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Evaluation of *in vitro* Antioxidant activity of Flowers of *Cassia fistula* Linn.

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Abstract: The present study was aimed to investigate the antioxidant activity of extracts of dried flower powder of *Cassia fistula* Linn.(Family: *Leguminosae)*. *Cassia fistula* Linn., a semi-wild India Labernum, is widely cultivated in various countries including Asia, Mauritius, South Africa, Mexico, China, West Indies, East Africa and Brazil as an ornamental tree for its beautiful bunches of yellow flowers and also used in traditional medicine for several indications. *Cassia fistula* Linn. has been reported to possess hypoglycaemic, anticancer, abortifacient, anticolic, antifertility, estrogenic, laxative, antimicrobial, antipyretic, anti-inflammatory, smooth muscle stimulant, antiarthritic, antitussive, purgative, analgesic, antiviral, hepatoprotective, and anti implantation activity. The primary phytochemical study and *in vitro* antioxidant study was performed on hydroalcoholic extract of shade dried flowers. Modern phytochemical screening of the plant has shown the presence of phenolic compounds, fatty acids, flavonoids, tannins and glycosides. Extracted plant were evaluated for their phenolic & antioxidant activity. Phenolic content was measured using Folin-Ciacalteu reagent & was calculated as Gallic acid equivalents. Antiradical activity of hydroalcoholic extract was measured by DPPH(2,2-diphenyl-1 picryl hydrazyl) assay and was compared to ascorbic acid(vitamin C), and Ferric reducing power of the extract was also evaluated by Oyaizu method. In the present study three methods used for evolution of antioxidant activity. The first two methods were for direct measurement of radical scavenging activity & remaining one method evaluated the reducing power. The present study revealed that the *Cassia fistula* hydroalcoholic extracts of flower has significant radical scavenging activity. In this study, *Cassia fistula* were identified as potentially novel source of free radical scavenging compound. Results indicates that hydroalcoholic flower extracts of *Cassia fistula* have marked amount of total phenols which could be responsible for the antioxidant activity, but the mechanism remains unclear and could be further investigated by detailed phytochemical investigation.
Keywords: *In vitro* Antioxidant activity, *Cassia fistuala*, free radical scavenging activity(DPPH assay), Reducing power, total phenol content.

INTRODUCTION

Natural antioxidants present in the plants scavenge harmful free radicals from our body. Free radical is any species capable of independent existence that contains one or more unpaired electrons which reacts with other molecule by taking or giving electrons, and involved in many pathological conditions[1]. Free radicals can be described as chemical species that have an unpaired electron and play very important role in human health and beneficial in combating against several diseases like cardiovascular disorders, lung damage, inflammation etc. These free radicals are highly unstable and when the amount of these free radicals exceed in the body, it can damage the cells

and tissues and may involved in several diseases. Thus there is the need of antioxidant of natural origin because they can protect the human body from the diseases caused by free radicals[2,3].

An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. The most common reactive oxygen species (ROS) include superoxide (02^-) anion, hydrogen peroxide (H202), peroxyl (ROO^-) radicals, and reactive hydroxyl (OH^-) radicals. The nitrogen derived free radicals are nitric oxide (NO) and peroxynitrite anion (ONOO)[4]. Under normal state of affairs, the ROS generated are detoxified by the antioxidants nearby in the body and there is symmetry between the ROS generated and the antioxidant present. However due to ROS over production and/or derisory antioxidant argument, this equilibrium is hindered favoring the ROS gain that culminates in oxidative hassle. The ROS readily attack and induce oxidative damage to various biomolecules including proteins, lipids, lipoproteins and DNA[5]. This oxidative damage is decisive etiological factor concerned in quite a lot of chronic human diseases such as diabetes mellitus, cancer, atherosclerosis, arthritis, and neurodegenerative diseases and also in the ageing course[6]. Based on growing interest in free radical biology and the lack of effective therapies for most chronic diseases, the expediency of antioxidants in protection against these diseases is defensible. Epidemiological studies have brought into being that the intake of antioxidants such as vitamin C reduces the risk of coronary heart diseases and cancer[7]. It is possible to reduce the risk of chronic diseases and prevent disease progression by either enhancing the body's natural antioxidant defenses or by supplementing with proven dietary antioxidants[8].

Currently available synthetic antioxidants like BHT, butylated hydroxyl anisole (BHA) and tertiary butylated hydroquinones have been suspected to cause or prompt negative health effects. Hence, strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants[9]. Several studies revealed that phenols, mainly the type of flavonoids, from some medicinal plants are safe and bioactive, and have antioxidant properties and exert anticarcinogenic, antimutagenic, antitumoral, antibacterial, antiviral and anti-inflammatory effects[10]. Therefore in current years, substantial attention has been directed towards credentials of plants with antioxidant ability that may be used for human expenditure.

Due to their redox properties, acting as reducing agents, hydrogen donors, singlet oxygen quenchers and chelating metals[11,12,13]. The medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent antioxidant activities, no side effects and economic viability[14], in recent years one of the areas, which attracted a great treaty of attention, is antioxidant in the control of degenerative disease in which oxidative dent has been implicated. Several plant extracts have been shown to antioxidant activity[15,16,17].

OBJECTIVE

Recently interest has increased considerably in finding natural occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants that are being restricted due to their side effects such as carcinogenicity. One among such natural plants is *Cassia fistula* Linn. Commonly known as Indian Laburnum, *Cassia fis*tula L.,(Leguminosae), a semi-wild Indian Labernum (also known as the Golden Shower), is distributed in various countries including Asia, Mauritius, South Africa, Mexico, China, West Indies, East Africa and Brazil as an ornamental tree for its beautiful bunches of yellow flowers. Recognize by the british pharmacopoeia[18].The flowers are reported to have demulcent and lubricating effect, bitter, acrid, cooling, emollient and useful in skin diseases, pruritus, burning sensation, dry cough and bronchitis[19].

Thus, present study was undertaken to evaluate the *in vitro* antioxidant effect of hydroalcoholic extract of *Cassia fistula* flower parts. The main constituents present in flowers are tannins, isoflavonoids, flavonoids, glycosides, anthraquinones, phenolic compounds. The objective of the present study was to evaluate the antioxidant potential and free radical scavenging activity of a hydroalcoholic extract of *Cassia fistula* Linn. The extract was examined for different reactive oxygen species (SOS) scavenging activities such as DPPH assay, ferrus Reducing capacity, and total phenol content.

MATERIAL AND METHODS

Plant material

The fresh *Cassia fistula* flowers were collected from the local vendor in Jamnagar, Gujarat, India in the month of March-April 2009. The plant was authenticated by the department of pharmacognosy, I.P.G.T.& R.A. Jamnagar, Gujarat, India. Plant parts were collected on the basis of the information provided in the ethanobotanical survey of India. Each specimen/plant material was labeled, numbered, annoted with the date of collection, locality and their medicinal uses were recorded.

Preparation of plant extract

The extraction of the *Cassia fistuala* flowers were carried out using known standard procedures[20]. The plant materials were dried in shade and powdered in a mechanical grinder. The powder(25.0gm) of the plant materials were initially de-fatted with petroleum ehrer (60-80°C), followed by 900 ml of hydroalcohol by

using a Soxhlet extractor for 72 hrs at a temp. not exceeding the boiling point of the solvent. The extracts were filtered using Whattman filter paper (No.1), while hot and concentrated in vacuum under reduced pressure using rotary flask evaporator and dried in a desiccator. The hydroalcoholic extract yield a dark brownish solid residue weighing 6.750 gm (27.0% w/w) respectively. the extracts were kept in sterile bottles, under refrigerated conditions, until further use. The dry weight of the plant extracts was obtained by the solvent evaporation and used to determine concentration in mg/ml. the extract was used directly for DPPH assay, total phenol and ferrus reducing power content and also for assessment of antioxidant capacity through various chemical assays.

Priliminary phytochemical testing

The extracts were subjected to Preliminary phytochemical testing to detect for the presence of different chemical groups of compounds. Air-dried and powdered plant materials were screened for the presence of saponins, tannins, alkaloids, flavonoids, triterpenoids, steroids, glycosides, anthraquinones, cumarin, saponins, gum, mucilage, carbohydrates, reducing sugars, starch, protein and amino acids as described in literatures[21,22,23].

Phytochemical evaluation

The hydroalcoholic extract of *Cassia fistula* flowers were subjected to the following chemical tests for the identification of various active constituents**.**

Chemicals & Instrument

Chemicals

2,2-Diphenyl-1-Picrylhydrazyl (DPPH, Lancaster-UK), Gallic acid (Loba-India), were purchased from Krishna scientific traders, Rajkot, Gujarat, India. *Folin Ciocalteu's* reagent, sodium carbonate, ascorbic acid, hydrogen peroxide, potassium ferricynide, trichloroacetica cid, ferric chloride, All other reagents were of analytical grade were obtained from the pharmaceutical chemistry laboratory of I.P.G.T & R.A., Jamnagar, Gujarat, India.

Instrument

UV spectrophotometer (Systronic double beam- UV-2201).

Centrifuge machine (Remi instruments-C24).

Table1. Phytochemical screening of *Cassia fistula* plant extracts.

Phytochemical composition of plant extracts

Serial No.	Secondary Metabolite	Flowers (Hydroalcoholic extract of *Cassia fistula*)
1.	Alkaloids	+
2.	Tanins	+
3.	Flavonoids	+
4.	Saponins	+
5.	Triterpenoids	+
6.	Steroids	+
7.	Glycosides	+
8.	Coumarin	-
9.	Anthraquinones	+
10.	Reducing Sugars	+
11.	Carbohydrates	+
12.	Gum & Mucilage	+
13.	Starch	-
14.	Proteins	+
15.	Amino acids	+

(+) Indicate Present and (-) Indicate Absent

Determination of total antioxidant activity

in-vitro antioxidant activity

Free radical scavenging activity(DPPH Assay) [24]

The anti-oxidant potential of any compound can be determined on the basis of its scavenging activity of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical as described by Sadhu et al[25] .DPPH is a stable free radical containing an odd electron in its structure and usually utilized for detection of the radical scavenging activity in chemical analysis.

The absorption maximum of a stable DPPH radical in methanol was at 517 nm. The decrease in absorbance of DPPH radical caused by antioxidants, because of the reaction between antioxidant molecules and radical progresses, which results in the scavenging of the radical by hydrogen donation.

Preparation of standard solution

Required quantity of Ascorbic acid was dissolved in methanol to give the concentration of 5, 10, 15, 25, 50 and 60 µg/ml.

Preparation of test sample

Stock solutions of samples were prepared by dissolving 10 mg of dried hydroalcoholic extract in 10 ml of methanol to give concentration of 1mg/ml. then prepared sample concentrations of 5, 10, 15, 25, 50 and 60 µg/ml.

Preparation of DPPH solution

3.9 mg of DPPH was dissolved in 3.0 ml methanol, it was protected from light by covering the test tubes with aluminum foil.

Protocol for estimation of DPPH scavenging activity

Antiradical activity was measured by a decrease in absorbance at 517 nm of a solution of colored DPPH in methanol brought about by the sample[26,27,28,29]. A stock solution of DPPH (1.3 mg/ml in methanol) was prepared such that 75 µl of it in 3 ml methanol gave an initial absorbance of 0.9. Decrease in the absorbance in the presence of sample extract and standard at different concentrations was noted after 30

Minutes.EC_{50} was calculated from% inhibition.A blank reading was taken using methanol instead of sample extract .Absorbance at 517 nm is determined after 30 min. using UV-visible Spectrometer(Systronic double beam- UV-2201), and IC50 (Inhibitory concentration to scavenge 50% free radicals) is also determined. Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity. IC50 value denotes the concentration of sample required to scavenge 50% of the DPPH free radicals.

The capability to scavenge the DPPH radical was calculated using the following equation.

$$\text{DPPH Scavenged (\%)} = \frac{\text{Acontrol-Atest}}{\text{Acontrol}} \times 100$$

Where A control = Absorbance of DPPH alone

A sample = Absorbance of DPPH along with different concentrations of extracts.

IC50 was calculated from equation of line obtained by plotting a graph of concentration versus % inhibition.

Reducing power assay

For the measurement of the reductive ability, we investigated the Fe+3 Fe+2 transforma-tions in the presence of *Cassia fistula* hydroalcoholic extract using the method of Oyaizu[30]. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Like the antioxidant activity, the reducing power of *Cassia fisutla* extract and standard increase with increasing concentration.

Preparation of standard solution

3 mg of ascorbic acid dissolved in 3 ml of distilled water. Dilutions of this solution with distilled water were prepared to give the concentration of 10, 25, 50, 75 and 100 µg/ml**.**

Preparation of test sample

Stock solutions of samples were prepared by dissolving 10 mg of dried methanolic extract in 10 ml of methanol to give concentration of 1mg/ml. then prepares sample concentrations of 10, 25, 50, 75 and 100 µg/ml.

Protocol for reducing power

According to this method, the aliquot of various concentrations of the standard and test sample extracts (10 to 100µg/ml) in 1.0 ml of deionized water were mixed with 2.5 ml of (pH 6.6) phosphate buffer and 2.5 ml of (1%) potassium ferricyanide .The mixture was incubated at 500°C in water bath for 20 min. after cooling, Aliquots of 2.5 ml of (10%) trichloroacetic acid were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution 2.5 ml was mixed with 2.5 ml distilled water and a freshly prepared 0.5 ml of (0.1%) ferric chloride solution. The absorbance was measured at 700 nm in UV-visible spectrometer (Systronic double beam-UV-2201) [31].A blank was prepared without adding extract. Ascorbic acid at various concentrations (10 to 100µg/ml) was used as standard. As illustrated in figures Fe^{+3} was transformed to Fe^{+2} in the presence of *Cassia fistula* extracts.This results indicates that

Increase in absorbance of the reaction mixture indicates increase in reducing power.

Total phenolic content

phenolic compounds are plant secondary metabolites produced either from phenylalanine or from its precursor shikimic acid[32]. The antioxidant potential of phenolic compounds has been shown in a number of in-vitro studies. They are capable of direct chain-breaking antioxidant action by radical scavenging. In addition to having potential for independent antioxidant action, polyphenols have been suggested to spare essential antioxidants[33].

Preparation of standard solution

3 mg of Gallic acid dissolved in 3 ml of distilled water. Dilutions of this solution with distilled water were prepared to give the concentration of 25, 50, 75, 100, 200 and 250 µg/ml**.**

Preparation of test sample

Stock solutions of samples were prepared by dissolving 10 mg of dried methanolic extract in 10 ml of methanol to give concentration of 1mg/ml. then prepares sample concentrations of 25, 50, 75, 100, 200 and 250 µg/ml**.**

Protocol for Total phenol

Total Phenolic content was determined using Folin-Ciocalteau was established according to the method described by singleton and Rossi[34]. The powdered extract of plant was dissolved in methanol to obtain a concentration of 1 mg/ml. The 100µl of this solution was taken in to 25ml volumetric flask, to which 10ml of water and 1.5ml of Folin-Ciocalteau reagent were added. The mixture was then kept for 5 min and to it 4 ml of 20% w/v sodium carbonate solution was added the volume was made up to 25ml with double distilled water. The mixture was kept for 30 minute until blue colour develops. The samples were then observed at 765 nm. The % of total phenolic was calculated from calibration curve of Gallic acid plotted by using similar procedure [35,36.]

RESULT AND DISCUSSION

Preliminary phytochemical screening

It was found that hydroalcoholic extract of *Cassia fistula* contained tannins, glycosides, phenolic compounds and flavonoids in higher amounts.

DPPH Free radical scavenging activity

In free radical scavenging activity, DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become stable diamagnetic molecule. The reduction capability of DPPH radical was determined by the decrease in its 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 anti oxidant activity with that of standard ascorbic acid at varying concentration tested (5,10, 15, 25, 50, 60 µg /ml). There was a dose dependant increase in the percentage antioxidant activity for all concentrations tested (Table2,3).

The extract at a concentration of 5µg/ml showed a percentage inhibition of 14.28±0.19 and for 60µg/ml it was 40.21±0.35. Ascorbic acid was used as the standard drug for the determination of the antioxidant activity by DPPH method. The concentration of ascorbic acid varied from 1 to 50 µg/ml. Ascorbic acid at a concentration of 5µg/ml exhibited a percentage inhibition of 44.50±0.59 and for 50 µg/ml 98.79±0.28 (Table 2,3). A graded increase in percentage of inhibition was observed for the increase in the concentration of ascorbic acid. The EC50 value of ascorbic acid was found to be 6.1 µg/ml. EC50 value of sample extracts could not be calculated because of lower values of inhibition than 50 % . All determinations were done in duplicate and the mean values were determined. Hence DPPH is usually used as a substance to evaluate the antioxidant activity.

Reducing power assay

Reducing power assay method is based on the principle that substances, which have reduction potential, react with potassium ferricyanide (Fe3+) to form potassium ferrocyanide (Fe2+), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm. The reducing power of the hydroalcoholic extracts and standards increases with the increase in amount of sample and standard concentrations.(Table 4,5). The Reducing power shows good lienear relation in both standard(R^2=0.981) as well as sample extract(R^2=0.977)(Fig.3,4).

Table 2: Shows percentage inhibition of standard at concentrations (µg/ml) in hydrogen peroxide scavenging model

Concentration (µg/ml)	% Inhibition	EC50
5	44.50±0.59	
10	52.74±0.22	
15	62.63±0.34	6.1 µg/ml
25	78.57±0.32	
50	98.79±0.28	
60		

Values are mean ± SD of three parallel measurements
STD-Ascorbic acid

Table 3: Shows percentage inhibition of hydroalcoholic extract of flowers at various concentrations (µg/ml) in hydrogen peroxide scavenging model

Concentration (µg/ml)	% Inhibition	EC50
10	14.28±0.19	
15	16.70±0.23	
25	25.27±0.38	-
50	30.54±0.28	
60	40.21±0.35	

Values are mean ± SD of three parallel measurements
MCF-Hydroalcoholic extract of flower.

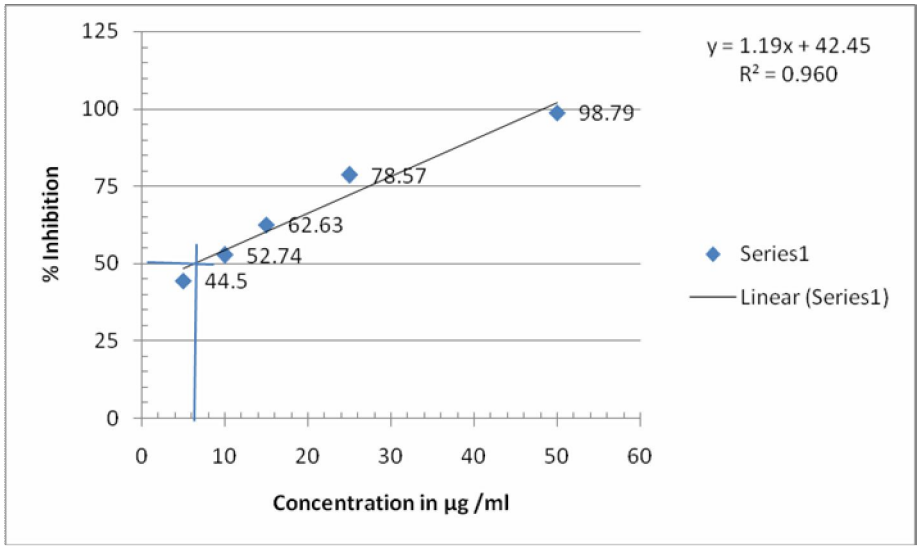

Fig.1 DPPH free radical scavenging activity of Std Ascorbic acid.

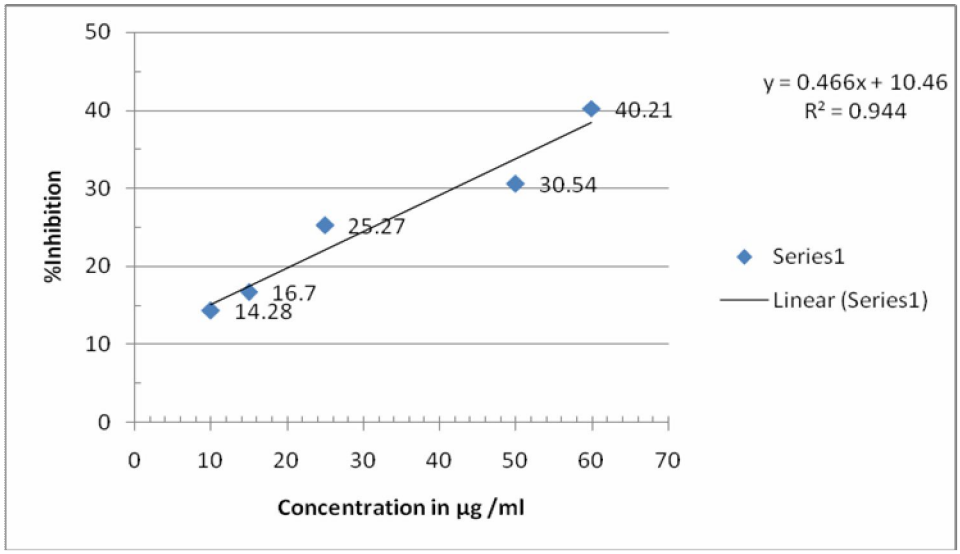

Fig.2 DPPH free radical scavenging activity of hydroalcoholic extracts of flower

Table 4: Shows the Absorbance of Standard at various concentrations (µg/ml) in ferric reducing power determination model.

Concentration (µg/ml)	Absorbance
10	0.090±0.002
25	0.122±0.003
50	0.240±0.002
75	0.352±0.004
100	0.471±0.001

Values are mean ± SD of three parallel measurements

Table 5: Shows the Absorbance of hydroalcoholic extract of flowers at various concentrations (µg/ml) in ferric reducing power determination model.

Concentration (µg/ml)	Absorbance
10	0.032±0.002
25	0.079±0.004
50	0.110±0.001
75	0.170±0.001
100	0.238±0.002

Values are mean ± SD of three parallel measurements

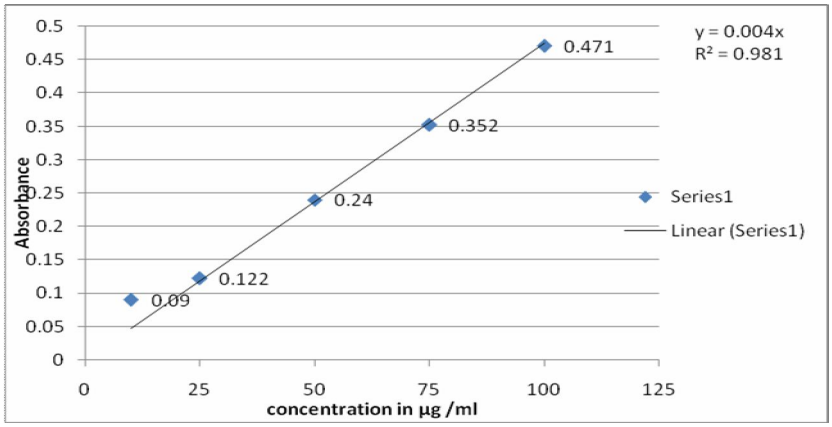

Fig.3 Ferric reducing power determination of standard ascorbic acid.

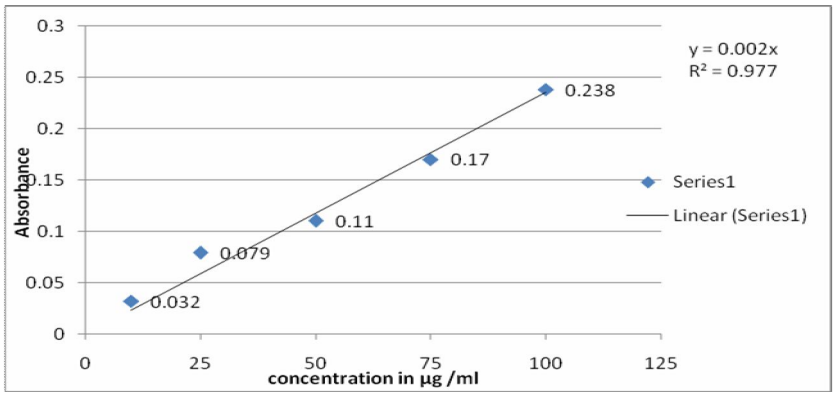

Fig.4 Ferric reducing power determination of hydroalcoholic extracts of flower

Total phenolic content

The total phenolic content of hydroalcoholic extract of *Cassia fistula* calculated as Gallic acid equivalent of phenols was detected.

Free radicals are produced under certain environmental conditions and during normal cellular function in the body; these molecules are missing in an electron, giving them an electric charge. To neutralize this, charge, free radicals try to withdraw an electron from, or donate an electron to, a neighbouring molecule. The newly create free radical, in turn, looks out for another molecules and withdraws or donates an electron, setting off a chain reaction that can damage hundred of molecules. The total phenol content shows good lienear relation in both standard as well as sample extracts(Fig.5,6). phenolic compounds are also very important plant constituents because of their hydroxyl groups confer scavenging ability.

Table 6: Shows the Absorbance of Standard Gallic acid at various concentrations (µg/ml) in total phenolic content determination model

Concentration (µg/ml)	Absorbance
25	0.194±0.012
50	0.395±0.010
75	0.652±0.009
100	0.790±0.015
200	1.192±0.010

Values are mean ± SD of three parallel measurements

Table 7: Shows the Absorbance of hydroalcoholic extract of flowers at various concentrations (µg/ml) in total phenolic content determination model

Concentration (µg/ml)	Absorbance
25	0.070±0.009
50	0.118±0.011
75	0.138±0.008
100	0.192±0.010
200	0.280±0.012
250	0.398±0.008

Values are mean ± SD of three parallel measurements

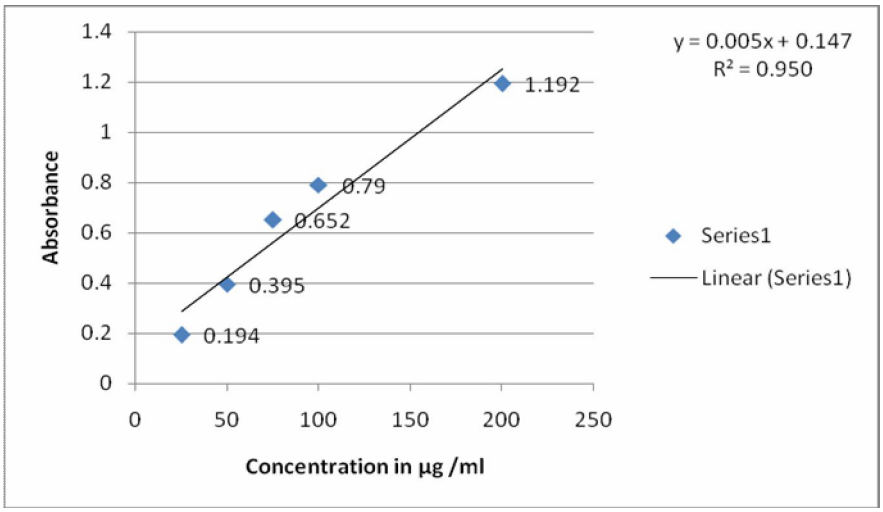

Fig.5 Total phenol content of standard Gallic acid.

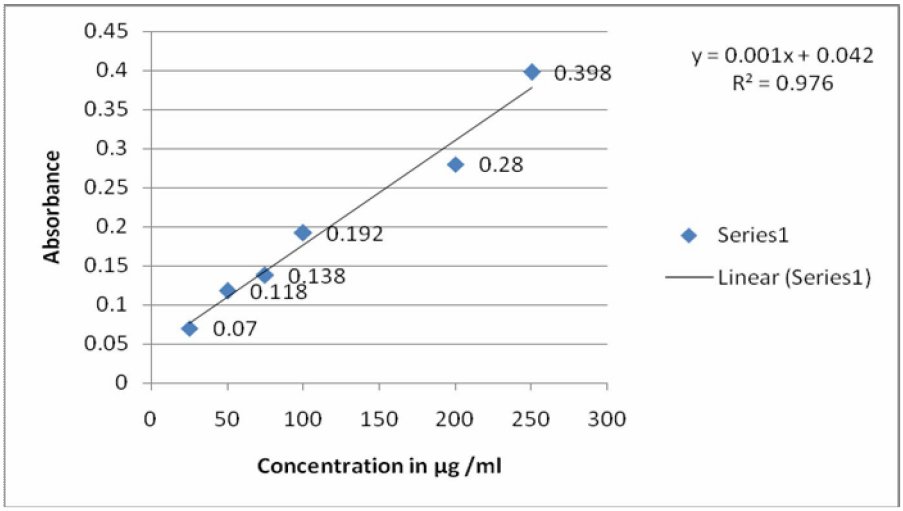

Fig.6 Total phenol content of hydroalcoholic extracts of flower

CONCLUSION

The hydroalcoholic extract of *Cassia fistula* showed antioxidant activity by inhibiting DPPH and hydroxyl radical, total phenol content and reducing power activities. The preliminary phytochemical investigation indicates the presence of phenols and flavonoids in the plant, In addition, the hydroalcoholic extract of *Cassia fistula* found to contain a noticeable amount of total phenols and tannins also flavonoids type components, which plays a major role in controlling antioxidants. The results of this study show that the hydroalcoholic 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 hydroalcoholic extract of *Cassia fistula* are currently unclear. Therefore, further works have been performed on the isolation and identification of the antioxidant components present in hydroalcoholic extract of *Cassia fistula.*

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REFERENCES

1. Madhavi D.L., Deshpande S.S. & Sulunkhe D.K., Food antioxidants: technological, toxicological and health perspectives; New york: Marcel dekker., 1996.

2. Upadhye M., Dhiman A., Shriwaikar A., Antioxidant activity of aqueous extract of Holostemma Annulare (Roxb) K Schum. Adv Pharmacol Toxicology., 2009, 10 (1), 127–131.

3. Mishra J., Srivastava R.K., Shukla S.V., Raghav C.S., Antioxidants in Aromatic and Medicinal plants. Science tech entrepreneur., 2007, 1–16.

4. Joyce D.A., Oxygen radicals in disease. Adv Drug React Bull., 1987, 127, 476–79.

5. Farber J. L., Mechanisms of cell injury by activated oxygen species. Environ. Health Pers., 1994, 102, 17-24.

6. Hogg N., Free radicals in diseases. Semin. In Reprod . Endocrin., 1998, 16, 241-88.

7. Marchioli R., Schweiger C., Levantesi G., Tavazzi L. and Valagussa F., Antioxidant vitamisn and prevention of cardiovascular disease:epidemiological and clinical trial data. Lipid., 2001, 36, 53-63

8. Stanner S.A., Hughes J., Kelly C.N., Buttriss J.A., Review of the epidemiological evidence for the antioxidant hypothesis. Public Health Nutrition., 2000, 7, 401-422.

9. Barlow S.M., Toxicological aspects of antioxidants used as food additives.Food antioxidants Elsevier London., 1990, 253–307.

10.Ozgová S., Hermánek J., Gut I., Different antioxidant effects of polyphenols on lipid peroxidation and hydroxyl radicals in the NAPH-, Feascorbate-, Fe-microsomal systems. Biochem Pharmacol., 2003, 66, 1127–1137.

11. Tung Y.T., Wub J.H., Huang C.Y., Chang S.T., Antioxidant activities and phytochemical characteristics of extracts from Acacia confuse bark. Bioresource Technology., 2009, 100, 509–514.

12.Frankel E., Nutritional benefits of flavonoids. International conference on food factors: Chemistry and cancer prevention. Hamamatsu, Japan: Abstracts., 1995, C, 2-6.

13. Larson R., The antioxidants of higher plants. Phytochemistry., 1988, 27, 969–978.

14.Auudy B., Ferreira F., Blasina L., Lafon F., Arredondo F., Dajas R., Screening of antioxidant activity of three Indian medicinal plants, traditionally used for the management of neurodegenerative diseases. J. Ethnopharmacol., 2003, 84, 131–138.

15. Larson R.A., The antioxidants of higher plants, Phytochem.,1998, 27, 969-78.

16. Tripathi Y.B., Chaurasia S., Tripathi E., Upadhyay A. and Dubey G.P., Bacopa monniera Linn. As an antioxidant: Mechanism of action . Ind. J. of Exp. Biol.,1996, 34, 523-26.

17. Sreejayan R.M.N.A.,Nitric oxide scavenging by curcuminoids. J. pharm. and Pharmacol., 1997, 49, 7-105.

18. Mukhopadhyay M., Saha A., Dutta A., De B., Mukherjee A., Genotoxicity of sennosides on the bone marrow cells of mice. FoodChem. Toxicol.,1998, 36, 937–940.

19. Sharma P.C., Yelne M.B., Dennis T.J.,Database on medicainal plants used in Ayurveda., 2005, Vol.2, 29-35.

20. Harborne J.B., Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. Chapmanand Hall., 1984.

21. Khandelwal K.R., Practical Pharmacognosy, Nirali Prakashan, Pune , Edition 2nd., 2009, 149-156.

22. Kokate C.K., Practical Pharmacognosy, New Gyan Offset Printers, Delhi., 2000, 107-109.

23.Kumar A., Ilavarasan R., Jayachandran, Decaraman, M., and Aravindhan P., Phytochemicals Investigation on a tropical plant in South India. Pakistan Journal of Nutrition., 2009, 8(1), 83-85.

24. Anandjiwala S., Bagul M.S., P Arabia M., Rajani M., Free radical scavenging activity of Panchvalkala, Indian Journal of Pharmaceutical Sciences, January-February., 2008, 31-35.

25.Sadhu S.K., Okuyama E., Fujimoto H, Ishibashi M., Separation of Leucas aspera, a medicinal plant of Bangladesh, guided by prostaglandin inhibitory and antioxidant activities. Chemical Pharmaceutical Bulletin., 2003, 51, 595-98.

26. Ravishankar M.N., Srivastava N., Padh H., Rajani M., Evaluation of antioxidant properties of root bark of Hemidesmus indicus, Phytomedicine 2002, 9, 153-60.

27.Vani T., Rajani M., Sarkar S., Shishoo C.J., Antioxidant properties of the Ayurvedic formulation Triphala and its constituents, Int J Pharmacog., 1997, 35, 313-7.

28. Navarro C.M., Montilla M.P., Martin A., Jimenez J., Utrilla M.P., Free radical scavenging and antihepatyotoxic activity of Rosamarinus tomentosus, Planta Med., 1993, 59, 312-4.

29. Bagul M.S., Ravishankar M.N., Padh H., Rajani M., Phytochemical evaluation and free radical scavenging activity of rhizome of Bergenia ciliate (Haw) Sternb: Forma ligulata Yeo, J Nat Rem., 2003, 3, 83-9.

30. Oyaizu M., Studies on product of browning reaction prepared from glucose amine. Japanese Journal of Nutrition., 1986, 44, 307-315.

31.Quisumbing E., Medicinal Plants of the Philippines., 1978.

32.Harbone, J.B., Methods of plant biochemistry: plant phenolics, Academic press, London, UK., 1989

33.Anna Nurmi., Antioxidant studies on selected Lamiaceae herbs in vitro and in humans, Ylio-pistopaino, University press, Helsinki, Finland, PDF, Http://www.ethesis.helsinki.fi/ISBN 978-952-10-4555-4. 2008.

34. Singleton V.L., Rossi J.A. Jr., Colorimetry of total phenolics with phosphomolybdic acid-phosphotungstic acid reagents. Am. J. Enol. Viticult., 1965, 16, 144-58.

35. Shahidi F., and Wanasundara P. K. J. P. D., Phenolic antioxidants. Critical Reviews in Food, Science and Nutrition., 1992, 32, 67–103.

36. Rice E., Miller C. A. and Paganga G., Free radical scavenging activity of plant polyphenolic. General Trends in Plant Science., 1997, 2(8), 152-159.

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