvent was then expelled using a rotary evaporator, and the solid was recovered. The residue was subsequently dried under vacuum and stored for further use in phytochemical analysis at 4°C in a refrigerator.

***Corresponding author:**
A. Uma Ravi Sankar
E-mail: drravigpcet@gmail.com

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2.3. Qualitative Phytochemical Analysis

CF flower extract was tested for the presence of bioactive compounds using the following standard methods [12-14].

2.3.1. Test for proteins millon's test

CF flower extract when mixed with 2 mL of Millon's reagent, a white precipitate appeared which turned red on gentle heating confirming the presence of protein.

2.3.2. Ninhydrin test

CF flower extract when boiled with 2 mL of 0.2% solution of Ninhydrin, the violet color appeared suggesting the presence of amino acids and proteins.

2.3.3. Test for carbohydrates Fehling's test

An equal volume of Fehling A and Fehling B reagents were mixed and 2 mL of it was added to CF extract and gently boiled. A brick-red precipitate appeared at the bottom of the test tube indicating the presence of reducing sugars.

2.3.4. Benedict's test

CF Flower extract when mixed with 2 mL of Benedict's reagent and boiled, a reddish-brown precipitate formed which indicated the presence of the carbohydrates.

2.3.5. Molisch's test

CF flower extract was mixed with 2 mL of Molisch's reagent and the mixture was shaken properly. After that, 2 mL of concentrated H_2SO_4 was poured carefully along the side of the test tube. The appearance of a violet ring at the interphase indicated the presence of carbohydrates.

2.3.6. Iodine test

CF Flower extract was mixed with 2 mL of iodine solution. A dark blue or purple coloration indicated the presence of the carbohydrate.

2.3.7. Test for phenols and tannins

CF flower extract was mixed with 2 mL of 2% solution of $FeCl_3$. A blue-green or black coloration indicated the presence of phenols and tannins.

2.3.8. Test for flavonoids Shinoda test

CF flower extract was mixed with a few fragments of magnesium ribbon, and concentrated HCl was added dropwise. The pink scarlet color appeared after a few minutes which indicated the presence of flavonoids.

2.3.9. Lead acetate test

10 mL of extract was taken and a few drops of 10% lead acetate solution was added. Appearance of yellow color precipitate indicates the presence of flavonoids.

2.3.10. Alkaline reagent test

CF flower extract was mixed with 2 mL of 2 % solution of NaOH. An intense yellow color was formed which turned colorless on the addition of a few drops of diluted acid which indicated the presence of flavonoids.

2.3.11. Frothing test for saponins

CF flower extract was mixed with 5 mL of distilled water in a test tube, and it was shaken vigorously. The formation of stable foam was taken as an indication of the presence of saponins.

2.3.12. Test for glycosides Liebermann's test

CF flower extract was mixed with each of 2 mL of chloroform and 2 mL of acetic acid. The mixture was cooled in ice. Carefully concentrated H_2SO_4 was added. A color change from violet to blue to green indicated the presence of a steroidal nucleus, that is, the glycone portion of the glycoside.

2.3.13. Salkowski's test

The CF extract was mixed with 2 mL of chloroform. Then, 2 mL of concentrated H_2SO_4 was added carefully and shaken gently. A reddish-brown color indicated the presence of a steroidal ring, that is, the glycone portion of the glycoside.

2.3.14. Keller-kilani test

CF flower extract was mixed with 2 mL of glacial acetic acid containing 1–2 drops of 2 % solution of $FeCl_3$. The mixture was then poured into another test tube containing 2 mL of concentrated H_2SO_4. A brown ring at the interphase indicated the presence of cardiac glycosides.

2.3.15. Test for steroid (Liebermann and Burchard reaction)

CF Flower extract was mixed with 2 mL of chloroform and concentrated H_2SO_4 was added sidewise. A red color produced in the lower chloroform layer indicated the presence of steroids. Another test was performed by mixing the CF extract with 2 mL of chloroform. Then, 2 mL of each of concentrated H_2SO_4 and acetic acid were poured into the mixture. The development of a greenish coloration indicated the presence of steroids.

2.3.16. Test for terpenoids

CF flower extract was dissolved in 2 mL of chloroform and evaporated to dryness. To this, 2 mL of concentrated H_2SO_4 was added and heated for about 2 min. A greyish color indicated the presence of terpenoids.

2.3.17. Test for alkaloids

CF flower extract was mixed with 2 mL of 1% HCl and heated gently. Mayer's and Wagner's reagents were then added to the mixture. The turbidity of the resulting precipitate was taken as evidence of the presence of alkaloids.

2.3.18. O-toluidine reaction for gum and mucilages

For this test, approximately 5 mg of each sugar was dissolved in 0.5 mL of deionized water in a 3 mL flat-bottomed vial. The vial was heated in a laboratory oven at 100°C for 5 min and allowed to cool. The liquid was then centrifuged for 1 min and three drops of the supernatant were mixed with 0.5 mL of O-toluidine solution. The solution was then heated in an oven for 10 min at 100°C. Gum exudates and mucilages are yield a brown color reaction.

2.3.19. Coumarins test

When sodium hydroxide solution is added to the test sample, it produces a strong yellow color that fades to colorless when hydrochloric acid is added, which may also indicate the presence of Coumarins.

3. QUANTITATIVE PHYTOCHEMICAL ANALYSIS

3.1. Total Phenolic Content

The amount of phenol in the aqueous extract was determined by the Folin–Ciocalteu reagent method with some modifications. 2.5 mL of 10% Folin–Ciocalteu reagent and 2 mL of 2% solution of Na_2CO_3 were added to 1 ml of flower extract. The resulting mixture was incubated for 15 min at room temperature. The absorbance of the sample was measured at 765 nm. Gallic acid was used as standard (1 mg/mL). All the tests were performed in triplicates. The results were determined from the standard curve and were expressed as gallic acid equivalent (mg/g of extracted compound) [15].

3.2. Total Flavonoid Content

The aluminum chloride colorimetric method was used with some modifications to determine flavonoid content. 1 mL of sample Flower extract was mixed with 3 mL of methanol, 0.2 mL of 10% aluminum chloride, 0.2 mL of 1M potassium acetate, and 5.6 mL of distilled water and remained at room temperature for 30 min. The absorbance was measured at 420 nm. Quercetin was used as standard (1 mg/mL). All the tests were performed in triplicates. Flavonoid contents were

determined from the standard curve and were expressed as quercetin equivalent (mg/g of extracted compound) [15].

4. RESULTS AND DISCUSSION

Golden shower or Indian laburnum is scientifically well known as CF and belongs to the Family Fabaceae, subfamily Caesalpinoidae. It has pronounced medicinal properties and is rich in bioactive metabolites with diversified biological activities. CF is an ornamental as well as an important component in the forest ecosystem which blooms in summer and gives an aesthetic view. All parts of the plant are medicinally useful in the treatment of fevers, heart diseases, biliousness, hemorrhages, ulcers, wounds, piles, fistula, and skin diseases, in traditional medicine.

Table 1 illustrates the results of phytochemical analysis of CF extracts of CF containing alkaloid, carbohydrate, flavonoid, proteins and diterpenes, fat and fixed oils, saponins, and tannins in CF. The phytoconstituents of biomolecules CF flower extracts such as flavonoids, saponins, alkaloids, steroids, tannins, and terpenoids. The examined enriched sources of biocomponents are given in [Table 1]. CF plant extracts play a major role because it contains various types of phytochemicals such as "CF" which generally produce several secondary metabolites such as alkaloids, flavonoids, phenols, tannins, saponins, and quinines which are important sources of biocides and many other pharmaceutical drugs. These significant secondary plant metabolites have typical biological activity. Moreover, traditionally, the powdered leaves are used to heal circumcision wounds and as an antiseptic for wounds, pruritus, skin rashes, and in the supervisory control of microbial infections.

Phytochemical analysis conducted on the CF extracts revealed the presence of constituents that are known to exhibit medicinal as well as physiological activities [12]. Analysis of the Flower extract revealed the presence of phytochemicals such as phenols, tannins, flavonoids, saponins, glycosides, steroids, terpenoids, and alkaloids. The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites [16]. They possess biological properties such as antiapoptosis, antiaging, anticarcinogen, anti-inflammation, antiatherosclerosis, cardiovascular protection, and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities [17]. Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds [18,19]. Natural antioxidants mainly come from plants in the form of phenolic compounds such as flavonoids, phenolic acids, and tocopherols. [20]. Tannins bind to proline-rich proteins and interfere with protein synthesis. Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they are antimicrobial substances against a wide array of microorganisms *in vitro*. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls [7]. They also are effective antioxidants and show strong anticancer activities [21-23]. The plant extracts were also revealed to contain saponins which are known to produce inhibitory effects on inflammation [24]. Saponins have the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include the formation of foams in aqueous solutions, hemolytic activity, cholesterol-binding properties, and bitterness [23,25]. Steroids have been reported to have antibacterial properties [26], and they are very important compounds especially due to their relationship with compounds such as sex hormones [27]. Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity [28]. Several workers have reported the analgesic [14,29], antispasmodic and antibacterial [30,31] properties of alkaloids. Glycosides are known to lower blood pressure according to many reports [32]. The results obtained in this study thus suggest the identified phytochemical compounds may be the bioactive constituents and this plant is proving to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit.

5. ANTIOXIDANT EVALUATION

5.1. 1, 1-diphenyl-2-picrylhydrazyl) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) Method

The radical scavenging activity of DPPH was measured. Extract solutions were prepared by dissolving different dry extracts in

Table 1: Phytochemical analysis of *Cassia Fistula* leaves, fruit pulp, and flower extracts.

S. No	Phytochemicals	Name of the test	C. F. extracts		
			CF.L	CF.FP	CF.F
1	Alkaloids	Mayer's and Wagner's test	+	+	+
2	Anthra quinones	Borntrager's test	-	+	-
3	Carbohydrates	Molisch's test	+	+	+
		Benedict's test	+	+	+
4	Coumarins	Alkali test (Using NaOH)	-	-	-
5	Fatty acids	Paper test	+	+	-
6	Flavonoids	Alkaline reagent test	+	+	+
		Lead acetate test	+	+	+
7	Glycosides	Keller-Killani test	-	-	+
8	Gum and Mucilage	O-Toluidine test	-	-	-
9	Phenols	Ferric chloride test	+	+	+
10	Protein and Amino acids	Ninhydrin test	+	+	+
11	Saponins	Frothing test	-	-	+
12	Steroids	Liebermann and Burchard reaction	-	+	+
13	Tannins	Using $FeCl_3$	-	+	+
14	Terpenoid's	Salkowski's test	+	+	+

+: Present, –: Absent

methanol to produce a solution of 10 mg/mL. In this experiment, 600 µM of DPPH was dissolved in 300 mL of methanol and used as a stock solution. The plant extract in methanol at various concentrations (1, 2, 3, 4, and 5 mg) whose final volume was maintained at 1 mL was mixed with an aliquot of 2 mL of 600 µM of DPPH solution in methanol and incubated at 25°C for 30 min. The absorbance of the test mixture was read at 517 nm using a spectrophotometer against a DPPH control containing only 1 mL of methanol in place of the extract. All experiments were performed thrice, and the results were averaged. Ascorbic acid was used as a standard. DPPH scavenging effect was calculated by the following equation given by [32];

$$DPPH\ scavenging\ effect = \left(\frac{The\ absorbance\ of\ sample - absorbance\ of\ blank}{Absorbance\ of\ sample}\right)\times 100$$

5.2. Hydrogen Peroxide H_2O_2

The optimum conditions were chosen as the best for the reaction catalyzed by the horse radish peroxidase. When plant extracts were added to these systems to test their ability to scavenge H_2O_2 molecules, these conditions were maintained. Phenol (12 mM) and 4-amino antipyrine (0.5 mM) were chosen and utilized for all of the aforementioned experiments since they were the most effective. The resulting chromophore had the highest intensity due to the concentrations. Plant extracts and conventional antioxidants were used to calculate the percentage inhibition (percent I) of H_2O_2 induced by them. The reaction mixture, which included the test sample (plant extract/standard antioxidant; 350 mL), phenol solution (12 mM, 350 mL), 4-amino antipyrine (0.5 mM, 100 mL), H_2O_2 (0.7 mM, 160 mL), and HRP (1 U/mL), was incubated at 37°C for 30 min. At 504 nm, the absorbances of the resultant solutions were compared to a reagent blank made out of phosphate buffer instead of plant extract/standard antioxidant and phenol. Plant extract was substituted with phosphate buffer in the control, which was prepared using the same reagents. The following steps were taken to reduce interference from plant extracts in the assay: Background subtraction samples were produced using plant extract with additional reagents substituting phenol with phosphate buffer for each concentration of plant extract. The original absorbance reading was subtracted from each of the resultant absorbance values. Different kinds of plant extracts that are known for their antioxidant characteristics were evaluated for their H_2O_2 scavenging capabilities. The percentage inhibition of hydrogen peroxide was calculated by the equation as described for many antioxidant assays.

$$\%\ Inhibition = \left(\frac{The\ absorbance\ of\ sample - absorbance\ of\ blank}{Absorbance\ of\ sample}\right)\times 100$$

5.3. Quantitative Phytochemical Analysis Experiment

5.3.1. DPPH inhibition assay of CF

Analysis of variance parameters revealed the percentage of DPPH inhibition in the flowers and fruit pulp of CF was statistically highly significant ($P < 0.01$). The results showed a significantly higher inhibition percentage in the flowers of CF (21.825A ± 0.069) as compared to its fruit pulp, which demonstrated a lower percentage (10.660B ± 0.533) in C.

Hydrogen peroxide (H_2O_2) scavenging activity of CF flowers and fruit pulp. Hydrogen peroxide (H_2O_2) scavenging activity in flowers and fruit pulp of CF was found to be highly significant ($P < 0.01$) as shown in Figure 2. The results showed that higher hydrogen peroxide (H_2O_2) scavenging activity was observed in CF flower (22.642A ± 0.146) and leaves (15.650B ± 0.127) as compared to fruit pulp (48.326C ± 0.163) CF leaves, which demonstrated less activity as indicated in Figure 2. Furthermore, the results confirmed that H_2O_2 scavenging activity was higher in fruit pulp than flower and leaves.

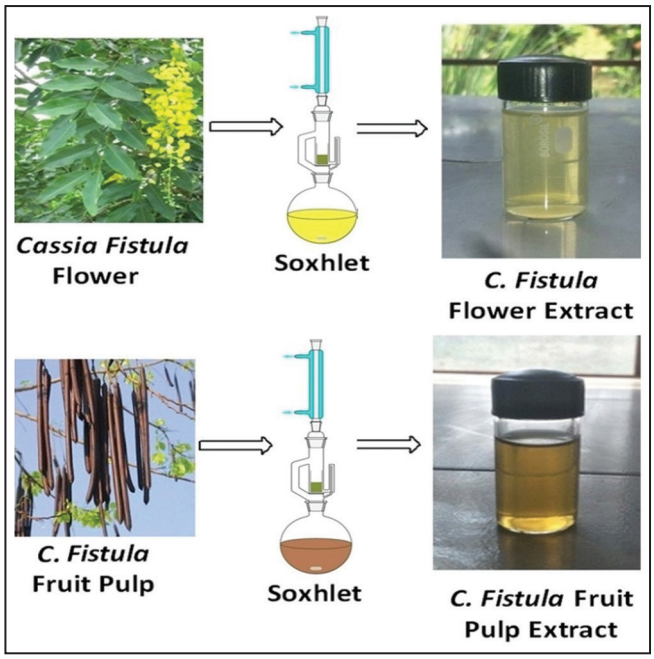

Figure 1: Extraction of *Cassia Fistula* Fruit pulp and flower.

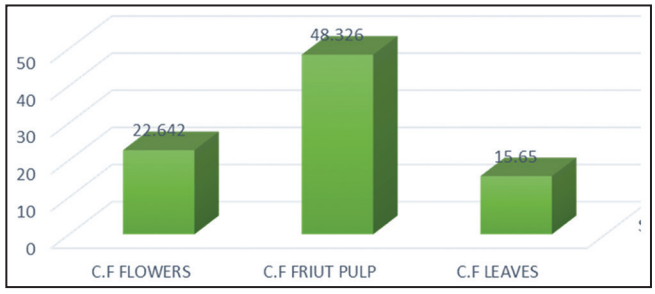

Figure 2: Graphical representation of Hydrogen peroxide (H_2O_2) scavenging activity in flowers, fruit pulp, and leaves of *Cassia fistula*.

6. CONCLUSION

The results revealed the presence of medicinally important constituents in the plants studied. Much evidence was gathered in earlier studies which confirmed the identified phytochemicals to be bioactive. Several studies confirmed the presence of these phytochemicals contributes medicinal as well as physiological properties to the plants studied in the treatment of different ailments. Therefore, extract from this plant could be seen as a good source of useful drugs. The traditional medicine practice is recommended strongly for these plants as well and it is suggested that further work should be carried out to isolate, purify, and characterize the active constituents responsible for the activity of these plants. Furthermore, additional work is encouraged to elucidate the possible mechanism of action of these extracts.

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***Bibliographical Sketch**

Dr. Arigala. Uma Ravi Sankar, presently working as a Assistant professor, Department of Chemistry in G. Pullaiah College of Engineering & Technology, Kurnool, A.P, India. He also worked as a Assistant professor of Chemistry in Sree Vidyanikethan Engineering College, Mohan Babu University, Tirupati, A.P, India. Before entering into this group, I worked a Research professor with Prof. Tae Jeong Kim, Kyungpook National University, South Korea during June 2012-May 2013 and successfully developed "New MRI contrast agents" and BK21 Research Professor with Prof. Kuk Ro Yoon, in Hannam University, Daejeon, South Korea, May, 2010-May 2012. I worked on the Synthesis of Nano Material. Before entering into this group, I worked a post doc with Prof. Mitsuji Yamashita, Shizuoka University, Japan during January 2008-March 2010 and successfully developed "New MRI contrast agents for diagnosis the early stage of cancer" and the results were published. I was awarded with Doctoral Degree in December 2007 by Sri Venkateswara University; Tirupati, India for my PhD thesis entitled "A Study on the Synthesis and Bio-Activity of New Organophosphorus Acyclic /Heterocyclic Compounds" and published nearly 15 reports during my PhD work and 20 reports during my Post.doc work. I am well experienced in the field of synthetic organic chemistry, transition metal chemistry and bioorganic chemistry of several organic substrates. He attended many regional seminars and International Conferences for presenting her research work at Japan 2010, South Korea, 2012, India 2023.

Dr. A.B.V. Kiran Kumar is an entrepreneur and researcher.He has 15 years of post-PhD experience, in nanomaterials, chemistry research and teaching. He has guided 15 master and one PhD student all of his students are good in doing publications. He has national and international collaborations. he published 38 publications and 4 patents (filed) on various fields such as nanocomposites, RGO Nanocomposites, supercapaciotrs, Photo- catalysis and nano anti-biotics. When he return to India, he joined at Amity Institute of Nanotechnology, Amity University Delhi. He is working on three emerging nanotechnology research areas; those are ENERGY, WATER and MEDICAL applications. As part of research in Energy, he is developing electrode materials for energy storage, he also working on cost-effective photo catalysts to degrade organic dyes and medical pollutants;. As part of medical field research, developing nanoantibiotics and membranes for Wound healing applications.