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Phytochemical analysis of *Cassia fistula* and its *in vitro* antimicrobial, antioxidant and anti-inflammatory activities

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ABSTRACT

Three solvent (methanol, aqueous and petroleum ether) extracts of *Cassia fistula* were used to analyze phytochemicals and evaluated for different in vitro biological activity. The methanol extracts of *Cassia fistula* yielded 10 different phytochemicals and TPC at higher concentration. The methanol extract has shown dominance in inhibition of all bacteria tested and except *Cercospora carthami, Fusarium solani* and *F. oxysporum* were not inhibited whereas all other fungi tested were inhibited strongly by methanol extract. The antioxidant activity in 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and Ferric ion Reducing Antioxidant Power (FRAP) assay was performed for all the three extracts, the methanol was showed high activity of free radical inhibition of 69 and 714.86 followed by aqueous (60, 448.14) and petroleum ether (25, 387.51). The methanol extract inhibited the heat induced albumin denaturation and red blood cell membrane stabilization with 88.61 and 79.33 g/ml. Proteinase activity was significantly inhibited by the methanol (83.88) extract followed by aqueous (66.21) and petroleum ether (48.61) was compared with standard Aspirin 92.87 g/ml. The methanol extract was inhibited the xanthine oxidase (44.83) and acetylcholinesterase (18.98) followed by aqueous and petroleum ether extracts. The highest antilipoxygenase activity was noticed from methanol extract (62.16) followed by aqueous (56.43) and petroleum ether extract (38.32). From all the results, we found that that phytochemicals (alkaloids, saponins, flavonoids, anthraquinone and phenolic compounds) present in *C. fistula* extract may be responsible for the antimicrobial, antioxidant and anti-inflammatory activity.

Keywords: *Cassia fistula*, phytochemicals, antimicrobial, antioxidant, anti-inflammatory.

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INTRODUCTION

Cassia fistula L. (Caesalpinioideae) a very common plant known for its medicinal properties is a semi-wild Indian Laburnum known as a the golden shower. It is distributed in various regions including Asia, South Africa, China, West Indies and Brazil (Kumar et al., 2006). Extracts relieve constipation, piles and detoxifier (Agarwal and Paridhavi, 2005). Many biologically important compounds were isolated and identified from different parts of the plant (Thirumal et al., 2012). The plant extracts were shown as potent antibacterial, antifungal, anti-inflammatory and antioxidant (Gupta, 2010) properties and the findings were done using different solvent

extracts and parts of the plant. The chemical analysis of different parts of *C. fistula* has been reported. It was found to contain flavonoids, phenolic compounds and proanthocyanidins (Luximon et al., 2002). *C. fistula* extracts have been reported for various pharmacological activities including anti-inflammatory (Rajeswari et al., 2006), antioxidant (Irshad et al., 2012), antimicrobial (Irshad et al., 2013), wound healing properties (Kumar et al., 2006) and anticancer activity (Irshad et al., 2014).

Based on the literature survey, the present investigation was aimed, used three different solvents subjected to phytochemical analysis and same extracts were used antimicrobial activity (which were not tried in previous reports), anti-inflammatory and antioxidant properties using different methods. These results will add up to useful properties of the plant *C. fistula*.

MATERIALS AND METHODS

Plant collection and extract preparation

The plant was collected in November 2013 from Western Ghat region of Udupi, Karnataka, India. The plant was identified by Dr SGK Bhat, Taxonomist and identified as *Cassia fistula*. The collect plant was air dried at room temperature for 4 weeks to get consistent weight. The dried all parts were later ground to powder. Dried parts were used to extract the phytochemicals using three different solvents (Govindappa et al., 2011). The extracts were filtered using Buckner Funnel and Whatmann No. 1 filter paper. Each filtrate was concentrated to dryness under reduced pressure at 40°C using a rotary evaporator. Each extract was resuspended in the solvents viz. aqueous, methanol and petroleum ether individually to yield a 50 mg/ml stock solution (Taylor et al., 1996; Adedapo et al., 2008).

Phytochemical analysis

Phytochemical analysis was carried out for saponins, flavonoids, cardiac glycosides, terpenoids, steroids, tannins, phenol, anthroquinone, alkaloids (Obdoni and Ochuko, 2001) and tannins (Kaur and Arora, 2009) was performed as described by the authors. Wagner's and Heger's reagents were used to alkaloid foam test for saponins, Mg-HCl and Zn-HCl for flavonoids, Keller-Killani test for cardiac glycosides, Salkonoski test for terpenoids, acetic anhydride and sulphuric acid for steroids, chloride and gelatin for tannins, ferric chloride for phenol, hexane and diluted ammonia for anthraquinone test. All these experiments were carried out for three solvent extracts (aqueous, methanol and petroleum ether) of dried plant individually.

Determination of total phenolic content

Total Phenol Content (TPC) in extracts was determined by Folin-Ciocalteu's colorimetric method as described by Adedapo et al. (2009b). Extracted solution (0.3 ml in triplicate) was mixed with 1.5 ml of 10% Folin-Ciocalteu's reagent and 1.2 ml of 7.5% (w/v) sodium carbonate. The mixture was kept in the dark for 30 min and absorbance was measured at 765 nm. Quantification was done on the basis of a standard curve of Gallic acid. The results were expressed as Gallic Acid Equivalent (GAE), that is, mg Gallic acid/100 ml. All tests were performed in triplicate.

Determination of antioxidant activity

In order to investigate the antioxidant properties of the three solvent extracts, ferric ion reducing antioxidant power (FRAP) and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay.

FRAP assay

FRAP reagents were freshly prepared by mixing 25 ml acetate buffer (300 mM, pH 3.6), 2.5 ml 2,4,6-Tris-(2-pyridyl)-S-triazine (TPTZ) solution (10 mM TPTZ in 40 mM/L HCl) and 2.5 ml $FeCl_3$ (20 mM) water solution. Each sample (150 µl) (0.5 mg/ml) dissolved in methanol was added in 4.5 ml of freshly prepared FRAP reagent and stirred and after 5 min, absorbance was measured at 593 nm, used FRAP working solution as blank (Szollosi and Szollosi Varga, 2002; Tomic et al., 2009). A calibration curve of ferrous sulfate (100 to 1000 µmol/L) was used and results were expressed in µmol Fe^{2+}/mg dry weight extract. The relative activity of the samples was compared to L-ascorbic acid.

DDPH radical assay

The effect of three different solvent extracts on DPPH radical was estimated using the method of Liyana-Pathirana and Shahidi (2005). DPPH solution was freshly prepared by dissolving 24 mg DPPH in 100 ml ethanol, stored at -20°C before use. 150 µl of sample (10 µl sample + 140 µl distil water) is allowed to react with 2850 µl of DPPH reagent (190 µl reagent + 2660 µl distil water) for 24 h in the dark condition. Absorbance was measured at 515 nm. Standard curve is linear between 25 to 800 µM ascorbic acid. Results expressed in µm AA/g fresh mass. Additional dilution needed if the DPPH value measured was over the linear range of the standard curve. Mix 10 ml of stock solution in a solution of 45 ml of methanol, to obtain an absorbance of 1.1 ± 0.02 units at 517 nm using spectrophotometer (Katalinic et al., 2006). All determinations were performed in triplicate. The percentage inhibition of DPPH radical by the samples was calculated according to formulas of Yen and Duh (1994):

% inhibition = [{Abs control - Abs sample}/Abs control] × 100

Where, Abs control is the absorbance of the DPPH radical+ ethanol, Abs sample is the absorbance of DPPH radical+ sample extract/standard.

Determination of antimicrobial activity

Antimicrobial assay

Bacillus subtilis, *Pseudomonas fluorescens*, *Clavibacter michiganensis* sub sp. *michiganensis*, *Xanthomonas oryzae* pv. *oryzae*, *Xanthomonas axanopodis* pv. *malvacearum* and strains of *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumonia* bacteria were obtained from stock cultures presented at -80°C at Department of Studies in Applied Botany, Seed pathology and Biotechnology, University of Mysore, Manasa Gangothri, Mysore, Karnataka, India and Department of Studies in Biotechnology and Microbiology, Bangalore University, Gnana Bharathi, Bangalore, India respectively. Three Gram positive bacteria tested were *B. subtilis*, *C. michiganensis* sub sp. *michiganensis*, *S. aureus* and six Gram negative bacterias tested were *P. fluorescens*, *X. oryzae* pv. *oryzae*, *X. axanopodis* pv. *malvacearum*, *E. coli*, *P. aeruginosa* and *K. pneumonia*. All bacteria

were grown on nutrient agar media.

Fungi (*Aspergillus flavus, Aspergillus niger, Aspergillus nidulans,Aspergillus flaviceps, Alternaria carthami, Alternaria helianthi,Cercospora carthami, Fusarium solani, Fusarium oxysporum,Fusarium verticilloides* and *Nigrospora oryzae* were obtained from Department of studies in Applied Botany, Seed pathology and Biotechnology, University of Mysore, Manasa Gangothri, Mysore, Karnataka, India and Department of studies in Microbiology, Bangalore University, Gnana Bharathi, Bangalore, India respectively. All fungi were grown on potato dextrose agar medium.

Paper disc method

Diameter of zone of inhibition was determined using the paper disc diffusion method as described by Lai et al. (2009) and Adedapo et al. (2008). A swab of the bacteria suspension containing 1 × 10^8 CFU/ml was spread on to Petri plates containing nutrient agar media. Each extract were dissolved in ethanol to final concentration of 10 mg/ml. Sterile filter paper discs (6 mm in diameter) impregnated with 1 mg of plant extracts were placed on culture plates. The plates were incubated at 37°C for 24 h. The ethanol served as negative control while the standard streptomycin (10 µg) discs were used as positive controls. Antimicrobial activity was indicated by the presence of clear inhibition zone around the discs. The assay was repeated thrice and mean of three experiments was recorded.

***In vitro* anti-inflammatory activity**

Inhibition of albumin denaturation

Methods of Mizushima and Kobayashi (1968) and Sakat et al. (2010) followed with minor modifications. The reaction mixture was consisting of test extracts and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted using small amounts at 37°C HCl. The sample extracts were incubated at 37°C for 20 min and then heated to 51°C for 20 min after cooling the samples the turbidity was measured spectrophotometrically at 660 nm. The experiment was performed in triplicate. Percent inhibition of protein denaturation was calculated as follows:

% inhibition= [{Abs control- Abs sample}/Abs control] × 100

where Abs control is the absorbance of the DPPH radical+ ethanol, Abs sample is the absorbance of DPPH radical+ sample extract/standard.

Membrane stabilization test

Preparation of Red Blood Cells (RBCs) suspension

Fresh whole human blood (10 ml) was collected and transferred to the centrifuge tubes. The tubes were centrifuged at 3000 rpm for 10 min and were washed three times with equal volume of normal saline. The volume of blood was measured and reconstituted as 10% v/v suspension with normal saline (Saket et al., 2010).

Heat induced hemolytic

The reaction mixture (2 ml) consisted of 1 ml of test sample and 1 ml of 10% RBCs suspension, instead of test sample only saline was added to control test tube. Aspirin was taken as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in a water bath at 56°C for 30 min. At the end of the incubation the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatant was taken at 560 nm. The experiment was performed in triplicates for all the test samples. Percent membrane stabilization activity was calculated by the formula mentioned above (Saket et al., 2010).

Protein inhibitory action

The test was performed according to the modified method of Oyedepo and Femurewas (1995) and Sakat et al. (2010). The reaction mixture (2 ml) was containing 0.06 mg trypsin, 1 ml of 20 mM Tris HCl buffer (pH 7.4) and 1 ml test sample of different concentrations. The reaction mixture was incubated at 37°C for 5 min and then 1 ml of 0.8% (W/V) casein was added. The mixture was inhibited for an additional 20 min, 2 ml of 70% perchloric acid was added to terminate the reaction. Cloudy suspension was centrifuged and the absorbance of the supernatant was read at 210 nm against buffer as blank. The experiment was performed in triplicate. The percentage of inhibition of proteinase inhibitory activity was calculated.

Anti-lipoxygenase activity

Anti-lipoxygenase activity was studied using linoleic acid as substrate and lipoxidase as enzyme (Shinde et al., 1999). Test samples were dissolved in 0.25 ml of 2 M borate buffer pH 9.0 and added 0.25 ml of lipoxidase enzyme solution (20,000 U/ml) and incubated for 5 min at 25°C. After which, 1.0 ml of lenoleic acid solution (0.6 mM) was added, mixed well and absorbance was measured at 234nm. Indomethcin was used as reference standard. The percent inhibition was calculated from the following equation:

% inhibition= [{Abs control- Abs sample}/Abs control] × 100

A dose response curve was plotted to determine the IC_{50} values. IC_{50} is defined as the concentration sufficient to obtain 50% of a maximum scavenging capacity. All tests and analyses were run in triplicate and averaged.

Xanthine oxidase assay

Xanthine oxidase activity was assayed spectrophotometrically at 300 nm as described by Yamamoto et al. (1993). Briefly, the reaction mixture consisting of 500 µl of solution A (0.1 M phosphate buffer containing 0.4 mM xanthine and 0.24 mM NBT), 500 µl of solution B (0.1 M phosphate buffer containing 0.0449 units/ml xanthine oxidase) and 50 µl of a 10% of each solvent extracts were incubated in a cuvette at 37°C for 20 min. The enzyme activity was expressed as the increment in absorption at 300 nm per unit time.

Acetylcholinesterase (AChE) Inhibitory activity

The AChE inhibitory assay and inhibition kinetics analysis was conducted according to the protocol described by Lopez et al. (2002) with some modifications. The assay mixture consisted of 200 µl of Tris-HCl 50 mM pH 8.0, 0.1% BSA buffer, 100 µl of extracts or fractions solution (final concentration: 100 µg ml^{-1}) was dissolved in buffer-MeOH (10%) and 100 µl of AChE (0.22 U ml^{-1}). The mixture

Table 1. Phytochemical analysis of different solvent extract of *Cassia fistula.*

Phytochemicals	Aqueous extract	Methanol extract	Petroleum ether extract
Alkaloids	+	++	+
Carbohydrates and glycosides	+	++	-
Fats	+	+	+
Saponins	+	++	-
Tannins	+	++	+
Flavonoids	+	++	-
Anthraquinones	-	+	-
Gums and mucilages	-	-	-
Phenolics compounds	++	++	-
Proteins and amino acids	++	++	-

Repeated the each experiment thrice, +: presence, -: Absence, ++: present at higher concentration.

was incubated at room temperature for 2 min before the addition of 500 µL of DTNB (5,5 dithiobis [2-nitrobenzoic acid] (3 mM) and 100 µl of substrate acetylthiocholine iodide (ATCI) (15 mM). The developing yellow color was measured at 405 nm after 4 min. Galantamine was used as positive control at a final concentration of 0.2 µg ml^{-1} in the assay mixture.

AChE inhibitory activity was expressed as percent inhibition of AChE, calculated as (1-B/A) × 100, where A is the change in absorbance of the assay without the plant extract (∆ abs. with enzyme- ∆ abs. without enzyme) and B is the change in absorbance of the assay with the plant extract (∆ abs. with enzyme -∆ abs. without enzyme).

Statistical analysis

Analysis of variance (ANOVA) was used to determine the significance of difference between treatment groups ($p < 0.05$). Means between treatment groups were compared for significance using Duncan's new Multiple Range post test.

RESULTS

Phytochemical analysis of three solvent extracts of *C. fistula* revealed the presence of 11 important phyto-constituents viz., alkaloids, saponins, flavonoids, anthraquinones, phenolic compounds, carbohydrate and glycosides, fats, gums and mucilages, proteins and amino acids. All the three different solvent extracts gave a variety of compounds. The methanol extract has shown presence of all compounds tested and alkaloids, saponins, tannins, flavonoids and phenolic compounds are present in high concentration. The methanol extract yielded all phytochemicals compared to other two solvent extracts (Table 1).

The TPC was determined using Folin-Ciocalteau reagent and expressed in terms of mg Gallic Acid Equivalent (GAE)/100 ml extract. The more TPC was found in methanol (9.66) followed by aqueous (7.21) and petroleum ether extract (5.11) (Figure 1).

The antimicrobial activity of aqueous, methanol and petroleum ether extracts of *C. fistula* gave different zones of inhibition on the organisms tested (Table 2). The methanol extract inhibited the growth of all most all the bacterial isolates, more activity was observed on *Staphylococcus aureus* and *X. axanopodis* pv. *malvacearum* and the medium activity was observed on *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia* and *Xanthomonass oryzae* pv. *oryzae*. Petroleum ether extract has shown very activity to tested bacteria. The methanol extract inhibits the growth of different fungi tested, all the *Aspergillus* and *Alternaria* species and *C. carthami* were inhibited by the same extract compared to other two extracts.

The antioxidant activity of three different solvent extracts was measured by the ability to scavenge DPPH free radical was compared with the standard, ascorbic acid. It was observed that methanol extract of *C. fistula* had higher activity than that of aqueous and petroleum ether extracts. Concentrations of 0.1 mg/ml, the scavenging activity of methanol extract reached 69, while aqueous and petroleum ether was 60 and 25, respectively. Though, the DPPH radical scavenging abilities of the extracts were less than those of ascorbic acid (80) at 0.1 mg/ml. This result clearly indicates that the extracts have proton donating ability and could serve as free radical inhibitors or scavenging acting possibly as primary antioxidants (Figure 2). The more scavenging activity was noticed in methanol extracts of *C. fistula*.

The FRAP reducing ability of three solvent extracts was ranged 714.86 to 387.51 µm Fe (II) /mg (Table 3). The antioxidant potentials of the methanol extracts of *C. fistula* was estimated by their ability to reduce TPRZ-Fe (III) complex to TPTZ-Fe (II). The FRAP activity values of methanol extract was significantly lower than that of standard ascorbic acid.

The cause of inflammation is demonstrated by denaturation of proteins. Denaturation of proteins were

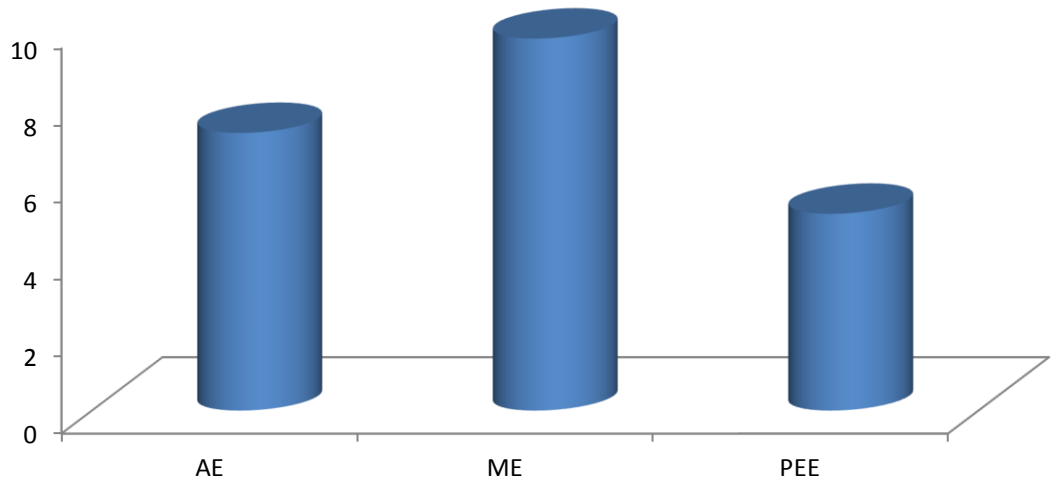

Figure 1. Determination of phenol content from different solvent extracts of *C. fistula.*

Table 2. *In vitro* inhibition of microbial growth from different solvent extracts of *Cassia fistula.*

Species names	AE	ME	PEE
Bacterial pathogens			
Escherichia coli	+	++	+
Pseudomonas aeruginosa	+	++	+
Staphylococcus aureus	+	+++	+
Klebsiella pneumonia	+	++	+
Pseudomonas fluorescens	+	+	+
Clavibacter michiganensis sub sp. *michiganensis*	+	+	+
Xanthomonass oryzae pv. *oryzae*	+	++	-
X. axanopodis pv. *malvacearum*	+	+++	-
Fungal pathogens			
Aspergillus flavus	+	++	+
A. niger	+	++	+
A. nidulans	+	++	-
A. flaviceps	+	++	-
Alternaria carthami	+	++	-
A. helianthi	+	++	-
Cercospora carthami	+	-	-
Fusarium solani	-	-	-
F. oxysporum	-	-	-
F. verticilloides	-	+	-
Nigrospora oryzae	-	++	-

+++ = maximum activity,++ = average, + = minimum activity, - = No activity. Repeated the experiments three times for each replicates. - Aqueous Extract, ME - Methanol Extract, PEE - Petroleum Ether Extract.

observed in extract treated blood. The possible mechanism of extracts was lead to stoped the protein denaturation. The extracts were effective in inhibiting heat induced albumin denaturation (Table 4). Maximum albumin denaturation inhibition 88.61 was observed with methanol extract followed by aqueous (62.88) and

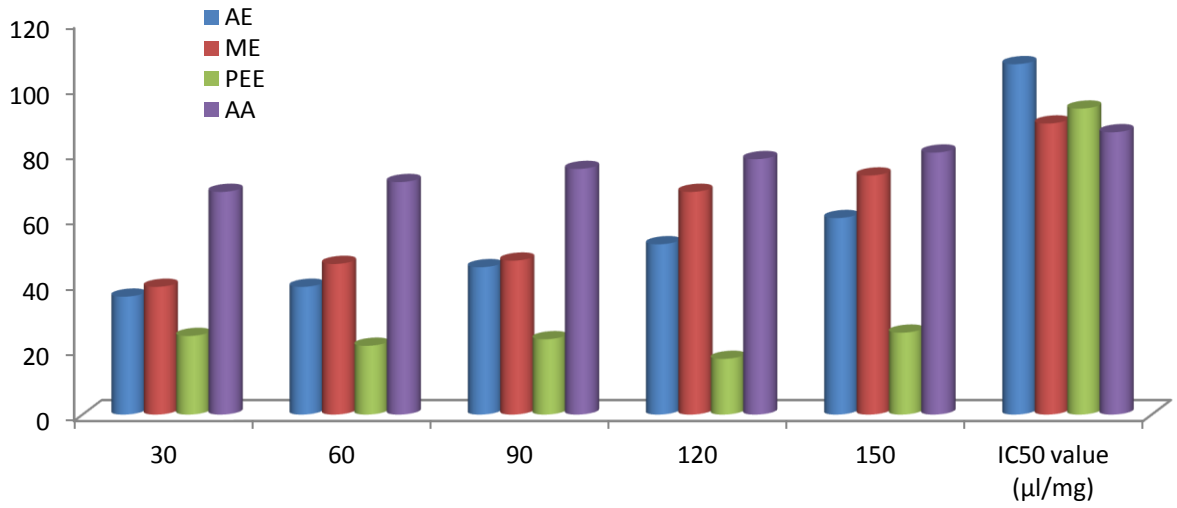

Figure 2. DPPH scavenging activities of three solvent extracts of *C. fistula.* AE - Aqueous Extract, ME - Methanol Extract, PEE- Petroleum Ether Extract, AA - Ascorbic Acid, Repeated each experiment thrice.

Table 3. Total antioxidant (FRAP) activities of three solvent extracts of *C. fistula.*

Extracts	FRAP
AE	448.14 ± 0.06^{c}
ME	714.86 ± 0.06^{b}
PEE	387.51 ± 0.06^{d}
AA	1648.52 ± 0.06^{a}

AE - Aqueous Extract, ME- Methanol Extract, PEE- Petroleum Ether Extract, AA- Ascorbic Acid, Repeated the each experiment thrice, Data represented the Arithmetic mean and standard error of three determinants. According DMRT (Duncan's multiple range test) the values provided with different superscripts remains significant at P ≤ 6 0.05.

Table 4. Effect of different solvent extracts of *Cassia fistula* on albumin denaturation, membrane stabilization and proteinase inhibitory activity percentage inhibition.

Test sample	Albumin denaturation	Membrane stabilization	Proteinase inhibition
AE	62.88 ± 0.006^{c}	67.54 ± 0.006^{c}	66.21 ± 0.006^{c}
ME	88.61 ± 0.006^{b}	79.33 ± 0.006^{b}	83.48 ± 0.006^{b}
PEE	45.26 ± 0.006^{d}	39.14 ± 0.006^{d}	48.61 ± 0.006^{d}
Aspirin (200 µg/ml)	75.89 ± 0.006^{a}	85.92 ± 0.03^{a}	92.87 ± 0.05^{a}

AE - Aqueous Extract, ME- Methanol Extract, PEE - Petroleum ether extract. Repeated the each experiment three times for each replicates. Data represented the Arithmetic mean and standard error of three determinants. According DMRT (Duncan's multiple range test) the values provided with different superscripts remains significant at P ≤ 6 0.05.

petroleum ether (45.26) extracts. Aspirin, a standard anti-inflammatory drug showed the maximum albumin inhibition 75.89 at the concentration of 200 µg/ml and this was less than methanol extract.

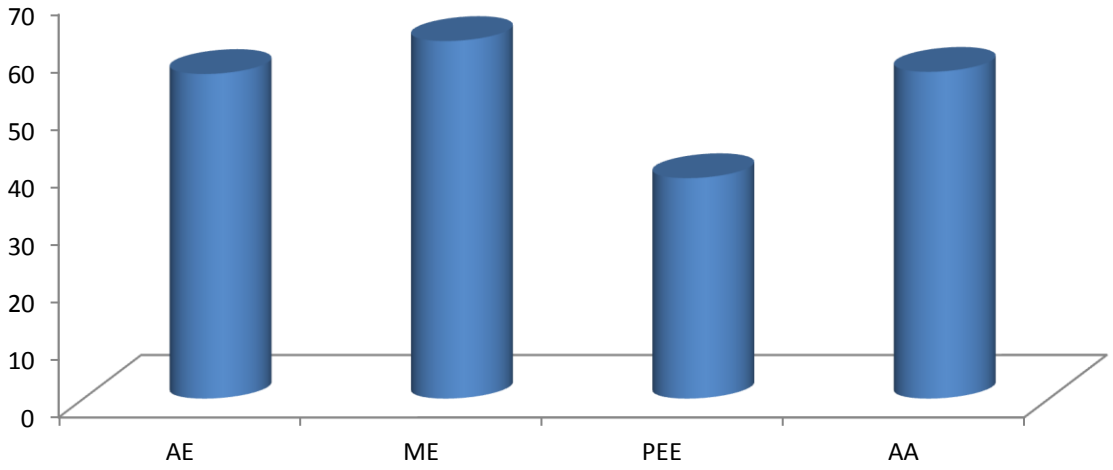

Figure 3. Anti-lipoxygenase activity of different solvent extracts of *C. fistula.*

Table 5. Inhibition of xanthine oxidase and acetyl cholinesterase activities from different solvent extracts of *Cassia fistula.*

Extracts	Inhibitors samples activities (%)	
	Xanthine oxidase (IC50 µg/ml)	Acetyl cholinesterase
AE	36.14 ± 1.26^{b}	14.63 ± 1.36^{c}
ME	44.83 ± 1.43^{a}	18.98 ± 1.35^{b}
PEE	29.47 ± 1.56^{c}	9.47 ± 1.05^{d}
Galanthamine (20 µg/ml)	--	50.00 ± 1.36^{a}

AE - Aqueous Extract, ME - Methanol Extract, PEE - Petroleum ether extract. Data represented the Arithmetic mean and standard error of three determinants. According DMRT (Duncan's multiple range test) the values provided with different superscripts remains significant at P ≤ 6 0.05.

RBCs membrane stabilization was studied to know the mechanism of anti-inflammatory action of different three solvent extracts of *C. fistula*. The methanol extract was effective in inhibiting the heat induced hemolysis. The effect may possibly inhibit the release of lysosomal content of neutrophils at the site of inflammation. The extracts inhibited the heat induced hemolysis of RBCs varying degree (Table 4). The highest inhibition was observed with methanol (79.33%) followed by aqueous (67.54%) and petroleum ether (39.14%) extracts. The standard drug, aspirin showed the highest inhibition of 85.92%.

The *C. fistula* methanol extract (83.48%) exhibited significant antiproteinase activity, in decreasing order aqueous (66.21%) and petroleum ether (48.61%). The aspirin (92.87%) showed the highest poteinase inhibitory activity (Table 4).

The methanol and aqueous extracts were significantly inhibited the lipoxygenase activity by 62.16 and 56.43 respectively, followed by petroleum ether (38.32). The standard indomethacin had shown 56.81% inhibition at a concentration of 60 µg/ml (Figure 3).

The maximum inhibition of xanthine oxidase was observed from methanol (44.83%) followed by aqueous (36.145) and petroleum ether (29.47%) (Table 5).

All the three solvent extracts were tested for *in vitro* AChE inhibition activity at a concentration of 100 µg/ml and in the assay mixture galanthamine used as a positive control. Among three solvent extracts the methanol (18.98) exhibited the best AChE inhibitory activity followed by aqueous (14.63) and petroleum ether (9.42) (Table 5).

DISCUSSION

In recent years, the search for phytochemicals possessing antioxidant, antimicrobial and anti-inflammatory properties have been on the rise due to their potential use in the therapy of various chronic and infectious diseases. Epidemiology and experimental studies have implicated oxidative cellular damage arising

from an imbalance between free radical generating and scavenging systems as the primary cause of cardiovascular, diseases, cancer, aging etc (Halliwell, 1996). Due to risk of adverse effects encountered with the use of synthetic antibiotics, medicinal plants may offer an alternative source for antimicrobial agent with significant activity against pathogenic and infective microorganisms. In addition, a number of antibiotics have lost their effectiveness due to the development of resistant strains, mostly through the expression of resistance genes (Berahou et al., 2007).

Our finding results we sugeested to use of *C. fistula* as traditional medicine. Hence, we found strong antioxidants, antimicrobial and anti-inflammatory activities in methanol extract of *C. fistula*. High TPC values found in methanol extract (9.66) mg GAE/100 ml imply the role of phenol compounds in inhibiting these activities. Plant phenol compounds have been found to possess potent antioxidants (Adedapo et al., 2009b; Adesegun et al., 2009; Lai et al., 2010), antimicrobial (Kaur and Arora, 2009; Lai et al., 2010) and anti-inflammatory activity (Sakat et al., 2010; Garg et al., 2010).

The flavonoids from plant extracts have been found to possess antioxidants, antimicrobial and anti inflammatory properties in various studies (Lopez-Lazaro, 2009; Yoshida et al., 2008). The presence of tannins in all extracts may explain its potent bioactivities as tannins are known to possess potent antimicrobial activities (Kaur and Arora, 2009), antioxidants (Zhang and Lin, 2008), and anti-inflammatory properties (Fawole et al., 2010). The saponins have already shown as antimicrobial activity (Mandal et al., 2005), antioxidant activity (Gulcin et al., 2004) and anti-inflammatory activity (Gepdireman et al., 2005).

Denaturation of proteins is a well documented cause of inflammation. The inflammatory drugs (salicylic acid, phenylbutazone, etc) have been shown to depend on their ability to thermally induced protein denaturation (Mizushima and Kobayashi, 1968). Similar results were observed from many reports from plant extract (Sakat et al., 2010). The extracts may possibly inhibit the release of lysosomal content of neutrophils at the site of inflammation.

These neutrophils lysosomal constituents include bactericidal enzymes and proteinases, which upon extracellular release cause further tissue inflammation and damage (Chou, 1997). The precise mechanism of this membrane stabilization is yet to be elucidated it is possible that the *C. fistula* produced this effects surface area/volume ratio of the cells, which could be brought about by an expansion of membrane or the shrinkage of cells and an interaction with membrane proteins (Shinde et al., 1999).

Proteinases have been implicated in arthritic reactions. Neutrophils are known to be a source of proteinase which carries in their lysosomal granules many serine proteinases. It was previously reported that leukocytes proteinase play important role in the development of tissue damage during in inflammatory reactions and significant level of protection was provided by proteinase inhibitors (Das and Chatterjee, 1995). Recent studies have shown that many flavonoids and related polyphenols contributed significantly to the antioxidant and anti-inflammatory activities of many plants (Fu et al., 2013). Hence, the presence of bioactive compounds in the methanolic extract of different parts of *C. fistula* may contribute to its, antimicrobial, antioxidant and anti-inflammatory activity. The present investigation has shown that the leaf and stem extract of *C. fistula* has active phytochemicals, which are able to inhibit plant and animal pathogenic bacteria and fungi. The methanol extract fractions showed significant antimicrobial activity against all Gram-positive and Gram-negative bacteria and different fungi tested. The antioxidant and anti-inflammatory properties were confirmed in the ethanol extract fractions. These activities may be due to the presence of polyphenolic compounds such as flavonoids, tannins, phenols and saponins.

LOXs are sensitive to antioxidants and the most of their action may be in inhibition of lipid hydroperoxide formation due to scavenging of lipidoxy or lipid peroxy-radical formed in course of enzyme peroxidation. This can limit the availability of lipid hydroperoxide substrate necessary for the catalytic cycle of LOX. The results obtained from our studies on *C. fistula* had shown its potential anti-inflammatory activity.

The *C. fistula* extracts inhibited the lipoxygenase enzyme activity. This indicates that plant *C. fistula* is more useful in studies of inflammation and in various related physiological studies and disease such as aging, cancer, etc.

The solvent fractions exhibited a moderate XO inhibitory activity and therefore may be due to presence of bioactive constituents and these may be useful in the treatment of XO induced diseases. The acetylcholinesterase inhibitory activity was noticed from above said solvent extracts, these can be used for the neurological diseases. These extracts also exhibited antioxidant and anti-inflammatory properties strongly. These extracts reduced the activity of lipoxygenase, xanthine oxidase and acetylcholinesterase activities. Purification of each bioactive compound can be needed and purified compound can also increase their activity.

The antioxidant activity and anti-inflammatory activity was comparable to standard ascorbic acid, BHT and aspirin. These findings provide scientific evidence to support traditional medicinal uses and indicate a promising potential for the development of an antimicrobial, antioxidant and anti-inflammatory agent of *C. fistula* plant. This medicinal plant by in vitro results appear as interesting and promising and may be effective as potential sources of novel antimicrobial, antioxidant

and anti-inflammatory drugs.

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