Research Article

Antioxidant effect and Phytochemical Analysis of Chloroform Extract of *Cassis fistula* using FT-IR, HPLC and GC-MS analysis

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ABSTRACT

The aim of the present investigation is to screening of phytochemical constituents for its free radical scavenging activity of chloroform extract of *Cassia fistula* leaves. The preliminary screening was carried out by standard chemical method. It evidenced that the presence of phytochemicals such as alkaloids, anthraquinones, saponins, phenols, tannins, flavonoids and terpenoids in the chloroform extract. Various functional groups like alcohols, phenols, carboxylic acid and nitro groups were associated with the extract were characterized using Fourier transform infrared spectroscopy (FTIR). A few common compounds available in the extract were identified by Gas chromatography – Mass spectrum (GC-MS) analysis. The DPPH free radical scavenging activity and 50 % inhibitory concentration (IC_{50}) was calculated and compared with the standard synthetic drug BHT. The IC_{50} value of leaf extract and BHT was found to be 20.01 µg/ml and 32.12 µg/ml, respectively. This study revealed that *C. fistula* chloroform extract of leaves has alternative drugs for free radical it could be acted as a novel source of free radical scavengers.

Keywords: Cassia fistula, chloroform extract, antioxidant, HPLC, GC-MS.

INTRODUCTION

Cassia fistula L. is a semi-wild Indian Laburnum commonly known as golden shower; it is belongs to the family Fabaceae. This plant is commonly grown in Asia, South Africa, China and Brazil and each part of this plant is well known for its medicinal properties[1].This plant cultivated as ornamental tree due to its beautiful yellow flowers in tropical areas[2]. *C. fistula* is medium sized tree and grown up to 20 m height. The bark of this plant is a grey colour. The leaves are dark green in colour and are arranged in alternate. The fruit of *C. fistula* is dark colour with 60 cm long. The whole part of *Cassia fistula* tree has medicinal properties like astringent, cooling, purgative, tonic, laxative, anthelmintic, antiperiodic[3], diuretic, anti-inflammatory, and antioxidant[4]. Moreover leaves are useful in treatment of skin diseases, burning sensation, dry cough and fever. Flowers are used in cardiac diseases and fever. The plant *C. fistula* has main chemical components are anthraquninone derivatives and tannins. Several investigations reported that *C. fistula* extract has therapeutically applications including anti-oxidant[5], anti-inflammatory [6], [7] and antimicrobial [8,9], anticancer[10], anti-dermatophytic and wound healing properties [1].

Free radicals are unstable and as chemical molecules independently having one or more unpaired electrons and they play role in metabolic activity. While exceeding the amount of free radicals in the body cause cell damage and tissues. This imbalance between free radical and antioxidant systems leads to cause of cardiovascular, diseases, cancer, aging etc[11]. Some of the common free radicals are nitric oxide, hydrogen peroxide, hydroxyl radical, superoxide anion radical etc. These are otherwise known as reactive oxygen species[12].

An antioxidant is defined as a molecule that preventing the oxidation of other molecule. Currently synthetic antioxidants like BHT and BHA are available to slowing or inhibit the free radicals formation and low effectiveness [13]. These drugs may cause negative health impact[14]. To overcome this problem plants are utilized in therapeutic applications. Plants are having natural antioxidants like phytochemicals such as phenols, flavonoids etc capable to scavenging harmful free radicals[15]. In this study chloroform extract of *Cassia fistula* leaves was screened and characterized by FTIR, GC-MS and HPLC for DPPH free radical scavenging activity.

MATERIALS AND METHODS

Preparation of chloroform extract

The leaves were washed with tap water and distilled water. Washed leaves were air dried at room temperature for 3 days. The dried leaves were pulverized into fine powder. The chloroform extract of the C. fistula leaf was prepared by soaking 10 g of fine powder in 100 ml of chloroform solvent for 24 hours. Then, the extract was filtered using Whatman No.1 filter paper and collects the filtrate. The collected filtrate was packed in airtight container and stored in dark conditions. The extract was concentrated by vacuum rotary evaporator for the study of phytochemical screening and antioxidant studies.

Phytochemical screening of chloroform extracts

Presence of bioactive phytochemicals like alkaloids, anthraquinones, glycosides, resins, quinines, saponins, phenols, tannins, flavonoids, steroids, terpenoids, proteins and sugars in chloroform extract was carried out by following standard methodologies as described by Harborne[16,17], Kokate, [18], Trease and Evans [19] and Edeogaet al [20].

Finally the plant extracts were analysed using FTIR analysis, GC-MS analysis and HPLC.

Antioxidant activity of chloroform extract of *C. fistula* leaves

Antioxidant activity of chloroform extract of leaf was analysed by Spectrophotometric method on the basis of determination of scavenging activity of DPPH free radical 10.BHT was prepared at different concentrations (5, 10, 20, 30, 40, and 50 µg/ml) and considered as standard. Stock of test sample was prepared by dissolving 10 mg in 10 ml chloroform at concentration of 1 mg/ml. From this stock solution, different concentration of 5, 10, 20, 30, 40, and 50 µg/ml was prepared. DPPH free radical was prepared by dissolving 1 mM DPPH in 3 ml methanol and kept in dark conditions to protect from sunlight by covering aluminium foil. A 3 ml of different concentrations of leaf extract and standard were mixed with 0.5 ml of DPPH solution and incubated in dark conditions for 30 min. After incubation, the absorbance at 517 nm is determined using UV-vis Spectrophotometer. Methanol was used as blank. The percentage of free radical scavenging of leaf extract was calculated by following equation

$$\%\ of Inhibition = \frac{Absorbance of control - Absorbance of test sample}{Absorbance of control} \times 100$$

The inhibition concentration to scavenge 50% free radical (IC50) is determined by plotting a graph of concentration (µg/ml) against % of free radical inhibition.

RESULTS AND DISCUSSION

Phytochemicals analysis

Medicinally valuable plants are having large number of pharmaceutically important compounds which are considered to able to be study for investigation of new herbal drugs for many harmful life threatening diseases like cancer, ulcer and tumours. Bioactive molecules from medicinal plants proved which inhibit microbial growth and radical scavenging activity. In this study preliminary phytochemical screening of chloroform extract of *C. fistula* leaves was analysed for antioxidant activity. The result of phytochemical screening of Chloroform extract of *C. fistula* leaves are shown in Table 1. It concluded that the presence of phytochemicals such as alkaloids, anthraquinones, saponins, phenols, tannins, flavonoids and terpenoids. Other phytochemicals such as glycosides, resins, quinines, steroids, proteins and sugars were not present in chloroform leaf extract of *C.fistula*. Alkaloids, saponins and flavonoids are attributed to the medicinal properties of plants.

Table 1: Phytoconstituents screening of chloroform extract of *C. fistula* leaves

Phytochemicals	Presence/Absence
Alkaloids	+
Glycosides	-
Anthraquinones	+
Resins	-
Quinones	-
Saponins	+
Phenols	+
Tannins	+
Flavonoids	+
Steroids	-
Terpenoids	+
Proteins	-
Sugars	-

FTIR

FTIR characterization studies are used to identify the functional molecules of the phytochemicals present in the plant extract or other materials. Figure 1 shows seven different absorption peaks at wave numbers which are corresponds to functional molecules of the chloroform extract of *C. fistula* leaves. The strong and broad band was observed at 3258 cm^{-1}corresponds to H–bonded O–H stretch alcohols, phenols. The weak band at 2980cm^{-1} indicates the presence of O-H stretching carboxylic acid groups. A very weak band was observed at 2868 cm^{-1} corresponds to C-H stretching alkanes. The bands 1625 and 1372 cm^{-1} are assigned to C=O stretch amides and N=O bend nitrogroups, respectively. The narrow bands shown at absorption peak 1042 and 878cm^{-1} are designated to C-N stretched aliphatic amines and N-H wag primary and secondary amines respectively. Hence this result concluded that the chloroform extract of C. fistula has active functional groups like carboxylic acids, amine, alcohol etc. These functional groups are associated with the bioactive phytochemicals in the leaf extract.

GC-MS

GC-MS analysis of leaves of *C. fistula* chloroform extract showed the presence of 13 components at the different retention time (Figure 2). 1-decanol, 2-ethyl-, Heptacosane, 1-chloro-, 3,7,11,15-tetramethyl-2-

hexadecen-1-ol, tritetracontane, tetradecane, 1-chloro-, 17-Pen#tatriacontene, Hexadecane, 1-chloro-, Tetratriacontane, 14-Heptadecenal, ctatriacontane, 1,38-dibromo- , Octadecanal, 2-T-Butyl-5-chloromethyl-3-methyl-4-oxoimidazolidine-1-carboxylic and Di-N-decylsulfone. The molecular weight and formula of 13 main components is presented in Table3.

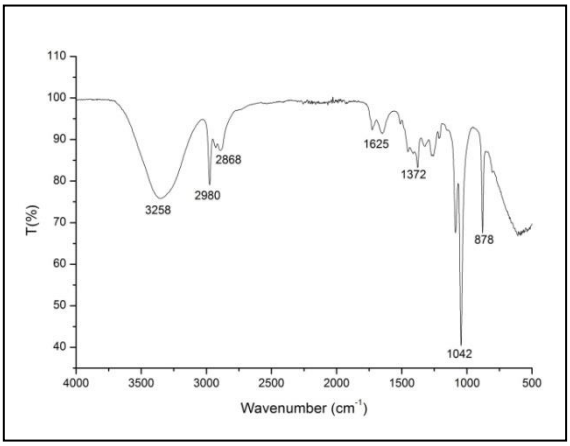

Figure 1: FTIR characterization shows the functional groups present in chloroform extract of *Cassia fistula* leaves

Table 2: Functional groups of chloroform extract of *Cassia fistula* leaves analysed by FTIR

S. No	Wave number (cm^{-1})	Functional groups
1	3258	H–bonded O–H stretch alcohols, phenols
2	2980	O–H stretch carboxylic acids
3	2868	C–H stretch alkanes
4	1625	C=O stretch amides
5	1372	N=O bend Nitro groups
6	1042	C–N stretch aliphatic amines
7	878	N-H wag primary and secondary amines

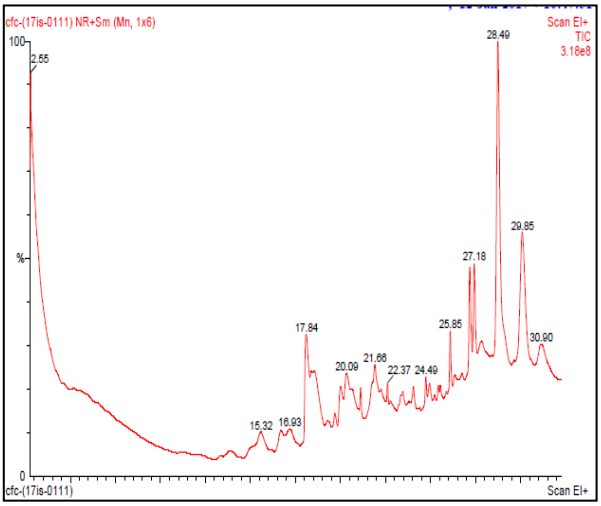

Figure 2: GC-MS analysis of chloroform extract of *Cassia fistula* leaves

Table 3: GC-MS analysis of chloroform extract of *Cassia fistula* leaves

Retention time	Name of the compound	Molecular weight	Molecular formula
15.32	1-Decanol, 2-ethyl-	186	$C_{12}H_{26}O$
16.93	Heptacosane, 1-chloro-	414	$C_{27}H_{55}Cl$
17.84	3,7,11,15-tetramethyl-2-hexadecen-1-ol	296	$C_{20}H_{40}O$
20.09	Tritetracontane	604	$C_{43}H_{88}$
21.66	Tetradecane, 1-chloro-	232	$C_{14}H_{29}Cl$
22.37	17-Pentatriacontene	490	$C_{35}H_{70}$
24.49	Hexadecane, 1-chloro-	260	$C_{16}H_{33}Cl$
25.85	Tetratriacontane	478	$C_{34}H_{70}$
26.94	14-Heptadecenal	252	$C_{17}H_{32}O$
27.18	Octatriacontane, 1,38-dibromo-	690	$C_{38}H_{76}Br_2$
28.49	Octadecanal	268	$C_{18}H_{36}O$
29.85	2-T-Butyl-5-chloromethyl-3-methyl-4-oxoimidazolidine-1-carboxylic	304	$C_{14}H_{25}O_3N_2Cl$
30.90	Di-N-decylsulfone	346	$C_{20}H_{42}O_2S$

HPLC

HPLC technique was used to identification of phyto-chemical components of the herbal plants. Each phyto-constituents of the plant extract exhibit a characteristic peak under certain retention times. Figure 3 shows four peaks for four compounds present in the chloroform extract of *C. fistula* leaves.

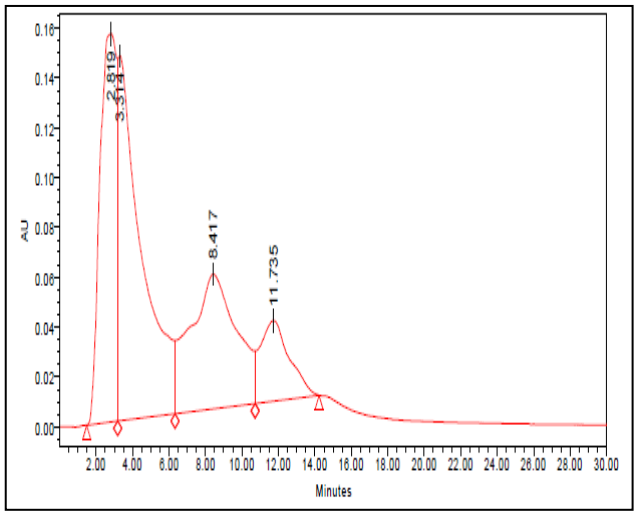

Figure 3: HPLC analysis of chloroform extract of *Cassia fistula* leaves

Antioxidant activity of chloroform extract of leaves of *C. fistula*

Chloroform leaf extract of *C. fistula* exhibited greater antioxidant activity compared to standard BHT at different concentration (5, 10, 20, 30, 40 and 50 µg /ml). In a dose dependent manner, percentage of the antioxidant activity of leaf extract was increased as increasing the concentration (Figure 4). The leaf extract at a concentration of 5µg/ml showed a percentage inhibition was found to be 20.13.28±1.24 and for 50µg/ml it was 91.43±1.45. The BHT at a concentration of 5µg/ml exhibited a percentage inhibition was found to be 13.21±1.63 and for 50µg/ml it was noted as75.23±1.23 (Table 4). The 50% inhibition concentration (IC_{50}) value of leaf extract and BHT was found to be 20.01 µg/ml and 32.12 µg/ml, respectively. Regression analysis shows the good linear relation in plant extract towards concentration and inhibition activity (Figure 4).

Antioxidant activity is determined on the basis of the stable DPPH free radical accepting an electron from molecules. It is visually identified by changing colour from purple to yellow. In this study, flavonoids, tannins, alkaloids phenols were may responsible antioxidant activity which they donate an electron to DPPH and neutralizes the free radicals. Similarly, Bhalodia et al [13] reported that antioxidant activity of flower extract of *C. fistula* plant.

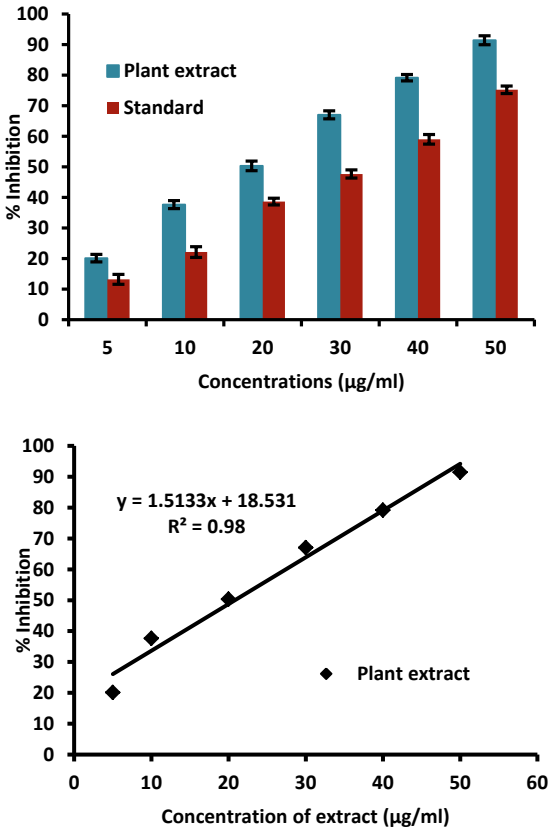

Figure 4: DPPH free radical scavenging activity and Regression graph of DPPH free radical scavenging activity of chloroform extract of *C. fistula*

Polterait[21] stated that the flavonoids, phenols and tannins may responsible for the free radical scavenging effects and they act as free radical scavengers. Some other reports demonstrated that phenol[22-24], flavanoids[25], saponins[26] and tannins[27] have been pounds have been found to possess potent antioxidants, antimicrobial and anti-inflammatory activity.

Table 4: DPPH free radical scavenging activity of chloroform extract of *Cassia fistula* leaves

Concentration of chloroform *C. fistula* leaf extract (µg/ml)	% Inhibition of DPPH free radical	
	Plant extract	Standard
5	20.13±1.24	13.21±1.63
10	37.65±1.33	22.13±1.76
20	50.33±1.56	38.65±1.09
30	67.03±1.33	47.66±1.33
40	79.18±1.03	59.03±1.56
50	91.43±1.45	75.25±1.23

± Standard deviation

CONCLUSION

The qualitative preliminary screening shows the presence of alkaloids, flavonoids, phenols, terpernoids and anthraquinone were established. FTIR shows the available functional bioactive molecules in the chloroform extract of C. fistula leaves. The 13 major components present in *Cassia fistula* chloroform extract of leaves were identified by GC-MS. HPLC demonstrated the four potential compounds present in the extract at different retention time (Rt). The potential antioxidant activity of *Cassia fistula* chloroform extract of leaves was established by measuring DPPH radical scavenging at different concentrations. The activity was compared with synthetic drug shows greater percentage of inhibition of free radical.

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