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Antioxidant Activity of *Cassia Fistula* Flower Extracts

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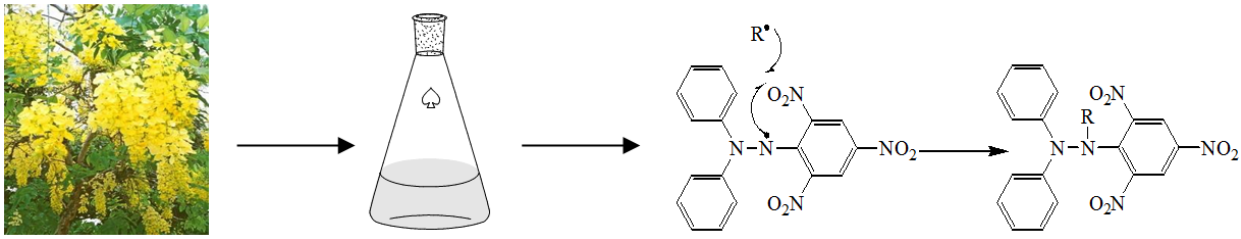
Abstract: The golden yellow flowers of *Cassia fistula* were extracted with n-hexane, dichloromethane and ethyl acetate. A few common compounds in the extracts were identified by GC MS analysis. Antioxidant activity of the crude extracts were compared by measuring DPPH radical scavenging activity, super oxide radical scavenging activity and lipid peroxidation inhibitory activity.

Keywords: Cassia fistula, antioxidants, antioxidant activity, DPPH, medicinal plants.

1 Introduction



Scheme 1

During the cellular respiration, oxygen generates energy in the form of ATP and some free radicals called Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) as the by-products. These reactive species play a dual role in human as both toxic and beneficial compounds. At low or moderate levels, reactive species exert beneficial effects on cellular redox signaling and immune function, but at high concentrations, they produce oxidative stress, a harmful process that can damage cell function and structures [1-2]. The delicate balance between their two opposite effects is undoubtedly a key aspect of life. Free radical induced oxidative stress is now believed to be a fundamental mechanism underlying a number of human cardiovascular, neurologic and other disorders. It has been estimated that approximately 5% of inhaled oxygen is converted into several damaging ROS [3]. These ROS may oxidize proteins, lipids or DNA and can initiate degenerative diseases.

Antioxidants that can trap free radicals are our crucial defense against free radical induced damages, and are critical for maintaining optimum health. They can reduce the risk for chronic diseases including cancer and heart diseases. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Owing to various side effects of synthetic antioxidants, naturally occurring antioxidants have considerable importance in medicine and in food processing. In this communication we report the antioxidant activity of *Cassia fistula* flower extracts. *Cassia fistula Linn.* (Leguminoseae) is a very common plant found in Kerala. The flowers of *Cassia fistula* has an attractive golden yellow colour (Figure 1) and is reputed as the official flower of the state. Different parts of the plant are reported to have a wide range of pharmacological applications [4-6].

Figure1. The flowers of *Cassia fistula*

2 Experimental

2.1 Chemicals

1,1-Diphenyl-2-picryl-hydrazyl (DPPH), nicotinamide adenine dinucleotide (NADH), butylated hydroxyl toluene(BHT) and trichloroacetic acid (TCA) nitro blue tetrazolium (NBT), phenazinemethosulphate (PMS) and thiobarbituric acid (TBA) were procured from Sigma Aldrich, USA. Solvents and other chemicals used were of analytical reagent grade.

2.2 Extraction of flower

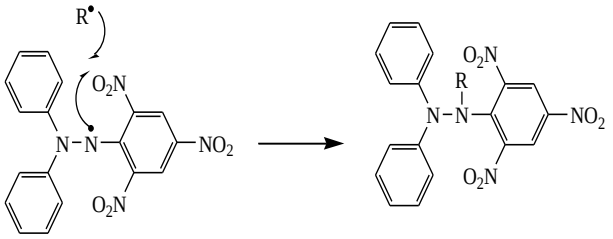
The extracts of *Cassia fistula* flower were prepared as per the general procedure given below. 5g petals of flower dried under shade was made into a paste in a mortar. The paste obtained was stirred with 50mL of organic solvent for nearly 3 hours, using a magnetic stirrer, in an Erlenmeyer flask. The yellow colored solution was decanted and the residue was again extracted twice with the same solvent. The extracts were combined together and concentrated in a rotary evaporator. The dried mass is collected and used as such for GC MS analysis and for antioxidant assay. The solvents used are dichloromethane, ethyl acetate and n-hexane.

2.3 GC-MS analysis

The GC-MS analysis was carried out at SAIF IIT Madras, Chennai using a gas–chromatography (make–Agilent, 7890) coupled with mass spectrometer (Joel AccuTOFGCV4G). It is a combined analyser (GC HRMS) that has a superior ability in analyzing organic compounds both quantitatively and qualitatively. The components were separated on Rtx – 5MS quartz capillary column (60m x 0.25mm) with diphenyl dimethylpolysiloxane stationary phase. The temperature increased to 180°C at a rate of 10°C/min and kept for 4 minutes, then with 15°C/min to 300°C and kept for 20 minutes. Sample injection volume was 0.3 mL with a split ratio 1:20, runtime 35 minutes and pressure at the column inlet 163kPa with Helium carrier gas at 1.21 mL/min flow rate. Compounds were identified by comparison of mass spectra with database of National Institute of Standard and Technology (NIST). The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library.

2.4 Determination of DPPH free radical scavenging activity

DPPH is a well-known radical to monitor chemical reactions involving radicals and recently it is most widely used for antioxidant assay [7]. When a solution of DPPH (1) having a strong absorption at 517 nm is mixed with that of a substance that can donate a hydrogen atom or a free radical, then DPPH is converted into its reduced form (2) which can be monitored by measuring the absorbance at 517 nm. Lower absorbance at 517 nm represents higher DPPH scavenging activity. The extracts were tested for the scavenging effect on DPPH radical according to the method of Pan *et al* [8].



(1) Diphenyl picryl hydrazyl free radical

(2) Diphenyl picryl hydrazyl non radical

In this method, 0.2 mL of extract solution in ethanol (95 %) at different concentrations (0.2, 0.5, 0.8 and 1.2 mg mL^{-1}) was added to 8 mL of 0.004 % (w/v) stock solution of DPPH in ethanol (95%). The scavenging activity on the DPPH radical was determined by measuring the absorbance at 517 nm until the reaction reached the steady state, using a UV–VISIBLE spectrometer (UV-160A, Shimadzu Co., Japan). All determinations were performed in triplicate. The DPPH radical scavenging activity (*S %*) was calculated as

$$S\% = ((A_{\mathbf{control}} - A_{\mathbf{sample}})/A_{\mathbf{control}}) \times 100$$

where $A_{\mathbf{control}}$ is the absorbance of the blank control (containing all reagents except the extract solution) and $A_{\mathbf{sample}}$ is the absorbance of the test sample. Radical scavenging potential of the extracts were compared from EC_{50} value, which represents the sample concentration at which 50% of the DPPH radicals scavenged.

2.5 Determination of Super oxide radical scavenging activity

The super oxide scavenging ability was assessed according to the method of Nishikimi, *et. al* [9]. The reaction mixture contained NBT (0.1 mM) and NADH(0.1 mM) with or without sample to be assayed in a total volume of 1 mL of Tris–HCl buffer (0.02 M, pH 8.3). The reaction was started by adding PMS (10 µM) to the mixture, and change in the absorbance was recorded at 560 nm every 30 s for 3 min. The percent inhibition was calculated against a control without test sample. Radical scavenging potential of the extracts were compared from EC_{50} value, which represents the sample concentration at which 50% of the radicals scavenged.

2.6 Determination of Lipid peroxidation inhibitory activity

Lipid peroxidation inhibitory activity was assessed according to the method of Yen, *et. al.*[10]. In this method, egg lecithin (3 mg/mL phosphate buffer, pH 7.4) was sonicated for 3 minutes .The test samples (10–100 µg/mL) were added to 1mL of liposome mixture, control was without test sample. Lipid peroxidation was induced by adding 10 µl $FeCl_3$ (400 mM) and 10µl-ascorbic acid (400 mM). After incubation for 1 h at 37^0C, the reaction was stopped by adding 2 mL of 0.25N HCl containing 15% TCA and 0.375%TBA and the reaction mixture was boiled for 15 min then cooled, centrifuged and absorbance of the supernatant was measured at 532 nm. Inhibitory activity of the extracts were compared from EC_{50} value, which is sample concentration inhibited 50% of lipid peroxidation.

3 Results and Discussion

10 mg of the dried extracts of *Cassia fistula* flower was subjected to GC MS analysis. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The common components in the extracts identified are 4-Dihydroxy-1-methoxyanthracene-9,10-dione or Purpurin-1-methyl ether (**1**) , Methyl 16-methylheptadecanoate (**2**), Butyl hexadecanoate or Butyl palmitate (**3**), 3-Methoxy-17-(2-methyl allyl)-estra-17-ol (**4**), 1,8-Ddihydroxy-3-methoxy-6-methylanthracene-9,10-dione or Parietin (**5**), 10-Methylundecanoate (**6**), 1,3,8-Trihydroxy-6-methylanthracene-9,10-dione (**7**), (*E*)-9-Tetradecenoic acid (**8**), 1,8-Dihydroxyanthracene-9,10-dione-3-carboxylicacid or Rhein (**9**), (2*E*,4*E*)-3,4-Dihydroxy-1,6-bis(3-methoxyphenyl)hexa-2,4-diene-1,6-dione (**10**), 1,8-Dihydroxy-3-methyl-9,10-anthracenedione (**11**) and Butyl 2-butoxyhexadec-4-ynoate (**12**).

The percentage composition of the above components in the extracts were calculated from the area in the chromatogram are presented in Table1. Structures of compounds are given in Figure 2.

Antioxidant activity of the crude extracts was compared by measuring DPPH radical scavenging activity, super oxide radical scavenging activity and lipid peroxidation inhibitory activity. The concentration of extract required to produce 50% scavenging activity (EC_{50} value) suggest that the ethyl acetate extract is having maximum radical scavenging activity and it is comparable with that of BHT. The n-hexane extract produced the same effect at higher concentrations. It is probably due to lower content of phenolic compounds in the extract. A comparison of the results is presented in Figure 3.

Table 1. Percentage composition of components in the extracts

Compounds identified	n-Hexane extract	Dichloromethane extract	Ethyl acetate extract
1	3.2	4.8	5.2
2	4.6	1.8	1.5
3	5.3	2.1	1.8
4	3.1	3.4	3.6
5	2.4	4.1	4.4
6	4.2	2.1	2.1
7	2.3	5.2	5.7
8	2.1	2.3	2.3
9	2.4	3.8	4.0
10	1.6	2.2	2.4
11	2.1	4.8	5.1
12	3.6	1.2	1.2

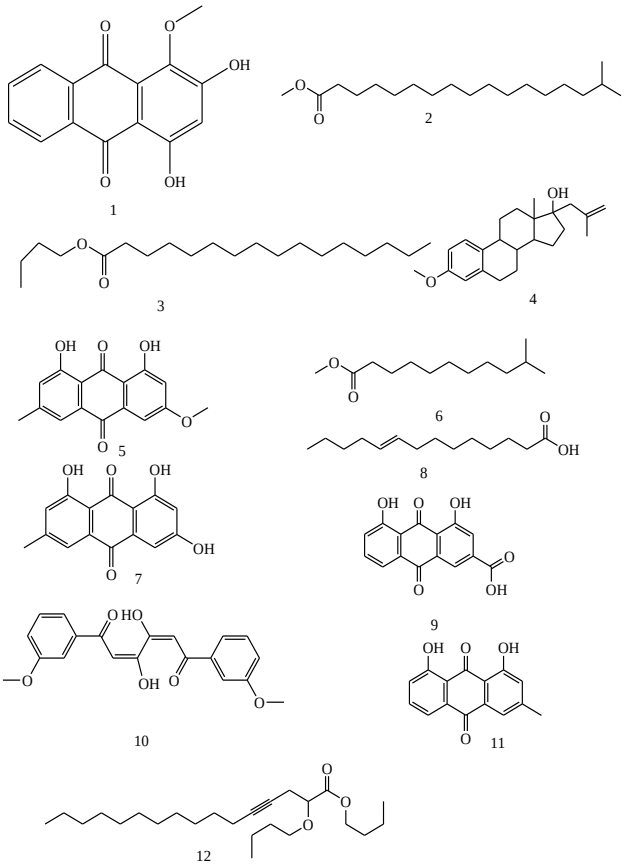


Figure 2. Phytochemicals in the extracts

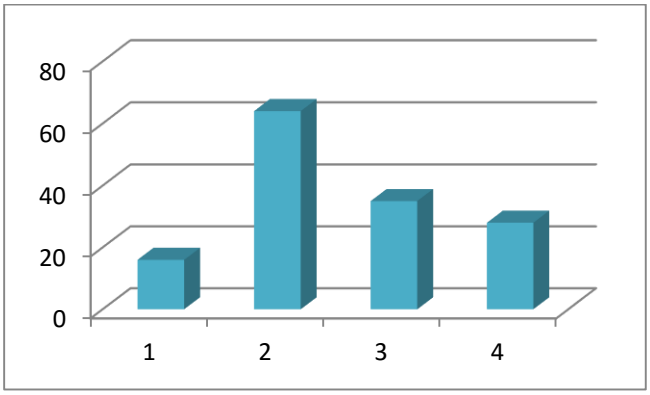


Figure 3.Comparison of EC_{50} value* of extracts and BHT
*1, 2, 3 and 4 are as in Table 2

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4 Conclusions

The major components present in *Cassia fistula* flower extracts were identified by GC MS. The potential antioxidant activity of *Cassia fistula* flower extracts was established by measuring DPPH radical scavenging, super oxide radical scavenging and lipid peroxidation inhibitory activities.Qualitative results reveal that ethyl acetate extract has the maximum antioxidant activity. It is probably due to the greater percentage of phenolic compounds in ethyl acetate extract which is more polar than the other solvents used in this study. Owing to the natural abundance of *Cassia fistula* and its wide pharmacological activities, further studies are required to isolate various phytochemicals from different parts of the plant and screening of each component for different biological activities.

Acknowledgements

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References

[1] M. Valko, D. Leibfrits, J. Moncol, M.T.D. Cronin, M. Mazur and J. Telser; *Int. J. Biochem. Cell Biol*.; **39**, 44-84(2007).

[2] Saiket Sen; Raja Chakraborty; *The Role of Antioxidants in Human Life;In Oxidative Stress: Diagnostics, Prevention and Therapy;*Andreescu. S; et al. (Editors); ACS Symposium series; *American Chemical Society: Washington*, DC, 2011.

[3] Q.Y. Zhu, R.M. Hackman,J.L. Ensunsa, R.R. Holt, C.L. Keen.; *Journal of Agricultural and Food Chemistry*, **50**, 6929 – 6934(2002).

[4] J.C. Chen, J.Y. Yeh, P.-C. Chen and C.K. Hsu; *Asian Journal of Health and Information Science*, **2**, 1-11(2007).

[5] A. H. Adnan Jehangir, Nagi, M. Shahzad and Azam Zia; *Biomedica*, **26**, 25-29(2010).

Table 2. Antioxidant activity of compounds

Compounds	EC_{50} value[a]($\mu g mL^{-1}$)		
	DPPH radical scavenging activity	Super oxide radical scavenging activity	Lipid peroxidation inhibitory activity
BHT (1)	16 ± 0.5	240 ± 3.2	100 ± 2.2
n-Hexane extract (2)	64 ± 4.2	262 ± 2.8	110 ± 2.5
Dichloromethane extract (3)	35 ± 3.6	190 ± 2.8	96 ± 2.2
Ethyl acetate extract (4)	28 ± 3.8	176 ± 2.2	78 ± 2.2

[a]Each value represents mean of three different observations ± S.D

[6] M. Kushawaha and R.C. Agrawal*; J. Sci. Res. Phar*., **1(3)**, 7-11(2012).

[7] P.L. Rajagopal, K. Premalatha, S.S. Kiron and K.R. Sreejith; *Int. J. Pharm.Che.Biol.Sci.*,**3(3)**, 672-679(2013).

[8] V. Bondet, W. Brand and W.C. Berset,. *J. Food Sci.Technol*.,**30**, 609–615(1997).

[9] M. Nishikimi, N.A. Rao and K. Yagi, *Biochem. Biophys. Res. Commun.*, **46**, 849-52(1972).

[10] P. D. Duh and G. H. Yen, *Food Chemistry*, **60**, 639 – 643(1997).