Biomedical & Pharmacology Journal, December 2024. Vol. **17**(4), p. 2515-2525

Phytochemicals, Anti-microbial and Anti-oxidant Investigations on *Cassia fistula* Fruit Pulp Extracts

Nirmala Arunachalam[1]*, Shivashankar Jayasankar[1], Jayaprakash Rajendran[2] and Radhamani Thamilarasan[1]

[1]Department of Biotechnology, Aarupadai Veedu Institute of Technology, Vinayaka Mission's Research Foundation (Deemed to be University), Paiyanoor, Chennai, Tamil Nadu, India.
[2]Department of Chemistry, School of Arts and Science, Vinayaka Mission's Chennai Campus, Vinayaka Mission's Research Foundation (Deemed to be University), Paiyanoor-603104, India.
*Corresponding Author E-mail: drnirmala81@gmail.com

https://dx.doi.org/10.13005/bpj/3044

(Received: 13 August 2024; accepted: 31 October 2024)

Plants are used as medicinal substances in many cultures and operate as a valuable reservoir for several powerful drugs owing to the existence of specific bioactive components. The process of discovering novel medications starts with the discovery of bioactive compounds derived from natural sources. *Cassia fistula* (Cf), also known as Konrai, is a natural plant belonging to the Caesalpiniaceae family. It is a significant tropical fruit and is renowned for its therapeutic characteristics. The current research aims to assess the phytochemical composition of the Cf fruit pulp by analysis of its aqueous and chloroform extracts. Additionally, the study will investigate the antioxidant and antimicrobial properties of these extracts. The analysis indicates the presence of beneficial phytochemicals except Cardiac glycosides, Polyphenol, and Anthroquinone. In FT-IR analysis, the phytochemical profile of the chloroform extracts demonstrated the existence of important phytochemical functional groups. Around 30 substances were found in the GCMS of the extract. Likely, the DPPH assay of the fruit pulp extracts exhibits the highest concentration (750μg/ml) and good antioxidant activity. Further, the antimicrobial activity was evaluated against disease-causing stains *Staphylococcus aureus, E.coli, A.niger* and *C. albicans*. The extract exhibited strong antibacterial activity in the chloroform extract compared to the aqueous extract. The findings indicated that chloroform extracts exhibited significant antifungal activity against *A.niger* in comparison to *C.albicans*. The aforementioned investigation validates that the chloroform extracts of Cf fruit pulp contains a diverse range of phytochemicals and other active functional groups, which are used for different therapeutic applications.

Keywords: Antioxidants activity; Cassia fistula; Medicinal properties; phytochemical analysis; Pulp extract.

Plants and the components they contain have been employed in traditional medicine for as long as humans have lived. Various medicinal plants with biological features such as antioxidant, anti-inflammatory, and anti-diabetic have shown therapeutic results in the management of health [1,2]. Different phytochemicals, sometimes referred to as secondary metabolites, are found in plants. Because they can work alone, in combination, or synergistically to promote health,

phytochemicals can be helpful in the treatment of many diseases [2]. Phytochemicals are essential for the pharmaceutical industries for formulation of novel medications and medicinal substance [3]. Various active phytochemicals of plant extracts are showing significant biological activities, such as anti-inflammatory, anti-diarrheal, anti-ulcer, and anticancer properties[4]. The golden shower tree, *Cassia fistula* Linn, is one such therapeutic plant found in nature. In tropical Africa, Bangladesh, Pakistan, and India, *Cassia fistula* (Cf) is a widely planted plant. The bulk of the naturally occurring bioactive compounds from Cf plant are secondary metabolites, and they are being used in medicines, dietary supplements, antibacterial properties and other useful industrial products [5,6]. Reports of medicinal plants' antimicrobial qualities are approaching from all over the world. By looking at Cf, this study seeks to increase the variety of antibacterial agents made from natural resources, a member of the Caesalpiniaceae subfamily of Leguminosae [7, 8]. It has been suggested that this plant can aid with skin diseases, liver issues, tuberculosis, haematemesis, pruritus, leucoderma, and diabetes[9].

Cf extract is used as an antiperiodic and to treat rheumatism. It has been established that plant components may be used as a medication to treat hyper cholesterolemia, in part due to their high fiber and mucilage content[10]. Hepato protective, anti-implantation, laxative, anti-inflammatory, smooth muscle stimulant, antiarthritic, antibacterial, antipyretic, antitussive, analgesic, antifungal, antiviral, and hypoglycemia are among the pharmacological effects of *Cassia fistula* [11, 12]. Fruits can help to treat diabetes, act as an antipyretic, abortifacient, demulcent, and reduce body heat. They can also help with chest pain, throat issues, liver problems, eye disorders, and grasping problems [13,14]. According to the previous literature, a lot of scientific studies were conducted on different parts of Cf plant but very limited study was witnessed in *Cassia fistula* fruit pulp. The current study examined the "Phytochemical analysis of Cf fruit pulp and its anti-oxidant and anti-microbial property" based on the previously mentioned information.

MATERIALS AND METHODS

Collection and Preparation of Sample

For the study purpose, Cf Fruit (Fig-1(a) was collected from the forest area in and around Chennai, Tamil Nadu, India, where it was found naturally (Fig-1(b). The plant specimen authentication was confirmed in Plant Anatomy Research Centre (PARC) Chennai, by denoting the placed specimen. The voucher number of the specimen is PARC/ 2022/ 4819. Segregation of fruit pulp from fruit pod (Fig -2a) has been done. They were cut into small pieces. It was shade-dried for 4 -5 days and ground to a fine powder using a Mixer or Blender. To facilitate additional analysis, the sample was kept in a sterile container.

The shade-dried powdered fruit pulp materials were extracted using two solvents -water and Chloroform at room temperature. The extract of the Cf fruit pulp was prepared by soaking 10 g of fine powder in 100 ml of water and chloroform respectively and kept in Rotary Shaker apparatus for 24 hours as shown in Fig-2(b).

Phytochemical Analysis

To identify the different preliminary phytochemical constituents (Hexane, petroleum ether and water extracts) such as Terpeniods, Carbohydrates, Phytosterol, Alkaloids, Quinone, Saponin, Steriods, Flavonoids, Cardiac Glycosides, Glycosides, Anthroquinones, Polyphenols. Chemical tests were carried out on the plant extracts using standard procedure [15].

Characterization of CF pulp extract

Fourier Transform Infrared (FTIR) Procedure

It is possible that Fourier Transform Infrared Spectrophotometer (FTIR) most efficient technique for identifying the different kinds of chemical bonds, commonly referred to as functional groups, present in various compounds. In this analysis, solvent extracts from dried powdered fruit pulp were employed. To prepare the samples, 10 ml of the extract was enclose within a 100 mg KBr pellet, resulting in translucent discs. Each pulverized samples from the plant specimens was then analyzed using an FTIR spectroscope (Shimadzu, IR Affinity 1, Japan), which operates with a resolution of cm^{-1} and a scanning range from 600 to 3600 cm^{-1}.

Gas Chromatography-Mass Spectrometer (GC-MS) Analysis

In the realm of phytochemical analysis and chemotaxonomic studies of medicinal plants containing biologically active compounds, the use of GCMS is essential. Before the Gas Chromatography-Mass Spectrometer (GC-MS) examination, roughly 1-gram dry extract was suspended in ethyl acetate, filtered through Whatman filter paper, and nitrogen-purged to eliminate any remaining ethanol. 1.0 µL sample was introduced into a Shimadzu GCMS-QP2010 Ultra fitted with a Restek fused silica capillary column (30 m in length, 0.25 mm in diameter, and 0.25 film thickness made entirely of diphenyl dimethyl polysiloxane). The oven temperature was adjusted at a rate of 5 °C per minute, ranging from 70 °C (2 minutes) to 280 °C (30 minutes). The carrier gas, helium (99.999%), was employed at a steady flow rate of 1.84 mL min^{-1}. 200°C was the ion source temperature, 119.2 kPa pressure, split injection mode (1:10), and injector temperature. Mass spectra were obtained between 40 and 600 m/z at a scan period of 0.5 s at 70 eV. For the extract, two duplicate injections total 35 minutes of GC running time were made. The relative percentage quantity of each component was ascertained by comparing its average peak area to the total area. The identification process involved in comparing the unknown component's spectrum to the recognized compound's spectra found in the National Institute Standard and Technique database.

DPPH assay

The total capacity of the extracts from various plant samples to scavenge free radicals was assessed based on a previously established method [16], with minor modifications, utilizing the stable DPPH radical. Prepare the 0.1 mM of DPPH solution in methanol and add 100 µl of this solution to 300 µl of the solution of samples respectively at different concentration (5, 10,15,20 µg/ml). The reaction mixture was allowed to incubate for 30 minutes in a dark environment at room temperature. Then the absorbance has to be measured at 517 nm using a UV-VIS spectrophotometer (Ascorbic acid can be used as the reference). Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. The free radical scavenging activity of the extracts was indicated by the reduction of the initial purple hue. The capability of scavenging the DPPH radical can be calculated by using the following formula.

$$\text{Scavenging activity (\%)} = [\text{Absorbance (control)} - \text{Absorbance (sample)} / \text{Absorbance (control)}] \times 100$$

Antibacterial Activity

Using the agar well-diffusion method, the antibacterial activity of the extracts was evaluated against Gram-positive bacteria *Staphylococcus aureus*, Gram-negative bacteria *Escherichia coli*, fungus *sps* of *Candida albicans* and *Aspergillus niger* . *Staphylococcus aureus* and *Escherichia coli* were grown on nutrient agar (Himedia, India); whereas, *Candida albicans* and *Aspergillus Niger* were grown on Sabouraud Dextrose Agar. Antibacterial activity of extracts against bacterial strains was performed on Mueller Hinton agar. After that, the culture plates were incubated for 24 hours at 37 °C. *Aspergillus niger* cultures plates were kept at room temperature for three days. After incubation, the zone of inhibition(mm) was measured and recorded in each plate. The mean value of the three replicate trials was used to express the results.

Determination of minimum inhibitory concentration

Based on the ZOI findings, the minimum inhibitory concentration (MIC) of the extracts against *Staphylococcus aureus* was determined using the broth dilution technique. In summary, a 0.5 McFarland: 1.5 × 108 CFU/mL *Staphylococcus aureus* culture was introduced to the nutritional broth, which contained extracts diluted at different concentrations (10^{-1} to 10^{-7}). The lowest concentration that prevents the organisms from growing visibly is known as the sample minimum inhibitory concentration or MIC. Using a UV-visible spectrophotometer, the growth of *Staphylococcus aureus* in the tubes was observed at 600 nm following a 24-hours incubation period. The minimum inhibitory concentration (MIC) was identified as the lowest concentration that demonstrated greater than 75% suppression of organism development.

Determination of minimum bactericidal concentration

For the purpose of measuring the

minimum bactericidal concentration (MBC) of the extracts against *Staphylococcus aureus*, fresh nutrient agar plates were cultured with the broth used for the MIC test. MBC is the lowest medication concentration at which 99.9% of the tested microorganisms are killed. Every experiment was run three times, and the mean values were used to express the results.

RESULTS AND DISCUSSION

Phyto constituents Screening of *C. fistula* fruit pulp extracts

To identify the presence of various chemical groups, the aqueous (A-CFFP) and chloroform (C-CFFP) extracts of the Cf fruit pulp were subjected to preliminary phytochemical testing. Terpenoids, carbohydrates, phytosterol, alkaloids, quinone, saponin, steroids, flavonoids, cardiac glycosides, glycosides, anthraquinones, and polyphenols were all phyto chemicals analyzed in both extracts.

Aqueous and Chloroform extracts of Cf fruit pulp were subjected to phytochemical analysis (Table -1). The finding shows the presence of bioactive phytochemicals like terpenoids, carbohydrates, phytosterol, quinone, saponin, and cardiac glycosides but lacks anthroquinone, polyphenols, and cardiac glycosides. Alkaloids, flavonoids, and terpenoids are thought to have significant antioxidant properties. According to previous report Cf fruit pulp contains more or less the same components of phytochemicals such as saponin, triterpenoids, steroids, glycosides, anthraquinone, flavonoids, gum, mucilage, proteins and amino acids[17].Another study reported that Cf plant is rich in phenolic compound, antioxidants such as anthraquinones, flavonoids and flavan

Fig. 1. (a) Collection of *C. fistula* Fruit, (b) *C. fistula* plant fruit with flower

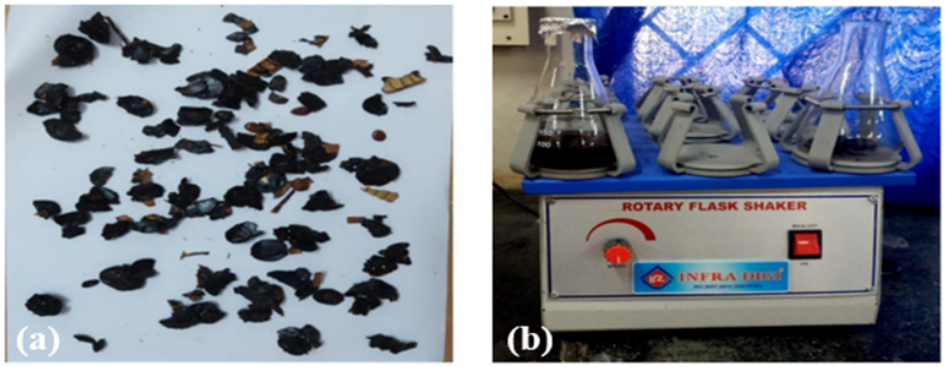

Fig. 2. a). Segregation of fruit pulp from fruit pod b). Extraction of *C. fistula* fruit pulp using chloroform and water

3-ol derivatives. The results shows positive for alkaloids, terpenoids, reducing sugars, saponins, tannins, carbonyl, phlobatanin, and steroids [18]. In the present study also, we observed the presence of many phytochemicals.

FTIR spectrum of *Cassia fistula* fruit pulp extracts

The functional molecules of the phytochemicals present in the plant extracts or other materials are identified using FTIR characterization studies. The peak values and most likely functional groups present in the fruit pulp of Cf are displayed in the FTIR spectrum of the chloroform extract. A peak was visible in the FT-IR spectra of chloroform extract of the Cf fruit pulp at 3286.70cm $^{-1}$ which indicates the O-H alcohol group stretching; the peak at 2314.58cm $^{-1}$ which indicates the Alkyne group; the peak at 1635.64cm $^{-1}$ which indicates the Alkene group C=C; the peak at 678.94 cm $^{-1}$ which indicates the

Table 1. Phytochemicals analysis of *Cassia fistula* fruit pulp extracts

Active Functional Group	Aqueous fruit pulp extract of *Cassia fistula*	Chloroform fruit pulp extract of *Cassia fistula*
Terpenoids Test	++	+++
Carbohydrate Test	++	++
Phytosterol Test	+	+++
Alkaloid Test	-	++
Quinone Test	++	++
Saponin Test	+	++
Steroids Test	++	++
Flavonoids Test	+	++
Cardiac Glycosides Test	+	-
Glycosides Test	-	+
Anthroquinone Test	-	-
Polyphenol Test	-	-

(-) Absent, (+) Present, (++) Moderate present , (+++) Highly present

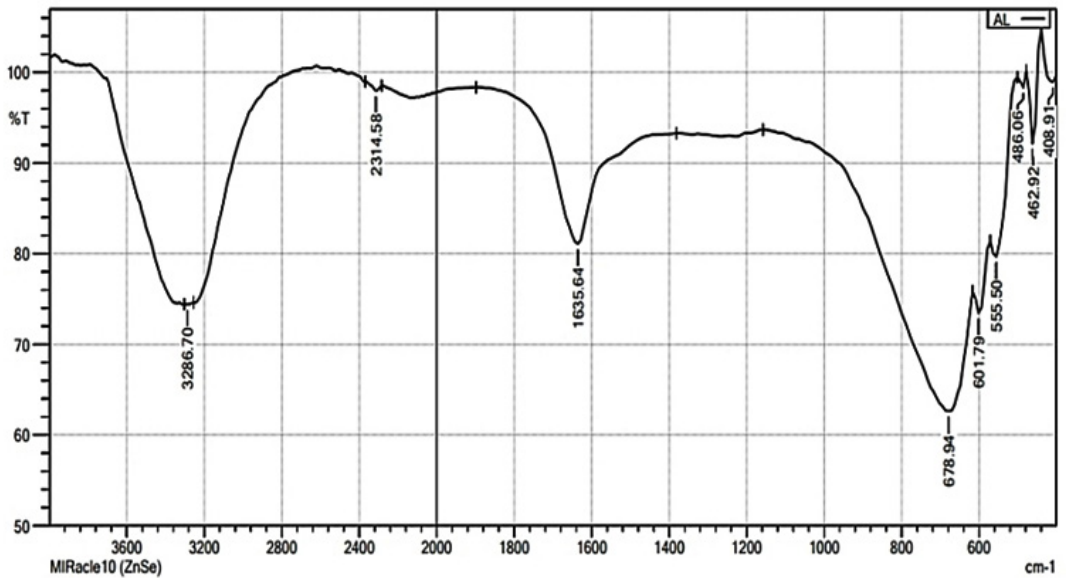

Fig. 3. FTIR spectrum of *Cassia fistula* fruit pulp extracts

halogen compound [iodo- compound CC-11]; the peak at 555.50 cm $^{-1}$ which indicates the halogen compound [chloro-compound CC-1]; The bending at 408.91 cm^{-1} which indicates the presence of NH functional group in the Chloroform extracts of Cf fruit pulp(Fig-3).

Functional molecules of the chloroform extract of Cf leaves were reported by previous study [18]. Phenols are O-H stretch alcohols with an H bond, and this is related to the strong and wide band observed at 3258 cm^{-1}. At 25 absorption peaks of 1042 and 878cm^{-1}, there are narrow bands that have been recognized as C-N stretched aliphatic amines and N-H primary and secondary amines, respectively. This work concludes that functional groups including carboxylic acids, amines, and alcohol are present in the chloroform extract of Cf. These functional groups are connected to the bioactive phytochemicals present in it. In the current study, we found that the chloroform extracts of Cf fruit pulp had a broadband, which indicates the stretching of the O-H alcohol group, and the least narrow band, which shows the presence of NH functional group. In previous study report says that the active functional groups found in *C. fistula* hexane extract include phenols, alcohols, carboxylic acids, ethers, and ester. The leaf extract's bioactive phytochemicals are linked to these functional groups[19].

Gas Chromatography & Mass Spectrometer (GC-MS) Analysis of Chloroform extracts of *Cassia Fistula* Fruit Pulp

Table 2. Phytochemical constituents identified in Chloroform extracts of *Cassia fistula* fruit pulp by using GC-MS analysis

Retention time	Area (mv.s)	Height (mv)	Compound Name	Molecular Formula
3.027	179.112	2.168	2-Ethoxyacetaldehyde	$C_4H_8O_2$
4.000	2.399	0.374	Cyclopent-one	C_5H_{10}
5.487	9418.607	1902.217	Not identified	Not identified
6.670	10.687	0.831	Tricosane	$C_{23}H_{48}$
7.247	22.024	1.073	Butanoic acid	$C_4H_8O_2$
7.587	27.746	1.578	Not identified	Not identified
8.373	10.625	0.378	(2E)-3,7-dimethylocta-2,6-dien-1-ol	$C_{10}H_{18}O$
9.040	22.233	1.400	Not identified	Not identified
9.913	32.067	1.844	3,7,11-trimethyldodeca-2,6,10-trien-1-ol	$C_{15}H_{26}O$
10.837	143.230	3.782	Undecanoic acid	$C_{11}H_{22}O_2$
11.697	9.358	0.572	Cyclonoxiloxane Octadecane	$C_{18}H_{38}$
12.823	2.176	0.183	6-methoxycyclohexane-1,2,3,4,5-pentol	$C_7H_{14}O_6$
13.637	105.977	3.925	1-Dodecanol	$C_{12}H_{26}O$
14.743	88.602	3.131	3,7,11,15-tetramethylhexadec-2-en-1-ol	$C_{20}H_{40}O$
15.187	102.252	3.183	Tetracontane	$C_{40}H_{82}$
16.247	45.955	1.215	n-Decanoic acid	$C_{10}H_{20}O_2$
17.570	26.613	1.116	1,4-ditert-butylbenzene	$C_{14}H_{22}$
18.613	375.956	20.481	Phytol-3,7,11,15-tetramethylhexadec-2-en-1-ol	$C_{20}H_{40}O$
19.527	76.354	2.602	Octadecanoic acid	$C_{18}H_{36}O_2$
20.027	44.018	1.993	3-hydroxy-3-methylpentanedioic acid	$C_6H_{10}O_5$
20.443	8.106	5.429	1,3-diaminothiourea	CH_6N_4S
22.040	160.128	5.266	4,11,11-trimethyl-8-methylidenebicyclo[7.2.0]undec-4-ene	$C_{15}H_{24}$
26.510	5964.756	47.243	5-isothiocyanatopentylbenzene	$C_{12}H_{15}NS$
28.067	4010.379	44.131	7,11,15-trimethyl-3-methylidenehexadec-1-ene	$C_{20}H_{38}$
28.710	8367.747	192.802	2,3-dihydroxypropyl octanoate	$C_{11}H_{22}O_4$
0.687	5.841	0.503	Not identified	Not identified
30.917	3062.129	143.482	Cholest-7-en-3-ol C27H46O	$C_{27}H_{46}O$
33.180	9864.300	136.368	Not identified	Not identified
34.460	2669.126	50.992	Phytol	$C_{20}H_{40}O$
36.040	117.332	5.059	n-propyl-9,12-octadecadienoate	$C_{21}H_{38}O_2$

A gas chromatogram derived from the chloroform extracts of Cf fruit pulp revealed many peaks, each of which represented a distinct chemical or functional component (Fig.4). Mass spectroscopy revealed the presence of 30 compounds in chloroform extracts of Cf fruit pulp(Table -2). Gas Chromatography and Mass Spectrometer (GC-MS) screening of the Chloroform extract of Cf fruit pulp revealed that the presence of compounds such 2-Ethoxyacetaldehyde, Cyclopent-one, tricosane, butanoic acid, (2E)-3,7-dimethylocta-2,6-dien-1-ol,Undecanoic acid,Cyclonoxiloxane Octadecane,6-methoxycyclohexane-1,2,3,4,5-pentol,1-Dodecanol,Tetracontane,n-Decanoicacid,1,4-ditert-butylbenzene,and Octadecanoic acid, remaining more components are present in it. The identification of compounds was based on their peak area (which represents the percentage of that compound), Retention time, Height, Compound name and molecular formula suggesting that Cf fruit pulp extracts showed the presence of many functional compounds and

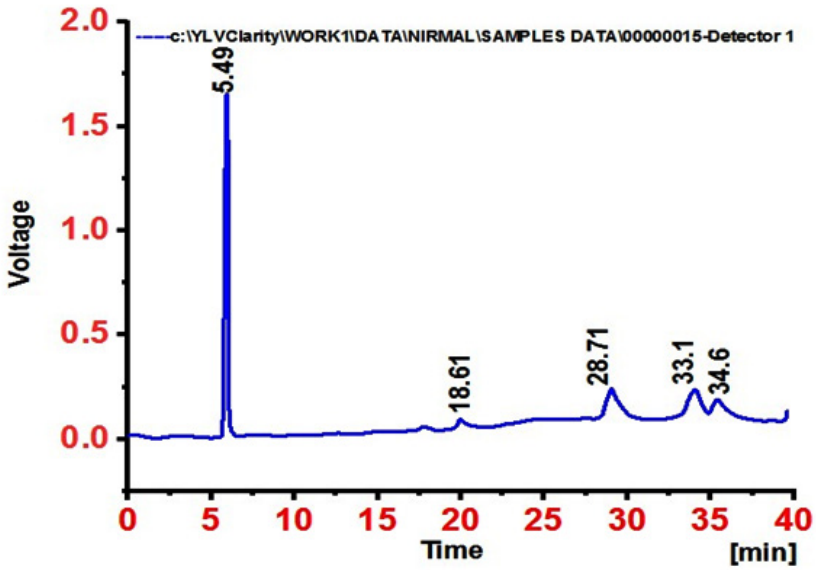

Fig. 4. Processed GC-MS- Spectrum *C. fistula* fruit pulp Chloroform extract with high voltage peaks

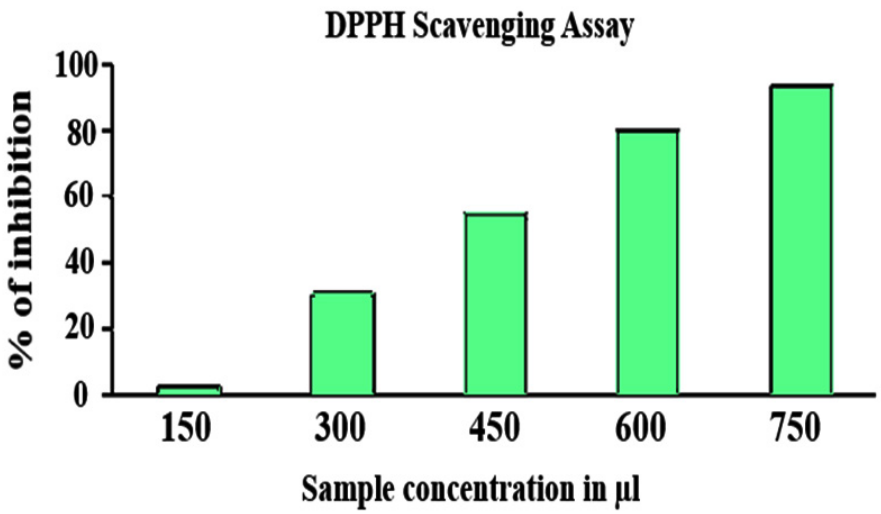

Fig. 5. DPPH assay (C-CFFP) shows a linear relation between concentration and % of inhibition

phytochemicals. It could be responsible for the therapeutic effects of the plant.

According to previous research GC-MS analysis of chloroform extracts of Cf leaf revealed the presence of 13 components at various retention times[20.] The another research reported that nearly thirty one chemicals were found in the n-hexane fraction of the methanolic flower extract of *C. fistula,* according to the GC-MS chromatogram. With a peak area of 17.81%, butanoic acid, methyl ester, was the main component in this fraction. This substance is part of the methyl ester group of fatty acids. The majority of this group's members have insecticidal[21] antifungal[22] and antibacterial properties[23]

Antioxidant activity of Chloroform extract of *Cassia fistula* fruit pulp

The percentage of the antioxidant activity of the fruit pulp of chloroform extracts shows increased (150, 300, 450, 600, and 750 µg/ml) antioxidant activity in a dose-dependent manner (Figure -5). At 150 µg/ml, the fruit pulp extract exhibited a percentage inhibition of 2.5, while at 450 µg/ml, it was 55. The fruit pulp extract's 50% inhibition concentration (IC50) values were determined to be 2.5 µg/ml and 55 µg/ml, respectively.

Table 3. Antibacterial activity of the *Cassia fistula* fruit pulp extracts (A- CFFP and C- CFFP)

S.no	Microorganism	Zone of Inhibition (mm)							
		Aqueous extract (µg/ml)				Chloroform extracts (µg/ml)			
		5	25	50	100	5	25	50	100
1.	S. *aureus*	-	13	14	16	-	15	16	20
2	E. *coli*	-	12	13	15	-	12	14	17

Table 4. Antibacterial activity of standard drugs

S.no	Drug (µg/mL)	Zone of Inhibition (mm)							
		S. aureus				*E. coli*			
		5	25	50	100	5	25	50	100
1.	Ampicillin	14	16	19	23	12	16	18	21
2	Chloramphenicol	13	15	17	20	13	16	18	19

Table 5. Antifungal activity of *Cassia fistula* fruit pulp extracts (A- CFFP and C- CFFP)

S.no	Microorganism	Zone of Inhibition (mm)							
		Aqueous extract (µg/ml)(A-CCFP)				Chloroform extracts (µg/ml)(C- CFFP)			
		5	25	50	100	5	25	50	100
1.	A. *niger*	-	13	14	17	-	16	18	19
2	C. *albicans*	-	15	16	18	-	12	15	17

Table 6. Antifungal properties of common medications

S.no	Drug (µg/mL)	Zone of Inhibition (mm) against selected micro-organisms(Fungal sps)							
		A. *niger*				C. *albicans*			
		5	25	50	100	5	25	50	100
1.	Greseofulvin	12	15	18	20	13	15	19	22
2	Nystatin	13	18	20	23	12	18	19	22

The plant extract has a good linear relationship between concentration and inhibitory activity, according to analysis. Based on the stable DPPH free radical absorbing an electron from molecules, antioxidant activity is calculated. It can be distinguished visually by turning from brown to yellow. The antioxidant activity in this study was attributed to flavonoids, tannins, alkaloids, and phenols; these compounds destroy free radicals by donating an electron to DPPH. As a result, it is evident from the graph that the maximum concentration of the Cf chloroform extract has a greater ability to scavenge free radicals, specifically DPPH. The antioxidant activity of the Cf plant's flower extract was mentioned in the early study[17] .In past study report says that, flavonoids, phenols, and tannins function as free radical scavengers and may be the cause of the effects[24]. Other studies have shown that tannins, flavonoids[25] phenol [23, 26, 27,] saponins, and tannins have strong anti-inflammatory, antibacterial, and antioxidant properties. To assess the antioxidant qualities of Cf bark, stem, leaf, and root, a separate study was carried out. The findings indicated that extracts from the bark of various age groups exhibited greater antioxidant activity compared to the other plant parts [28].

A recent study assessed Cf Linn.'s antioxidant potential and ability to shield erythrocytes from hydrogen peroxide-induced oxidative damage. According to the results, Cf aqueous extract had a 75% antioxidant and protective efficacy, while its ethanolic extract had a strong antioxidant activity and shielded erythrocytes from damage by over 90% [24]. We found that the A- CFFP and C-CFFP extracts from Cf exhibit strong antioxidant properties as concentrations rise in this investigation as well.

Zone of inhibition (ZOI) against selected bacteria

At varying dosages, such as 5 µg/ml, 25 µg/ml, 50 µg/ml, and 100 µg/ml, the antibacterial activity of the Cf fruit pulp extract (A-CFFP and C-CFFP) was evaluated against Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*). The extracts' antibacterial activity dose in a linear fashion as their concentration (µg/ml) increased (Table 3). When the obtained results were contrasted with those of prescription medications, it became clear that both extracts showed strong antibacterial activity (Table -4).

Zone of inhibition (ZOI) against selected Fungus

The fruit of Cf has antibacterial action, according to earlier study report [29.] Cf at a 500 µg/ml concentration using the agar dilution streak method. Just *E. coli* was only slightly inhibited, although *B. subtilis* as well as S. *epidermidis* no inhibition. Nonetheless, our study demonstrated strong antibacterial efficacy against the two selected bacterial species. Earlier scientific study reported that use of disc diffusion and MIC techniques to examine the antibacterial qualities of Cf [30]. The inhibition against *S. aureus* was stronger in alcoholic extracts as compared to aqueous extract. Furthermore, we found that, in comparison to aqueous extract, the fruit pulp of Cf exhibited higher inhibition in chloroform extract.

The efficacy of the Cf extracts (aqueous and chloroform) against certain fungus species was evaluated by measuring their zone of inhibition. As the quantities of the extracts (5 µg/ml, 25 µg/ml, 50 µg/ml, and 100 µg/ml) increased, their antifungal activity increased in a linear fashion. (Table -5). When the results were contrasted with those of common antifungal medications, it became clear that both extracts showed strong antifungal action (Table -6).

Finding of minimum inhibitory concentration (MIC)

From the ZOI results, MIC of both Cf fruit pulp extract (A- CFFP and C- CFFP) against *Staphylococcus aureus* were determined by microdilution method. The MIC result represents that the growth of *Staphylococcus aureus* was inhibited (above 75%) by A- CFFP and C-CFFP at a dilution factor of 10^{-3} to 10^{-4} and 10^{-4} to 10^{-5}, respectively. It confirms that C- CFFP exhibits high efficiency (high inhibition at low concentration) compared to A-CFFP.

CONCLUSION

From the current investigations concluded that the fruit pulp of Cf includes a variety of active phytochemicals. The results of this study demonstrate the existence of certain phytochemicals with biological activity that may have a significant therapeutic index. When creating novel medication formulations, the plant may be

a safer substitute. Except for cardiac glycosides, polyphenol, and anthraquinone, a qualitative phytochemical examination disclosed the existence of alkaloids, saponins, flavonoids, carbohydrates, steroids, quinone, phytosterol, and terpenoids. FT-IR and GC-MS analyses were used to observe and confirm their active functional groups and phytoconstituents. The DPPH assay of the fruit pulp extracts exhibits the highest concentration (750 µg/ml) and good antioxidant activity. The extract exhibited strong antibacterial activity in the chloroform extract compared to the aqueous extract. However, toxicological research and pharmacological activity testing should be done after isolating the pure chemical before use in humans. However, more research is required to more accurately assess the potential efficacy of the crude extracts.

ACKNOWLEDGMENT

The authors are grateful to Aarupadai Veedu Institute of Technology, Tamil Nadu, India for providing lab facilities and technical administrative support to complete the work successfully.

Funding Sources

The author(s) received no financial support for the research, authorship, and/or publication of this article

Conflict of Interest

The author(s) do not have any conflict of interest.

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials

Authors contribution

Nirmala Arunachalam- work design , guiding the entire work , Manuscript preparation; Shivashankar Jayasankar Research Work done by the candidate; Jayaprakash Rajendran- Manuscript preparation and technical support; Radhamani Thamilarasan- Manuscript preparation and support

REFERENCES

1. Rahmani A.H and Aly S.M. *Nigella sativa* and its active constituents thymoquinon shows pivotal role in the diseases prevention and treatment. *Asian. J. Pharm. Clin Res*., 2015; 8 : 48–53.
2. Rahmani A.H, Aly S.M, Ali H, Babiker A.Y, Srikar S and Khan A.A. Therapeutic effects of date fruits (Phoenix dactylifera) in the prevention of diseases via modulation of anti-inflammatory, anti-oxidant and anti-tumour activity. *Int. J. Clin. Exp. Med*.,2014;7 : 83–91.
3. Al-Bukhari M.I. Division 71 on medicine. In: Al-Bukhari S, editor. The Collection of Authentic Sayings of Prophet Mohammad (Peace be Upon Him) 2nd ed. Ankara, Turkey: Hilal Yayinlari .1976;81: 435-73.
4. Satyavati G.Y and Sharma M. In Medicinal plant in India ICMR. NEW DELHI .1989;12: 5-32.
5. Vashista P.C. Taxonomy of Agisosperme. P.B.M Press, New DELHI, India balunas M.J.and A.P. Kingholn .2005; 193.
6. Dahanukar S.A, Kulkarani R.A and Rege N.N. Pharamacology of medicine plants and natural produce. *Indian.J. Pharmacol*.2000; 32: S81.
7. Collett Henry. col. Sir. Flora simlensis (Flowering plants of simala) Bengal army New Connaught place , deharadun: *Bishen singh mahandrapal singh* . 1971; 108.
8. Duuta A.C. Botany for degree students 6 th ed. Calcutta Oxford university press. 2002; 558.
9. Asolkar L.V, Kakkar K.K and Chakre O.J. Second supplement to glossary of Indian medicinal plants with active principles. New Delhi publication and information directorate, CSIR. 1992; 177.
10. EL-Saadany S.S, BL –massry R.A, Labib S.M and Sitohy M.Z. The biochemical role and hypo cholesterolaemic potential of the legume cassia fistula in hypercholesterolaemia rats. *Die. Nahrung.* 1991; 35: 807-15.
11. Pandey G, Dravyaguna vijiana 3 rd ed. Reprint part 3. Varanasi: chowkhamba krishnadas Academy. 2005; 167.
12. Lavekar G.S. Database on medicinal plants in Ayurveda and siddha . vo; 6. Central for research in Ayurveda and siddha department of AYUSH, Ministry of health and family welfare , government of India .2009; 183.
13. Kirtikar K.R and Basu B.D. Indian medicinal plants. International book distributors, 2006.

14. Padma S. *Indian .J .of Microbiol.* 2006; 46(26): 169-170.
15. Harborne J.B. Textbook of Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis. 5th Edition. Chapman and Hall Ltd, London,1998; 21-72.
16. Dahanukar S.A, Kulkarni R.A and Rege N.N. Pharmacology of Medicinal Plants and Natural Products. *Indian J Pharmacol.* 2000; 32: S81-118.
17. Bhalodia N.R, Nariya P.B, Acharya R.N and Shukla V.J. Evaluation of in vitro Antioxidant activity of Flowers of *Cassia fistula* Linn . *Inter. Journ. of Pharm Tech Research*., 2011; 3(1): 589-599.
18. Ashraf Ali, *Cassia fistula* linn: A review of phytochemical and pharmacological studies,Inter. Jour. of Pharm. Science and Research ., 2014; Vol. 5(6): 2125-2130.
19. Sujatha J and Asokan S.Phytochemical analysis and Antioxidant effect of hexane extract of Cassia fistula using FT-IR and GC-MS analysis, Int. J. pharmaceutics & drug analysis, Vol.6 ISSUE 2, 2018; 173 – 179 ;
20. Sujatha. Antioxidant effect and Phytochemical Analysis of Chloroform Extract of Cassis fistula using FT-IR, HPLC and GC-MS analysis. *Int. J. Pharm. Sci. Rev. Res.* 2017 ; 46(1): 129-133.
21. De Meloa A.R, Garciaa I.J.P, Serrãob J.E, Santosa H.L, Limaa L.A.R.S and Alves S.N. Toxicity of different fatty acids and methyl esters on Culex quinquefasciatus larvae. Ecotoxicol. Environ. Saf. 2018;154: 1-5.
22. Suresh A, Praveenkumar R, Thangaraj R, Oscar FL, Baldev E, Dhanasekaran D and Thajuddin N. Microalgal fatty acid methyl ester a new source of bioactive compounds with antimicrobial activity. Asian Pac. J. Trop. Dis., 2014,4: S979-S984.
23. Ali A, Javaid A and Shoaib A . GC-MS analysis and antifungal activity of methanolic root extract of Chenopodium album against Sclerotium rolfsii. Planta Daninha 35: Article ID e017164713, 2017.
24. Kaur G.J and Arora D.S. Antibacterial and phytochemical screening of Anethumgraveolens, Foeniculumvulgare and Trachyspermumammi. BMC Compl Altern Med. 2009; 9: 30.
25. Lai H.Y, Yau Y.Y and Kim K.H. Blechnumorientale Linn - a fern with potential as antioxidant, anticancer and antibacterial agent. BMC. Complem. Altern. Med. 2010;10: 15.
26. Garg V.K.R, Jain M, Sharma P.K.R and Garg G. Anti-inflammatory activity of Spinaciaoleracea. *Int .J Pharma. Prof Res*. 2010; 1(1):1-4.
27. Lopez-Lazaro M. Distribution and biological activities of the flavonoid luteolin. *Mini .Rev .Med Chem.* 2009; 9: 31-59.
28. Mandal P, Babu S.S.P and Mandal N.C. Antimicrobial activity of saponins from Acacia auriculiformis. *Fitoterapia.*, 2005;76: 462-465.
29. Kumar A, Pande C.S and Kaul R.K. Chemical examination of *Cassia fistula* flowers. *Indian. J. Chem.* 1966; 4: 460.
30. Vimalraj T.R, Saravanakumar S, Vadivel S, Ramesh S and Thejomoorthy P. Antibcaterial effects of *Cassia fistula* extracts on pathogenic bacteria of veterinary importance . *Tamilnadu J Veter Anim Sci.* 2009; 5: 109–1