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Research Article

EXTRACTIONS, PHYTOCHEMICAL SCREENING AND IN-VITRO ANTIOXIDANT ACTIVITY OF *CASSIA FISTULA* EXTRACTS

Rohit Kumar Bargah* and Pradeep Kumar Kushwaha

Department of Chemistry, Govt.S.P.M. College
Sitapur Dist.-Surguja, Chhattisgarh, India-4971 11.

ABSTRACT

Cassia fistula Linn used extensively in various parts of the world against a wide range of climate, the synergistic action of its metabolite production being most probably responsible for the plant's beneficial effects. These plants has been reported to possess antipyretic, anti-analgesic, smooth muscle stimulant, hepatoprotective, laxative, antimicrobial, andante implantation activity. To explore the phytochemical constituents of the ethanol and aqueous extracts of stem bark and leaves of *Cassia fistula*. Preliminary phytochemical screening was performed by the standard method. Screening of the stem bark and leaves of *Cassia fistula* proved the presence of bioactive constituents such as alkaloid, phenolic compound, flavonoids, saponins, glycosides, steroids, carbohydrates, protein and amino acid. Coumarin, Anthraquinone, anthocynosides , terpenoids were absent in the stem bark and leaves both extracts. The primary phytochemical study and in-vitro antioxidant study was performed on ethanol and aqueous extract of shade dried stem bark and leaves. Various extracts of *Cassia fistula* was determined by the DPPH screening assay. Ethanol and aqueous extracts showed the highest amount of phenolic and flavonoid content and reducing capacity. The anti-oxidant potential of *Cassia fistula* extracts was measured by DPPH (1, 1-diphenyl -2- picryl hydrazyl) assay and was compared to ascorbic acid. The antioxidant potential of *Cassia fistula* extracts significantly correlated ($P<0.023$).

The present study revealed that the *Cassia fistula* hydroalcoholic extract and aqueous extract of stem bark and leaves high significant radical scavenging activity. Result indicates that plant extract of *Cassia fistula* have marked amount of total phenols which could be responsible for the antioxidant activity and needs further exploration for their effective use in modern and traditional system medicines.

Keywords: Cassia fistula, antioxidant activity, Phytochemical Screening, Scavenging activity, DPPH.

1. INTRODUCTION

Cassia fistula L. (Caesalpinioideae), a very common plant known for its medicinal properties is a semi wild Indian Labernum Known as the golden shower tree [1]. It has great therapeutic implication in India system of medicine. The extracts derived from different parts of this plant have anti-bacterial, antipyretics, analgesic, anti-inflammatory and hypoglycemic properties and are used in the treatment of various disorders such as haematemesis, rheumatism, skin diseases , eye and liver ailments[2-4]. It is also one of the ingredients of the preparations known as pilex, Purian for piles and detoxifier respectively[5]. Phytochemical investigation of crude plant extracts shown the presences of bioactive principles in the plant parts like flowers, roots, fruits, seeds, leaves and barks etc. Phytochemical are non-nutritive plant chemicals that have protective or disease preventive properties. Various parts of this plants are known to be important sources of secondary metabolites mainly phenolic compounds such as epicatechin, procyanidin, biflavonoids, rhein glycosides, from the leaves[6-7] kaempferol, alkaloids, triterpenes from the flower[8] and lupeol, Beta-sitosterol,

oxyanthraquinone, xanthone glycoside from the stem bark[9-10], leucine, tryptophan, rhein from the pulp pod and fruits.[11] Antioxidant components are micro-constituents that inhibit lipid oxidation by inhibiting the initiation or propagation of oxidizing chain reactions and are also involved in scavenging of free radicals. The recent time for the management and therapeutic implication of neuro degenerative disorder's aging and chronic degenerative diseases.[12] The present study was conducted to evaluate the physic chemical studies, and preliminary phytochemical studies and antioxidant efficiency of *Cassia fistula* a medicinal plants(flower and stem bark) using polar solvents as a ethanolic and aqueous solvent.

2. METHODS AND MATERIALS

2.1 Collection of Plant Material

The fresh flower and stem bark plants parts were collected from the Mainpat forest region of Surguja, District of Chhattishgarh India in the month of April-June 2016 and the plant material was authenticated by the taxonomist Dr. N. K. Singh from the department of Botany, Govt. College Sargoan [C.G.].

2.2 Preparation of extract

250gm. of powdered of Cassia *fistula* were packed in soxhlet apparatus separately and extracted with solvent ethanol and aqueous. The extracts were filtered while hot and the solvents were removed by distillation and the last traces of solvent being removed under reduced pressure. The ethanolic and aqueous extracts were stored in refrigerator for further experimental work.

2.3 Preliminary Phytochemical Screening

Preliminary phytochemical screening was performed by using standard method.[13-15]

I. TESTS FOR ALKALOIDS

i. Dragendorff's test: 1 ml of extract. + 1 ml KBr. An orange-red ppt. (alkaloids present).
ii. Mayer's test: 1 ml of extract + 1 ml of KHgI. Whitish yellow or cream colored ppt. (alkaloids present).

II. GLYCOSIDES

i. Legal's test: (1 ml Ext. + Pyridine + Na_2 [Fe (CN) $_5$NO]). No colour (Glycoside absent).
ii. Baljet's test: (1ml ext. + 1ml $C_6H_2KN_3O_7$) yellow to orange colour (glycoside present).

III. TESTS FOR CARBOHYDRATE

i. Benedict's test: (5ml Benedict's reagent. + 1ml extract) Boil 2 min. and cool. (Red ppt., sugars present).
ii. Molisch's test: Extracts in ethanol separately + drops of 20% w/v solution of α naphthal in ethanol (90%). Shake well + add from side of test tube 1 ml of Conc. H_2SO_4 was Reddish violet ring between junction of the layers (carbohydrates present)

IV. FOR STEROIDS

i. Salkowski test: (Extract + $CHCl_3$ + equal volume of conc. H_2SO_4) was added. Bluish red to cherry in $CHCl_3$ and green fluorescence in the acid (steroidal present).
ii. Liebermann-Burchard test: (Extract + 1 ml of acetic anhydride and dissolved) by warming. The contents were cooled and a few drops of conc. H_2SO_4 were from sides of the test tube. (Blue colour) sterols present.

V. TEST FOR PROTEINS

i. Biuret test : 1ml 40% NaOH + 2 drops 1% $CuSO_4$ soln. till a blue color appear + 1ml extract. Pinkish / purple violet color (protein present).

VI. TEST FOR SAPONINS

i. Extracts boiled with 1 ml of distilled water and shaken. Foam formed (saponins present).
ii. Extract + 2 ml of DW + sodium carbonate and shake. The Foam formed (saponins present).

VII. TEST FOR TANNINS

i. Extract + lead acetate solution. White precipitates (tannins present).

VIII. TESTS FOR FLAVONOIDS

i. Shinoda test: Test solution + magnesium turnings Conc. HCl drops pink scarlet.

2.4 Quantitative Phytochemical Screening

2.4.1 Determination of total phenolics

The total amount of phenolic content of plant extract was determined by Folin Ciocalteu method. The standard solutions were prepared by taking 1, 2, 3, 4, 5µl sample from the stock of 100mg/ml and maintain final volume of 1ml. to this 1ml standard solution, 1ml Folin Ciocalteu´s reagent, previously dilute (1:4) was added. To the mixture, 4ml of sodium carbonate (75g/L) and 10 ml of distilled (1:4) were added and mixed well. The mixture was allowed to stand for 2 Hrs. at room

temperature. Contents were then centrifuged at 2000g for 5 min and the absorbance of the supernatant was taken at 760 nm. A standard curve was obtained using various concentrations of Gallic acid equivalents (GAE).

2.4.2 Determination of total flavonols

The total flavonols in the plant extract were estimated using the method of Kumaran et,.al. In this method again 1, 2, 3, 4, 5µl samples were taken from stock of 100mg/ml and final volume of 2ml was maintained. To this 2ml $AlCl_3$ ethanol and 3.0 ml (50 g/l) sodium acetate solutions were added to 1 ml standard solution of different concentration. The total flavonoids content were expressed as rutin equivalents.

2.5 DPPH (1, 1- diphenyl -2 piccryl hydrazyl) radical scavenging activity

1,1-diphenyl 1-2 picryl hydrazyl (DPPH) radical scavenging activity was measured according to the method of llhami et.al 2005. Extract solution were prepared by dissolving of different dry extract in methanol to produce a solution of 10mg/ml. 600 µM DPPH was dissolved in 300ml methanol and used as stock solution. The plant extract in methanol at various concentration (1, 2, 3, 4 and 5mg) whose final volume was maintained 1ml and were mixed with an aliquot of 2ml of 600µM DPPH solution in methanol and incubated at 25^{o}C for 30 min. 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 experiment was performed thrice and the results were averaged. Ascorbic acid was used as a standard.

DPPH scavenging effect=

$$\frac{A_{Control} - A_{Sample}}{A_{Control}} \times 100$$

Where, $A_{Control}$ and A_{Sample} standard for absorbance of the control and absorbance of tested extract solution respectively.

4. RESULT AND DISCUSSION

The preliminary phytochemical constituents detected in the flower are known to be beneficial in the treatment of infected diseases[19-20]. Recently, a number of studies has been carries out on the various phytochemical of plant across the worlds[21-23]. Evolutions of phytochemical such as alkaloids, flavonoids, carbohydrates, glycosides, protein and amino acid, saponin and triterpenoids revealed the presence of most of the constituents in polar extracts. In the present investigation steam bark and leaves of *Cassia fistula* ethanolic and aqueous extract of showed the presence of flavonoids derivative, alkaloids, terpenoids, reducing sugars, tannins, saponins, steroids, glycosides, phenolic compound , protein and amino acid. [Table.1]. Phenols, mainly the type of flavanoids from some medicinal plants are safe and bioactive and have antioxidants, antitumor, antibacterial and anti -inflammatory properties. Bioactive compound formed in various plant parts possess multiple biological effects on human and non-human biota[24].

Table1: Phytochemical analysis of different solvent extracts of *Cassia fistula*

phytochemical constituents	stem barks		leaves	
	ethanolic	aqueous	ethanolic	aqueous
alkaloids	+	+	+ +	+
tannin	+	+	++	+
saponin	+	-	++	+
anthraquinone	-	-	-	-
phenolic compound	+	+	++	++
flavonoids	+	+	++	++
carbohydrates	+	+	++	+
glycoside	+	+	++	+
steroids	+	-	++	+
terpenoids	-	-	-	-
amino acid and protien	+	+	+	+
coumarin	-	-	-	-

(+): presence, (++) -presence of higher concentration, (-) absence

DPPH Free Radical Scavenging Activity

In free radical scavenging activity, DPPH is a stable free radical at room temperature and accepts and electron or hydrogen radical to become stable diamagnetic molecule. The reduction capability of DPPH radical was determined by the decrease in its [Table2] absorbance at 517 nm, which is induced by different antioxidants .The decrease in absorbance of DPPH radical caused by antioxidants because of the reaction between antioxidant molecules and radical progress which results in the scavenging of the radical by hydrogen donation. It is visually noticeable as a change in colour from purple to yellow. *Cassia fistula* exhibited a comparable antioxidant activity with that of standard ascorbic acid at varying concentration tested (50, 100, 150, 200, 250, IC_{50} µg/ml). There was a dose dependant increase in the percentage antioxidant activity for all concentrations tested [Table 2.1, 2.2, 2.3, 2.4] for stem bark and leaves of ethanolic and aqueous extracts.

The ethanolic and aqueous extracts of stem bark at a concentration of 50µg/ml showed a percentage inhibition of 39.21 ± 0.68 and 49.62± 0.73 and for 250µg/ml it was 99.17 ±0.38 and 111.52 ± 0.37. Ascorbic acid was used as the standard drug for the determination of the antioxidant activity by DPPH method. The concentration of standard ascorbic acid of 91.53 ± 0.81. A graded increase in percentage of inhibition was observed for the increasing in the concentration of ascorbic acid. As shown in table [2.1 and 2.2] *Cassia fistula* of ethanolic extract and aqueous extracts stem bark strongly scavenged DPPH radical with the IC_{50} being 75.82 and 135.08. The standard drug ascorbic acid scavenged DPPH radical was found to be 91.53 0±.81.Hence DPPH is usually used as a substance to evaluate the more antioxidant activity of plant extract.

Table 2.1: Free radical scavenging capacity of ethanol extract of *Cassia fistula* stem barks

Concentration (µg/ml)	DPPH Scavenging %	
	Ethanol Extract	Ascorbic Acid
50	39.21±0.68	91.53±0.81
100	60.35±0.92	-
150	73.14±0.47	-
200	86.45±0.52	-
250	99.17±0.38	-
IC_{50}	75.82	-

Values are mean ± SEM of three determinations

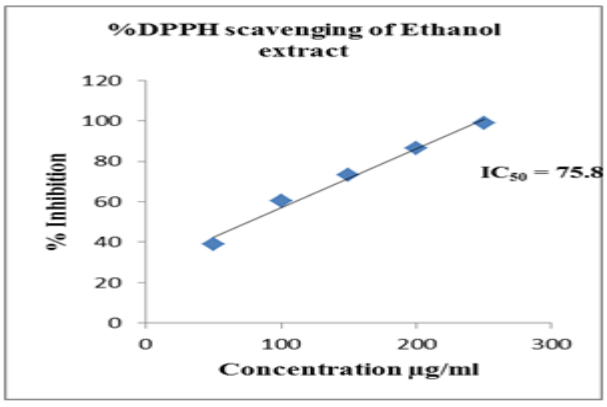


Table 2.2: Free radical scavenging capacity of aqueous extract of *Cassia fistula* stem barks

Concentration (µg/ml)	DPPH Scavenging %	
	Aqueous Extract	Ascorbic Acid
50	24.73±0.58	91.53±0.81
100	42.32±0.42	-
150	54.62±0.25	-
200	67.58±0.67	-
250	81.39±0.73	-
IC_{50}	135.08	-

Values are mean ± SEM of three determinations

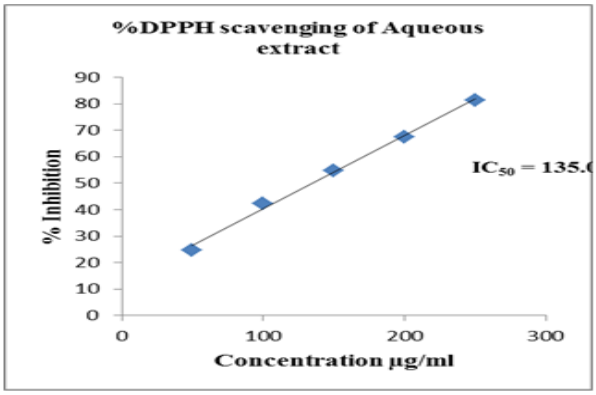


Table 2.3: Free radical scavenging capacity of ethanol extract of *Cassia fistula* leaves

Concentration (µg/ml)	DPPH Scavenging %	
	Ethanol Extract	Ascorbic Acid
50	38.17±1.05	91.53±0.81
100	53.62±0.62	-
150	68.12±0.84	-
200	84.36±0.42	-
250	95.11±0.64	-
IC_{50}	88.20	-

Values are mean ± SEM of three determinations

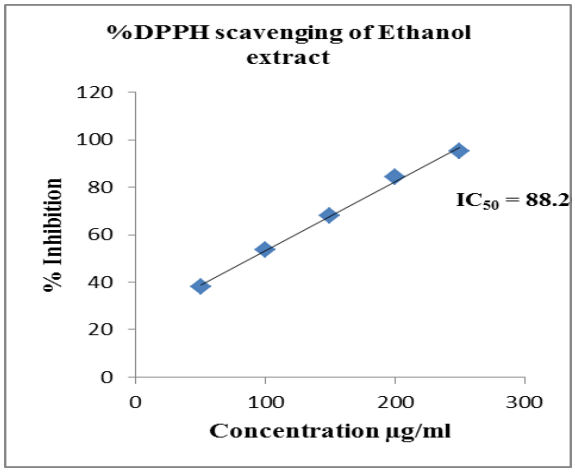
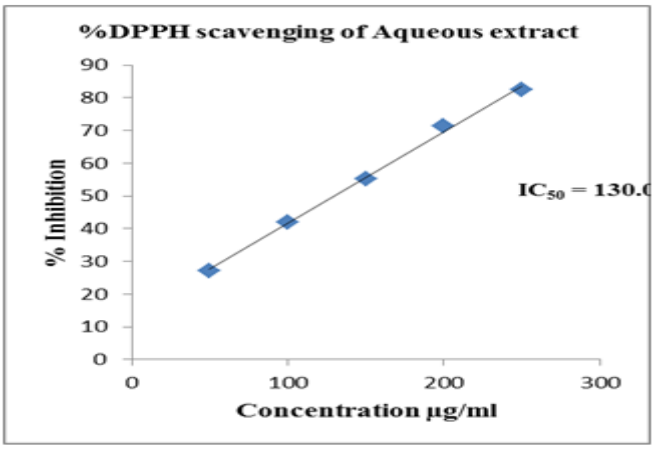


Table 2.4: Free radical scavenging capacity of aqueous extract of *Cassia fistula* leaves

Concentration (µg/ml)	DPPH Scavenging %	
	Aqueous Extract	Ascorbic Acid
50	27.14±0.38	91.53±0.81
100	41.85±0.72	-
150	55.24±0.49	-
200	71.30±0.35	-
250	82.43±0.63	-
IC_{50}	130.01	-

Values are mean ± SEM of three determinations



5. CONLUSION

Form this study it has been clear that crude extract of *Cassia fistula* contain some medicinal active components. The ethanolic and aqueous extract of *Cassia fistula* showed antioxidant activity by inhibiting DPPH and hydroxyl radical and total phenol content. The preliminary phytochemical investigation indicates the presence of phenol and flavonoids in the plant, and other bioactive constituent flavonoids type components which plays a major role in controlling antioxidants. The result of this study show that the ethanolic and aqueous extract of *Cassia fistula* can be used as easily accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical industry. However, the components responsible for the antioxidant activity of ethanolic and aqueous extracts of *Cassia fistula* are currently unclear. Therefore, further works have been performed on the isolation and identification of the antioxidant compounds present in extracts of plants.

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REFERENCES

1. Prashanth Kumar V, Chauhan NS, Padh H and Rajani M. Search for Antibacterial, Anti-fungal agents from selected Indian Medicinal Plants. J Ethanopharmacol. 2006;107:182-188.
2. Hahorun T, Neergheen VS, Aruoma OI. African J Biotech. 2005;4:1530-1540.
3. Duraipandiyan V, Ignacimuthu V. J Ethanopharmacol. 2007;1:590-594.
4. Sangeetha SN, Zuraini Z, Sashidharan S, Sunaini S. J Mol Biol Biotech. 2008;1:1-4.
5. Agrawal SS and Paridhavi M. Clinically useful herbal drugs. Ahuja Publishing Home. 2005;281-282.
6. Kaji NN, Khorana ML and Sanghavi MM. Indian J Pharma. 1968;30: 8-11
7. Mahesh VK, Sharma R and Singh RS. Anthaquinone and kaempferol from Cassia fistula species. J Nat Prod. 1984;47:733-751.
8. Tzakou O, Loukis A and Said A. Journal of essential Oil Research. 2007;19(4):360-361.
9. Anonymous. The Wealth of India first supplement series (Raw Material), National Institute of Science Communication and Resources (NISCAR), CSIR. 2007;1:234.
10. Rastogi RP and Malhotra BN.Compendium Indian Medicinal Plant. CDRI, Lucknow. 2004;4: 155-156.
11. Kumar A, Pande CS, Kaul RK. Chemical Examination of Cassia fistula. Indian J Chem. 1966; (4):460-463.
12. Larson R. The antioxidant of Higher Plants. Phytochemistry. 1988;27:967-978.
13. Evans WC. 16th edn. London; Sauders Elsevier,Trease and Evans, Pharmacognosy. 2009.
14. Harborne JB. A Guide to Modern Techniques of plant analysis, 3rd edn., London New York; Champan and Hall Phytochemical Method. 1998.
15. Farnsworth NR. Biological and Phytochemical Screening of plant. J Pharma Sci. 1966;, 5:525-576.

16. Polat AA, Caliskan O, Serce S, Saracoghi O, Kaya C and Ozgen M. Determining total phenolic content and total antioxidant capacity of Loquat Cultivars grown in Hatay. Pharmacogn Mag. 2010;6:5-8.
17. Chang C, Yong M and Wen H. J Chem Estimation of total flavonoid content in propelis by two complementary colorimetric method. J Food Drug Anal. 2002;10:178-182.
18. Villano D, Fernandez Pachon MS, Maya ML, Tronaco AM and Crareia-Parrilla MC. Radical Scavenging ability of polyphenolic compounds towards DPPH for radical. Talanta. 2000;71: 230.
19. Joselin J, Brintha TSS, Florence AR and Jeeva S. Asian Pacific Journal of Tropical Disease. 2012;2(S1):260-264.
20. Joselin J, Brinin TSS and Florence AR. Journal of Chemical Pharmaceutical Research. 2013; 5(4):106-111
21. Suresh, Kumar P. J Basic Applied Bio. 2008;2(1):9-13.
22. Asolkar LV, Kakekar KK and Chakre OJ. Second Supplement to glossary of Indian Medicinal Plant with active principles. New Delhi, Publication and Information Directorate, CSIR. 1992; 414-418.
23. Valsaraj R, Pushpangadan P, Smitt UW, Adsersen A and Nyman U. Antimicrobial Screening of Selected Medicinal Plants from India. J Ethanopharmacol. 1997;58:75-83.
24. Bargah RK. Phytochemical Screening Analysis and Therapeutic Potential of Tecoma stans (L.) International Journal of Applied Chemistry. 2017;13(1):129-134.