IARJSET ISSN (O) 2393-8021, ISSN (P) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

Impact Factor 7.105 ⁜ Vol. 9, Issue 2, February 2022

DOI: 10.17148/IARJSET.2022.9240

Pharmacological Activities of Cassia fistula leaves extracts

Dr. Premalatha S J

Assistant Professor, Government Science College, Chitradurga, Karnataka, India

Abstract: Plants are the best gifts that the Mother Nature has given us. They not only give us food but also nurture us while we fall sick. Since past decades, plants are proved to be the repertoires of myriads of pharmacologically important molecules that have been isolated, characterized, identified and applied for the treatment of various number of diseases. The scientific community has successfully employed the plants as biomedicines. This research work is the outcome of interest towards understanding the alchemical units in medicinal plant and its associated fungi that have the ability to combat the disease cancer. The study was carried to evaluate the crude extracts' antioxidant activities, estimation of total phenolics and free radical scavenging activity. Total antioxidant capacity of the extracts of Cassia fistula was evaluated by the phosphomolybdenum method and was expressed as µg of ascorbic acid equivalents (AAE) per ml of plant extract. Total antioxidant capacity of the test samples was calculated using the standard curve of ascorbic acid. Methanol and aqueous extracts of Cassia fistula were found to possess the highest total antioxidant capacity compared to chloroform extracts.

INTRODUCTION

The fact that inflammation and pain are closely linked to cancer has been well accepted by the studies and research. Above all the risk of cancer is increased in chronic inflammation and hence elimination of inflammation and pain are considered to be important strategies in cancer treatments [1]. Various malignancies are demonstrated to be significantly related to molecular mediators that induce inflammation like various cytokines. Hence the study on cancer treatment and drug development include the studies on anti-inflammatory and analgesic substances that reduce the inflammation and pain respectively [2].

Also the antioxidant and free radical scavenging activities are carried out in our studies using extracts of Cassia fistula leaves to validate these pharmacological parameters that are important in cancer research. Antioxidants are the molecules that protect the cells from the damage caused by free radical [3]. These free radicals possess incomplete electron shells and hence are highly reactive. In humans common form of free radical is oxygen molecule and becomes reactive oxygen species causing irreversible cell damage. The natural products are found be repertoires of antioxidants and free radical scavengers and hence prevent or protect from cancer**.**

This chapter comprises the secondary studies of Cassia fistula leaves extracts. By using TLC, alkaloids were partially from chloroform, methanol and water extracts of Cassia fistula. The study was carried to evaluate the crude extracts' antioxidant activities, estimation of total phenolics and free radical scavenging activity [4]. Total antioxidant capacity of the extracts of Cassia fistula was evaluated by the phosphomolybdenum method and was expressed as µg of ascorbic acid equivalents (AAE) per ml of plant extract. Total antioxidant capacity of the test samples was calculated using the standard curve of ascorbic acid. Methanol and aqueous extracts of Cassia fistula were found to possess the highest total antioxidant capacity compared to chloroform extracts.

The methanolic crude extract the showed the highest free radical scavenging activity in the concentration range of 10 – 100 microgram. It scavenged the DPPH in a concentration dependent manner.

MATERIALS AND METHODS

Chemicals and reagents

All the chemicals were of the standard grades and procured from Sigma Aldrich.

Compound preparation

Stock solutions were prepared by dissolving 200mg/ml of the crude extracts in DMSO (0.5% by volume). The stock solution was then diluted to the required concentration during treatment

In vitro studies

Antioxidant activity

Various concentrations of samples (10µg, 50µg and 100 µg of plant extracts in different solvents) were taken in a series

IARJSET ISSN (O) 2393-8021, ISSN (P) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

Impact Factor 7.105 ⁜ Vol. 9, Issue 2, February 2022

DOI: 10.17148/IARJSET.2022.9240

of test tubes. To this, 1.9ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were incubated at 95°C for 90 minutes and allowed to cool. The absorbance of the aqueous solution of each was measured at 695 nm against a blank. Antioxidant capacities were expressed as µg equivalents of ascorbic acid. Butylatedhydroxy anisole (BHA) was used as reference standard [10]. The values were expressed as ascorbic acid equivalents in µg per ml of extract.

Free radical scavenging activity

Different concentrations (10, 50 and 100µg of plant extracts in different solvents) of samples in Dimethyl sulfoxide (DMSO), were taken in a series of test tubes [11]. The volume was adjusted to 500µl by adding methanol. 5ml of 0.1mM methanolic solution of 1,1-diphenyl-2-picryl hydrazyl was added to these tubes and shaken vigorously. A control without the test compound, but with an equivalent amount of methanol was maintained. The tubes were allowed to stand at room temperature for 20 minutes. The absorbance of the samples was measured at 517nm. Butylated Hydroxy Anisole (BHA) was used as reference standard. Free Radical scavenging activity was calculated using the following formula:

$$\%\ \text{radical scavenging activity} = \frac{\text{Control OD} - \text{Sample OD}}{\text{control OD}} \text{ X } 100$$

Estimation of flavonoids

A standard curve of rut in was constructed using the following procedure. 0 to 100 µg/ml of rutin in six different test tubes was pipetted and the volume was made up to 0.5ml with distilled water. Sodium nitrite (5%; 0.03ml) was added to each tube and incubated for 5 minutes at room temperature, Aluminium chloride solution (10%; 0.06ml) solution was added and incubated for 5 minutes at room temperature.. Sodium hydroxide solution (1 M; 0.2ml) solution was added and total volume was made up to 1ml with distilled water. Absorbance was measured at 510nm against a reagent blank. Rutin calibration curve using different concentrations of rutin was prepared.

The sample extracts prepared using separately chloroform, methanol and water from Cassia fistula leaves were pipetted in 0 to 100µg/ml and the rutin equivalents were determined following the same procedure as above. From the standard curve, concentrations of flavonoids in the test samples were determined and expressed as µg of rutin equivalent. This gives the total flavonoid contents in different extracts. A bar diagram was constructed by taking samples on x axis and rutin equivalents on y axis.

Estimation of total phenolics

0 to 2.5 µg/ml of catechol was pipetted out in series of six test tubes and volume was made up to 3ml with distilled water. Folin-Ciocalteau reagent (0.5ml) was added to each tube and incubated for 3 minutes. at room temperature. Sodium carbonate (20%; 2ml) solution was added, mixed thoroughly and the tubes were incubated for 1 minute. in boiling water bath [12]. Absorbance was measured at 650nm against a reagent blank. Standard curve using different concentrations of standard phenolic-catechol was prepared. From the standard curve, concentration of phenols in the test samples was determined and expressed as µg/ml of catechol equivalent.

Statistical analysis

For in vitro pharmacological studies, all the values are expressed as Mean ± SEM. The experiments were conducted in triplicates. One way ANOVA was done using Graph pad Prism 5.1. Differences were regarded as significant when p value was less than 0.05.

RESULTS AND DISCUSSIONS

Total antioxidant activity of crude extracts

A standard ascorbic acid curve was constructed using butylatedhydroxyanisole as a reference standard by plotting the concentration versus absorbance. A linear scale was constructed. (Figure 1). This was followed by the antioxidant activity determination for the chloroform, methanol and water extracts of Cassia fistula leaves using the. Using the standard graph (Figure 1), of ascorbic acid equivalents for these extracts were expressed as µg/mg.. Methanol and water extracts had comparatively greater ascorbic equivalents than chloroform extracts at all the concentrations studies. The no.of µg equivalents for the extracts for all the solvents occurred in a dose dependent manner (Figure 2).

IARJSET ISSN (O) 2393-8021, ISSN (P) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

Impact Factor 7.105 ⁜ Vol. 9, Issue 2, February 2022

DOI: 10.17148/IARJSET.2022.9240

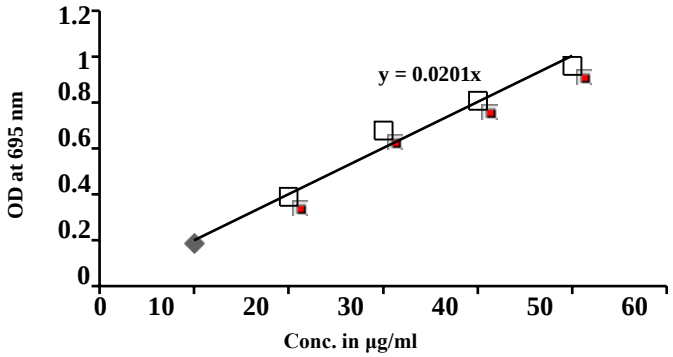


Fig. 1. Standard ascorbic acid curve

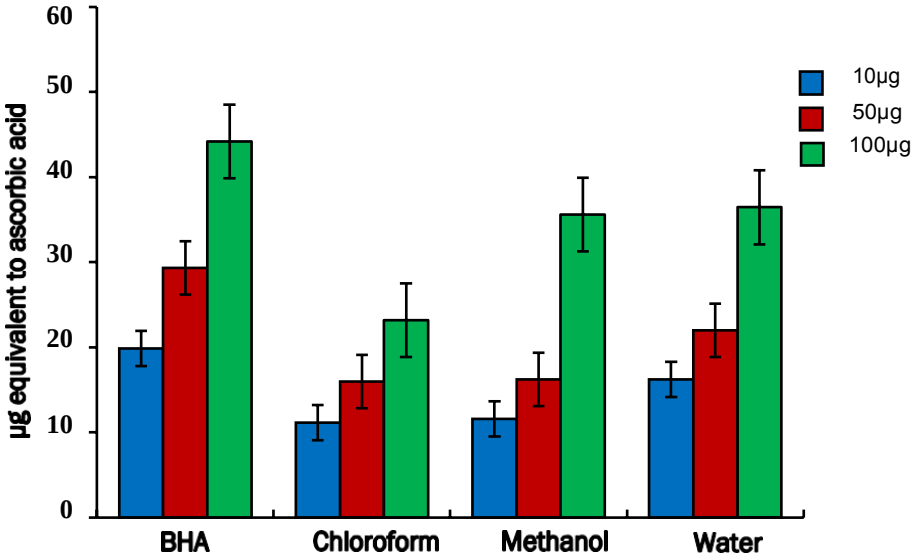


Fig. 2. µg equivalents of ascorbic acid in Cassia fistula leaves for the different extracts. All the values are expressed as mean ± SEM. The results were analyzed ANOVA test ($p<0.05$).

Free radical scavenging activity

Using the different concentrations of plant extracts in DMSO, the free radical scavenging activity of was calculated by DPPH method. A plot of absorbance versus concentration was constructed and the % radical scavenging activity was calculated using the formula. A bar diagram was constructed to assess the comparative free radical scavenging activity using BHA as a standard reference (Figure 3).

The methanol extract had the highest free radical activity of 25% of all the extracts but the results were much less than the standard compound BHA.

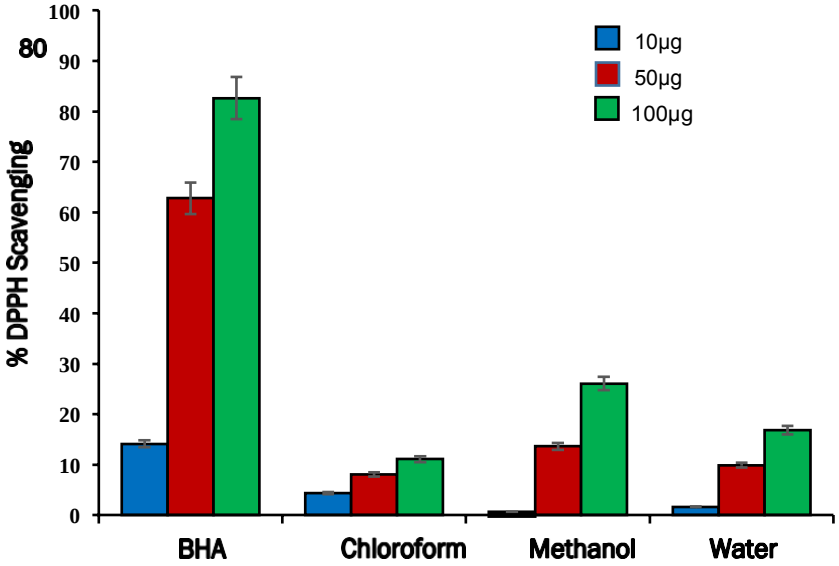
Fig.3. % Free radical scavenging activity of extracts of Cassia fistula leaves in comparison with standard BHA. The standard and plant extracts were used at 10, 50 and 100µg/ml concentrations. The results were analyzed ANOVA test ($p<0.05$). All the values are expressed as mean ± SEM.

IARJSET ISSN (O) 2393-8021, ISSN (P) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

Impact Factor 7.105 ⁜ Vol. 9, Issue 2, February 2022

DOI: 10.17148/IARJSET.2022.9240



Estimation of flavonoids

The chloroform, methanol and water extracts from the leaves of Cassia fistula were found to possess a significant amount of flavonoids. This observation was based on the construction of rutin calibration curve (Figure 4) and using the same for the determination of rutin equivalents in the samples taken. Methanol fraction had the highest flavonoid content than chloroform and water extracts (Figure 5).

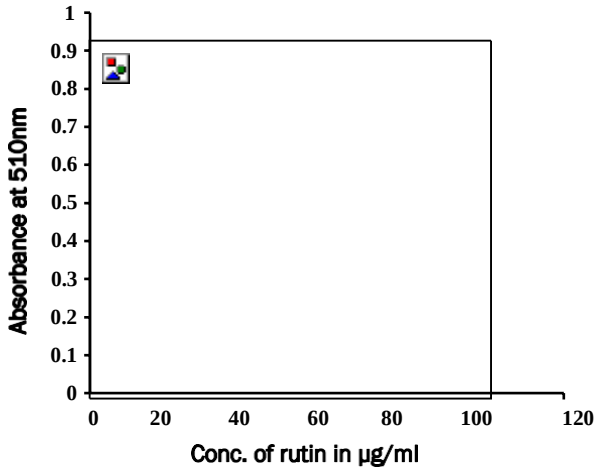
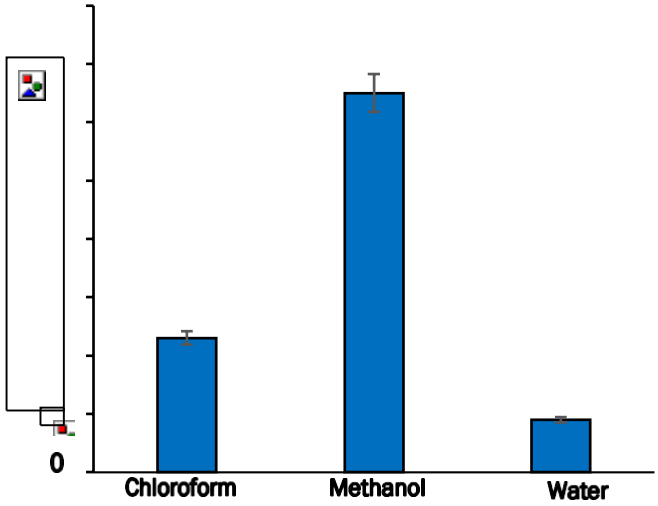


Fig.4. Rutin calibration curve constructed using different concentrations of rutin



IARJSET ISSN (O) 2393-8021, ISSN (P) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

Impact Factor 7.105 ⁜ Vol. 9, Issue 2, February 2022

DOI: 10.17148/IARJSET.2022.9240

Fig 5. Total flavonoids in Cassia fistula leaves extracted using three different solvents. The results were analyzed ANOVA test ($p<0.05$). All the values are expressed as mean ± SEM.

Estimation of total phenolics

A calibration curve was constructed using catechol as a reference standard for the phenolics (Figure 6). Then using the different concentrations Cassia fistula leaf extracts, the total phenolic content was determined as catechol equivalents (Figure 7). The total phenolics contents revealed that highest phenolics were found in methanol extracts. This was followed by water and very low in chloroform extracts.

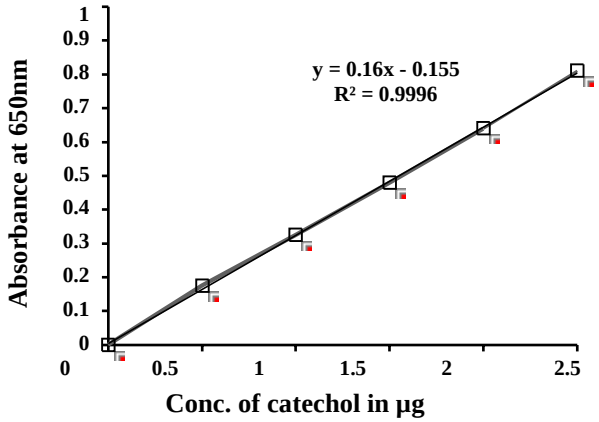


Fig. 6. Catechol calibration curve was constructed plotting concentrations of rutin in µg on x-axis and absorbance at 650nm All the values are expressed as mean± SEM.

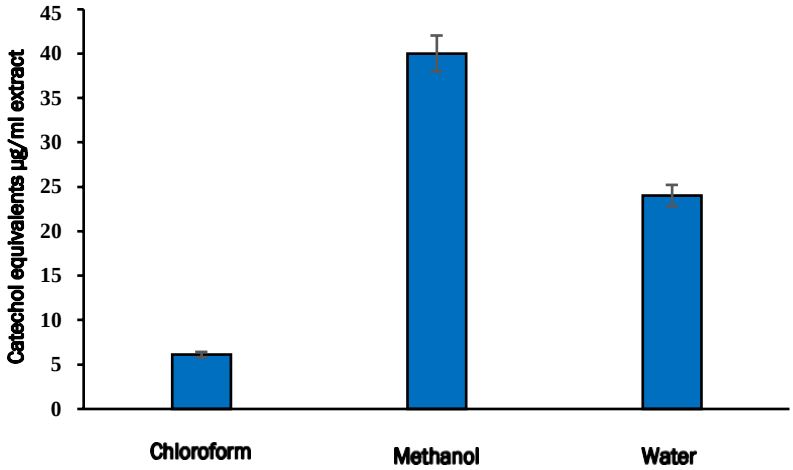


Fig 7. Total phenolic content of the different extracts of Cassia fistula leaves. The results were analyzed ANOVA test ($p<0.05$). All the values are expressed as mean ± SEM.

Discussion

A common symptom associated with the progression of cancer is inflammation. The cells that exhibit inflammation are genetically stable. There are other extrinsic factors which stimulate inflammation like alcohol intake, tobacco smoking but these factors in turn are connected to cancer triggering. Inflammation is systemic and local tissue response [13].

The findings were focused to assess the in vitro antioxidant activity of crude extracts from leaves of Cassia fistula in terms of DPPH free radical scavenging activity. The antioxidant activity was studied in reference with the standard butylated hydroxyl anisole (BHA). Of the three extracts at 100µg/ml, methanol extract at had the highest free radical scavenging among all extracts followed by water and chloroform extracts at the same concentration. The antioxidant capacities were not significant as compared to standard antioxidant compound BHA which can be attributed to the fact that the extract is crude and requires further purification of the extracts to characterize the actual phytochemicals involved in this activity. The chloroform extract had an insignificant activity compared to methanol and water extracts. The free radical scavenging activity occurred in a dose dependent manner in all the extracts and the standard.

Pathogenesis is associated with the generation of highly reactive oxygen species (ROS) which has a lone unpaired electron and induces oxidative stress. This is observed in various pathological conditions like cancer which leads to cell

DOI: 10.17148/IARJSET.2022.9240

injury. Though cells possess endogenous antioxidant enzymes like superoxide dismutase, catalase and glutathione peroxidase, this seems to be insufficient drive the scavenging of free radicals as observed in case of cancer. This disease has increased oxidative stress and requires assistance of exogenous molecules to scavenge the free radicals. The recent studies have proved the presence of polyphenols from plant extracts has antioxidant activities and can be used to forage the reactive oxygen species. Phenolic compounds are good electron donors because of the presence of hydroxyl groups. The extracts of leaves of Cassia fistula were used to assess the in vitro antioxidant and total phenolics content. To estimate the antioxidant activity, free radical scavenging activity using DPPH (2, 2-diphenyl-1-picrylhydrazyl) was adopted. Total antioxidant capacity of the extracts of Cassia fistula was evaluated by the phosphomolybdenum method and was expressed as µg ascorbic acid equivalents (AAE) per ml of plant extract. Total antioxidant capacity of the test samples was calculated using the standard curve of ascorbic acid. The methanol extract had the highest antioxidant activity as compared with water and chloroform extracts though all had an insignificant antioxidant activities compared to the standard reference compound BHA. The methanolic extract of the showed the highest free radical scavenging activity in the concentration range of 10 – 100 microgram. It scavenged the DPPH in a concentration dependent manner. Hence further purification of the extract has to be taken up to account for the antioxidant property of the extract.

CONCLUSION

By analyzing the above results and discussion we can conclude the Cassia fistula harbors the phytochemicals that can be anti-inflammatory, analgesic, muscle relaxant and anti-oxidants. The anti-inflammatory activity of the plant extracts shows that the further purification of the extracts need to be taken up to find out the active principles responsible for these pharmacologically important activities. According to recent reports, a highly positive relationship between total phenols and antioxidant activity appears to be the trend in many plant species. The statement has been justified in the current study where the methanol extract of Cassia fistula showed maximum total antioxidant capacity (in term of ascorbic acid equivalent) with maximum phenol content. Free radical scavenging shows the possible presence of anticancer agents.

No conflict of interest

REFERENCES

1. Balkwill F and Mantovani A. Inflammation and cancer: 357:539–545, 2001.
2. Won Ho Y and Keyong Ho L. Anti-inflammatory, Anti-arthritic and Analgesic Effect of the Herbal Extract made from Bacopa mannieriis, Cassia fistula and Phyllamnthus polyphyllus. Natural Product Sciences. 23(2):108-112. 2017.
3. Nayan RB, Acharya RN and Shukla VJ. Evaluation of in vitro Antioxidant Activity of hydroalcoholic seed extratcs of Cassia fistula linn. Free Radicals and Antioxidants. 68(1):233-241, 2011.
4. Alagoju P, Dinesh BJ and Latha P. Free Radicals: Properties, Sources, Targets, and Their Implication in Various Diseases. Free Radicals and Antioxidants. 30(1):11– 26, 2015.
5. Simon F and Robin W. Point on the use of animals in scientific research. The ethics of animal research. Science and Society. 8(6):526–530, 2007.
6. OECD (2008), Test No. 425: Acute Oral Toxicity: Up-and-Down Procedure, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris. 2008.
7. Manjit S, Vijender K, Ishpinder S, Vinod G and Ajudhia NK. Anti-inflammatory activity of aqueous extract of Mirabilis jalapa Linn. Leaves. Pharmacognosy. 2(6): 364–367, 2010.
8. Asie S, Majid M, Sima N and Manijeh M. Evaluation of Anti-inflammatory and Analgesic Activity of the Extract and Fractions of Astragalus hamosus in Animal Models. Iran J Pharmacology. 14(1):263–269, 2016.
9. Jayasree T, Maulik P, Ubedulla S, Harini K and Shankar J. Evaluation of skeletal muscle relaxant activity of aqueous extract of Nerium oleander flowers in Albino rats. Indian Journal of Pharmacology. 47(4):409–413, 2015.
10. Badakhshan MP, Subramanion LJ, Lachimanan YL, Yeng C and Sreenivasan S. Antioxidant activity of methanol extracts of different parts of Lantana camara. Asian Pac J Trop Biomed. 2(12):960–965, 2012.
11. Kandhasamy S and Sun CK. Free radical scavenging activity from different extracts of leaves of Bauhinia vahlii Wight & Arn. Saudi J Biol Sci. 20(4):319–325, 2013.
12. Ajaykumar S, Manoj RK and Rajendra DW. Estimation of Total Phenolic and Total Flavonoid Content and Assessment of in vitro Antioxidant Activity of Extracts of Hamelia patens. Research Journal of Phytochemistry. 10(2):67-74, 2016.
13. Aggarwal BB, Shishodia S, Sandur SK, Pandey MK, Sethi G. Inflammation and Cancer. Biochem Pharmacol. 72(11):1605-21, 2006.

