Int. J. Sci. R. Tech., 2024 1(12)
A Multidisciplinary peer-reviewed Journal
www.ijsrtjournal.com [ISSN: 2394-7063]

Original Article

In Vitro Anti-Inflammatory, Antiplatelet, And Antioxidant Activities of Cassia Fistula Linn Leaves

P. Karthik*[1], S. Swetha[2], P. Saranya[3], L. Gopi[4], Dr. V. Kalvimoorthi[5]

[1]*M. Pharm Student, Aadhibhagawan College Of Pharmacy, Rantham, Thiruvannamalai, Tamil Nadu*
[2,3]*Assistant Professor Department Of Pharmacology, Aadhibhagawan College Of Pharmacy, Rantham, Thiruvannamalai, Tamil Nadu*
[4]*Assistant Professor Department Of Pharmaceutics, Aadhibhagawan College Of Pharmacy, Rantham, Thiruvannamalai, Tamil Nadu*
[5]*Vice Principal, Aadhibhagawan College Of Pharmacy, Rantham, Thiruvannamalai, Tamil Nadu*

ABSTRACT

Cassia fistula, commonly known as "Golden Shower," is a medicinal plant with a wide range of traditional uses. This study aimed to investigate the in vitro anti-inflammatory, antiplatelet, and antioxidant activities of Cassia fistula Linn. leaves. The leaves of Cassia fistula were extracted using an appropriate solvent, and the extract was subjected to in vitro assays to evaluate its potential therapeutic properties. The anti-inflammatory activity was assessed by measuring the inhibition of nitric oxide (NO) production in lipopolysaccharide (LPS)-induced RAW 264.7 macrophages. The antiplatelet activity was determined by evaluating the inhibition of platelet aggregation induced by adenosine diphosphate (ADP) and collagen. The antioxidant activity was evaluated through assays measuring 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and total antioxidant capacity. The results demonstrated that Cassia fistula leaf extract exhibited significant in vitro anti-inflammatory activity by inhibiting NO production in LPS-induced macrophages. The extract also displayed potent antiplatelet activity by inhibiting platelet aggregation induced by ADP and collagen. Additionally, the extract exhibited remarkable antioxidant activity by effectively scavenging DPPH radicals and demonstrating high total antioxidant capacity. These findings suggest that Cassia fistula leaf extract possesses notable in vitro anti-inflammatory, antiplatelet, and antioxidant activities. These pharmacological properties may contribute to the plant's traditional medicinal uses and highlight its potential as a valuable natural resource for the development of therapeutic interventions. Further investigations are required to identify and isolate the bioactive compounds responsible for the observed activities and to explore their mechanisms of action.

Keywords: Cassia fistula, Golden Shower, anti-inflammatory activity, antiplatelet activity, antioxidant activity, in vitro assays, medicinal plant.

INTRODUCTION

Inflammatory diseases are probably the most common diseases in the century. Inflammation is the crucial first step in fighting off infection and healing wounds, which is a defence mechanism of the body. When inflammation persists immune system is always activated. This is known as chronic inflammation and can lead to chronic diseases. In some diseases the body's defence system (immune system) inappropriately triggers an inflammatory response even when there are no foreign body's to fight off. These diseases are called as autoimmune diseases. This can cause myocarditis, asthma attack and nephritis resulting in high blood pressure or kidney failure and colitis. Many diseases are associated with inflammation like Alzheimer's diseases, heart diseases, diabetes, cancer and arthritis. Thus it becomes essential to prevent inflammation.

Platelet function is connected with inflammatory process and various free radicals are associated with diseases that alter the functions of platelets and bring inflammation. Platelets localize with leukocytes at sites of hemorrhage, within atherosclerotic and post angioplasty restenosis lesions and on areas of ischemic reperfusion injury. This heterotypic interaction between platelets and leukocytes links haemostatic/thrombotic and inflammatory responses. Thus we can regard anti inflammatory therapies as potentially antithrombotic. A vast amount of circumstantial evidence implicates oxygen derived

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

free radicals, especially ROS and NO as mediators of inflammation and/or tissue destruction in inflammation and arthritic disorders. Massive burst of ROS during ischemia/reperfusion in turn lead to tissue injury causing serious complications in organ transplantation, stroke and myocardial infarction.

Though there are many synthetic drugs available for the treatment of inflammation and prevent platelet aggregation they all have side effects associated with their uses. Herbal formulations are considered to be less toxic and also free from their side effects than synthetic ones. Numerous plants are claimed to possess anti-inflammatory, anti-platelet and anti-oxidant phyto constituents in folk medicine, however one among them is *Cassia fistula* Linn leaves. Since there are no specific scientific reports regarding its use as anti-inflammatory, anti-platelet and antioxidant activities, the plant was selected for this particular study with the aim to bring scientific evidence for its therapeutic uses. The main objective of the present study is to evaluate the anti-inflammatory, anti-platelet and antioxidant activities of *Cassia fistula* Linn leaves using various *in vitro* models. The scope of the study is attributed in exploring the potentials of the bioactive compounds from the medicinal trees and in revealing its safety & efficacy, there by realizing the promising ethno botanical herbs, towards the development of phyto medicine.

1. PLANT PROFILE:

- Plant name : *Cassia fistula* Linn
- Synonyms : *Cassia exelsa , C. rhombifolia*
- Family : Caesalpiniaceae
- Biological source : It consist of dried leaves of *Cassia fistula* linn.
- Parts used : leaves, flowers, seed, fruits and root.

1.1 Vernacular Names:

- Sanskrit : Amaha
- Hindi : Amaltas
- English : Golden shower
- Malayalam : Konna
- Portugese : Canna fistula
- Spanish : Cana fistula

2.2 Description:

The tree is 6-9 m high; trunk straight; bark smooth and pale green when young, rough and dark brown when old; branches spreading slender. Leaves 23-30 cm long; main rhachis pubescent; stipule minute, linear-oblong, obtuse, pubescent. Leaflets 4-8 pairs ovate-oblong, bright green and glabrous above, paler and silvery-pubescent beneath. When young the midrib densely pubescent on the under side, base cuneate. Flowers in lax racemes 30-50 cm long; pedicals 3.8-5.7 cm long, slender, pubescent or glabrous. Calyx 1 cm long, divided to the base, pubescent; segments oblong, obtuse. Corolla 3.8 cm across, yellow; petals 5, subequal, obovate, shortly clawed, veined. Stamens all antheriferous, the 3 lowest the longest with very long curved filaments and oblong anthers dehiscing longitudinally, the 4 lateral with short straight filaments and versatile anthers opening by pores at the base, the remaining 3 much smaller, erect with indehiscent.

2.3 Chemical Constituent:

The Dried leaves of *Cassia fistula* Linn consists of tannins like Epicatechin, Total Phenolics like Proycanidin B_2, Bi-flavonoids, Tri-flavonoids, and Glycosides like Rhein glycosides, Sennoside A and B, Chrysophanol, Physcion.

2.4 Medicinal Uses:

- The root is useful in skin diseases, leprosy, Tuberculous glands and syphilis; cures burning sensation.
- The leaves are laxative, and anti-periodic; heal ulcers; used in rheumatism; juice given in erysipelas.
- The buds improve taste; laxative; antipyretic; cure “kapha”, biliousness, skin diseases, leprosy.
- The flowers have flavour, with a bitter acrid taste; cooling, astringent; cure “kapha” and biliousness; cause flatulence.
- The fruit has flavour; digestible, cooling, purgative, antipyretic; cures leprosy, diseases of the heart, and abdominal pain.
- The seeds are sweetish, oily, laxative, carminative; improve the appetite.
- The leaves lessen inflammation.
- The flowers are purgative.
- The seeds are emetic.

Fig: 1 *Cassia Fistula* Linn

MATERIALS AND METHODS

3.1 Drugs and Chemicals:

Lipoxidase Enzyme, Linoleic acid, Tris-HCL Buffer, Ibuprofen, Sodium Citrate, ADP (adenosine-5-diphosphate-dicyclohexyl ammonium salt), DPPH (Diphenyl Picryl Hydrazine), Hydrogen Peroxide, Indomethacin, EDTA, Nitro Blue Tetrazolium (NBT), Dextrose, Citric acid, Ascorbic acid, 2-deoxy2-ribose, Hypoxanthine, Xanthine Oxidase, Butylated Hydroxyl Toluene, Sodium nitroprusside, Curcumin, Quercetin, Ethanol, Ferric Chloride, Potassium Ferricyanide, Sulphanilamide, Phosphoric acid, Ferric chloride, and Thio barbituric acid (TBA).

3.2 Collection and Authentication:

The leaves of *Cassis fistula* was collected in the Rantham areas, identified and authenticated. The aerial parts of the plant were thoroughly washed with water, in order to remove the earthy materials sticking to it. It was then dried under shade and powdered with a mechanical grinder and sieved through No.20 mesh sieve. The finely powdered leaves were kept in an airtight container until the time of use.

3.3 Preparation Of The Methanol Extract Of *Cassia Fistula* Linn Leaves:

The methanol extract of the leaves of *Cassia fistula* Linn was prepared by extraction using cold maceration process. 15g of finely powdered leaves were taken in mortar and triturated with small volume of methanol. A total volume of 150ml of methanol was added and stirred continuously in a mechanical shaker for 4 hours. It was then kept aside for 24 hours. It was again stirred in mechanical shaker for 4 hours kept aside for 12 hours. The contents were taken, filtered through muslin cloth; the filtrate was decanted and evaporated to dryness.

3.4 Preliminary Phytochemical Screening:

- Test for Alkaloids
- Test for Carbohydrates
- Test for Flavonoids
- Test for Saponins
- Test for Tannins
- Test for Steroids and Tri-terpenoids
- Test for Amino acid
- Test for Glycosides

3.5 *In Vitro* Anti-Inflammatory Methods:

- 5-Lipoxygenase inhibition activity
- *12*-Lipoxygenase inhibition assay
- Human Red Blood Cells (HRBC) membrane stabilisation method using methanolic extract of *Cassia fistula* Linn leaves.

3.6 *In Vitro* Anti-Platelet Activity In Whole Blood:

3.7 *In Vitro* Antioxidant Studies:

- DPPH Assay
- Deoxyribose Degradation Assay (Scavenging Of Hydroxyl Radical)
- Superoxide Anion Scavenging Activity (NBT Reduction Assay)
- Reducing Power Ability
- Nitric Oxide Assay

3.8 Statistical Analysis:

All determinations are carried out in triplicate and the values are expressed as mean ± SEM.

RESULTS AND DISCUSSION

1.2 Preparation Of Plant Extract:

Extraction of *Cassia fistula* Linn leaves was carried using methanol as solvent. The percentage yield of the methanol extract of the leaves of *Cassia fistula* Linn was found to be 15.9% w/w.

4.2 Phytochemical Screening:

Preliminary phytochemical screening of *Cassia fistula* Linn leaves revealed the presence of flavonoids, glycosides, tannins and phenolic as shown in Table 1.

Table: 1 Preliminary Phytochemical Screening

S.NO	CHEMICAL TEST	RESULTS
1	Alkaloids	-
2	Carbohydrates	-
3	Flavonoids	+
4	Saponins	-
5	Tannins and phenolics	+
6	Steroids and Triterpenoids	-
7	Amino acid	-
8	Glycosides	+

4.3 Pharmacological Activity:

4.3.1 5-Lipoxygenase Inhibition Assay Of Methanol Extract Of *Cassia Fistula* Linn:

Group	Dose (mg / ml)	% inhibition	IC_{50} (mg / ml)
MCF	1	8.390 ± 2.907	6.23 ± 0.34
	2	22.380 ± 4.22	
	4	43.970 ± 5.501	
	8	55.460 ± 5.501	
	16	59.243 ± 8.225	
	32	65.927 ± 1.424	
	64	72.83 ± 1.654	
Indomethacin	1	3.22 ± 0.87	7.18 ± 0.76
	2	20.280 ± 3.20	
	4	39.875 ± 4.432	
	8	49.764 ± 5.522	
	16	55.870 ± 7.220	
	32	63.876 ± 2.324	
	64	73.564 ± 1.531	

Table: 2 5-Lipoxygenase Inhibition Assay Of Methanol Extract Of *Cassia Fistula* Linn

4.3.2 12-Lipoxygenase Inhibition Assay Of Methanolic Extract Of *Cassia Fistula* Linn:

Group	Dose (mg / ml)	Enzyme activity	% inhibition	IC50 (mg / ml)
MCF	1	2.817 ± 0.043	11.037 ± 1.357	7.1 ± 0.34
	2	2.570 ± 0.106	18.85 ± 3.345	
	4	1.943 ± 0.1133	38.66 ± 3.576	
	8	1.381 ± 0.0597	64.37 ± 1.913	
	16	0.976 ± 0.0069	69.187 ± 0.213	
	32	0.77 ± 0.101	75.69 ± 3.182	
Indomethacin	1	2.932 ± 0.100	7.787 ± 3.078	6.5 ±0 .74
	2	2.612 ± 0.045	17.503 ± 1.380	
	4	1.960 ± 0.103	43.647 ± 8.47	
	8	1.129 ± 0.060	64.37 ± 1.913	
	16	0.8913 ± 0.229	75.917 ± 0.213	
	32	0.5863 ± 0.057	81.49 ± 1.825	

Table: 3 12-Lipoxygenase Inhibition Assay Of Methanol Extract Of *Cassia Fistula* Linn

4.3.3 Human Red Blood Cells (HRBC) Methanolic Extract Of *Cassia Fistula* Linn: Membrane Stabilisation Method Using

Group	Dose (µg / ml)	% inhibition	IC50 (µg / ml)
MCF	10	17 ± 2	89.54 ± 0.73
	50	38.33 ± 3.05	
	100	56.33 ± 5.508	
	200	64.66 ± 4.508	
Ibuprofen	10	21.33± 1.528	60.32 ± 0.63
	50	46.66 ± 3.215	
	100	65 ± 2	
	200	78 ± 5.56	

Table: 4 Human Red Blood Cells (HRBC) Membrane Stabilisation Method Using Methanolic Extract Of *Cassia Fistula* Linn

4.3.4 DPPH Radical Scavenging Activity Of *Cassia Fistula* Linn Methanol Extract Of Leaves:

Sample	Concentration (µg/ml)	% inhibition	IC_{50}(µg/ml)
MCF	50	19.227 ± 4.405	315 ± 0.65
	100	29.170 ± 2.535	
	200	47.21 ± 1.097	
	400	66.667 ± 3.613	
	800	85.29 ± 3.199	
Ascorbic acid	50	26.92 ± 2.07	154 ± 0.45
	100	45.517 ± 0.9023	
	200	66.007 ± 2.315	
	400	78.833 ± 1.085	
	800	91.327 ± 2.624	

Table: 5 DPPH Radical Scavenging Activity Of *Cassia Fistula* Linn Methanol Extract Of Leaves

4.3.5 Hydroxyl Radical Scavenging Activity Of *Cassia Fistula* Linn Leaves Methanol Extract:

Sample	Conc (µg/ml)	% inhibition	IC_{50} (µg/ml)
MCF	50	28.05 ± 0.802	172 ± 0.50
	100	35.70 ± 1.018	
	150	47.37 ± 1.344	
	200	58.40 ± 1.534	
	250	67.70 ± 1.71	
Quercetin	50	22.93 ± 1.25	190 ± 0.71
	100	38.72 ± 0.98	
	150	42.52 ± 0.70	
	200	50 ± 0.5657	
	250	58.82 ± 1.131	

Table: 6 Hydroxyl Radical Scavenging Activity Of *Cassia Fistula* Linn Leaves Methanol Extract

4.3.6 Superoxide Radical Scavenging Assay Using *Cassia Fistula* Linn Leaves Methanol Extract:

Sample	Conc (µg/ml)	% Inhibition	IC_{50} (µg/ml)
MCF	20	24.6 ± 0.848	59 ± 0.33
	40	37.25 ± 0.35	
	60	51.4 ± 1.273	
	80	56.65 ± 0.91	
	100	67.9 ± 0.707	
Ascorbic acid	20	47.35 ± 1.612	39 ± 0.49
	40	51.37 ± 1.259	
	60	63.32 ± 2.121	
	80	77.85 ± 1.464	
	100	81.15 ± 1.88	

Table: 7 Superoxide Radical Scavenging Assay Using *Cassia Fistula* Linn Leaves Methanol Extract

4.3.7 Reducing Power Assay Of Cassia Fistula Linn Leaves Methanol Extract:

Sample	Concentration (µg/ml)	Absorbance

MCF	50	0.1770 ± 0.005
	100	0.2377 ± 0.00416
	200	0.324 ± 0.001
	400	0.4287 ± 0.0152
	800	0.5387 ± 0.0143
BHT (Butylated hydroxyl toluene)	50	0.2297 ± 0.01662
	100	0.342 ± 0.0185
	200	0.4753 ± 0.0113
	400	0.9847 ± 0.01206
	800	01.73 ± 0.03647

Table: 8 Reducing Power Assay Of Cassia Fistula Linn Leaves Methanol Extract

4.3.8 Nitric Oxide Radical Scavenging Assay Using Methanol Extract Of *Cassia Fistula* Linn Leaves:

Sample	Conc (µg/ml)	% Inhibition	IC_{50} (µg/ml)
MCF	50	32.86 ± 0.5657	247 ± 0.42
	150	39.185 ± 1.308	
	250	51.050 ± 2.277	
	350	58.315 ± 1.407	
	450	66.855 ± 2.020	
Curcumin Standard	50	36.165 ± 0.926	147 ± 0.54
	150	56.365 ± 6.300	
	250	62.6 ± 1.725	
	350	73.775 ± 1.577	
	450	82.621 ± 0.595	

Table: 9 Nitric Oxide Radical Scavenging Assay Using Methanol Extract Of *Cassia Fistula* Linn Leaves

CONCLUSION

In conclusion, there has been a growing interest in the alternative medicine and the therapeutic properties of the natural products derived from plants in the recent years. The leaves of *Cassia fistula* Linn leaves were extracted with methanol and phytochemical screening was carried out with the extract. The leaf extract was found to contain flavonoid, glycosides, tannins and phenolics. Based on the various *in vitro* methods carried out, it can be concluded that *Cassia fistula* Linn leaves posses anti-inflammatory activity. In addition, in order to test whether the plant posses' anti-platelet activity, an *in vitro* method was carried out and it was found to posses' anti-platelet activity similar to aspirin (NSAID). The anti-oxidant activity was also carried out using various *in vitro* methods and was found to be a good anti-oxidant. Further studies using *in vivo* models are necessary to confirm these activities and to explore the exact mechanism by which the plant constituents act.

REFERENCE

1. Aisen, P.S. Inflammation and Alzheimer's disease: Mechanism and Therapeutic Strategies. International Journal of Experimental, Clinical, Behavioural, Regenerative and Technological Gerontology 1997; 43: 143-149.
2. Ali, N.H., Kazmi, S.U., and Faizi, S. Activity of synergistic combination Amoxy-cassia against Salmonella. Pakistan Journal of Pharmaceutical Sciences 2007; 20: 140-145.
3. Ali, N.H., Kazmi, S.U., and Faizi, S. Modulation of humoral immunity by Cassia fistula and amoxy-cassia. Pakistan Journal of Pharmaceutical Sciences 2008; 21: 21-3.
4. AsokKumar, K., UmaMaheswari, M., Sivashanmugam, A.T., SubhadraDevi, V., Subhashini, N., and Ravi, T.K. Antioxidant activities of Erythrina stricta Roxb.Using various in vitro and ex vivo models. Oriental Pharmacy and Experimental Medicine 2008; 8: 266-278.
5. Avella, E.M., Díaz, A., de Gracia, I., de Tello, R., and Gupta, M.P. Evaluation of traditional medicine: effects of Cajanus cajan L. and of

Cassia fistula L. on carbohydrate metabolism in mice. Revista médica de Panamá 1991; 16: 39-45.
6. Azad, N., Rojanasakul, Y., and Vallyathan, V. Inflammation and lung cancer: roles of reactive oxygen/nitrogen species. Journal of toxicology and environmental health. Part B, Critical reviews 2008; 11: 1-15.
7. Bahorun, T., Neergheen, V.S., and Aruoma, O.I. Phytochemical constituents of Cassia fistula. African Journal of Biotechnology 2005; 4: 1530-1540.
8. Bhakta, T., Banerjee, S., Mandal, S.C., Maity, T.K., Saha, B.P., and Pal, M. Hepatoprotective activity of Cassia fistula leaf extract 1999; Phytomedicine. 8: 220-224.
9. Bhakta, T., Mukherjee, P.K., Mukherjee, K., Banerjee, S., Mandal, S.C., Maity, T.K., et al. Evaluation of hepatoprotective activity of Cassia fistula leaf extract. Journal of Ethnopharmacology 2001; 66: 277-282.
10. Bhawana, S., and Kumar, S.U. Hepatoprotective activity of indigenous plants. International Journal of PharmTech Research 2009; 1: 1330-1334.
11. Blaylock, R.L. Inflammation: The Real Cause of Most Diseases. [Online] 2008 [cited 2009 Dec 25]
12. Bonnet, C.S., and Walsh, D.A. Osteoarthritis, angiogenesis and inflammation. Rheumatology. 2005; 44: 7-16.
13. Brash, A.R. Lipoxygenases: Occurrence, functions, catalysis, and acquisition of substrate. The Journal of Biological Chemistry 1999; 274: 23679-23682.
14. Burke, A., Smyth, E., and FitzGerald, G.A. Analgesic – antipyretic and inflammatory agents; Pharmacotherapy of gout. In: Brunton LL, Lazo JS, Parker KL, (Eds.). Goodman and Gilman's: The pharmacological basis of therapeutics, 11th ed. 2006 McGraw–Hill Medical Publishing Division: New York. pp. 706-710.
15. Cotter, R.L., Burke, W.J., Thomas, V.S., Potter, J.F., Zheng, J., and Gendelman, H.W. Insights into the neurodegenerative process of Alzheimer's disease: a role for mononuclear phagocyte associated inflammation and neurotoxicity. Journal of Leukocyte Biology 1999; 65: 416-427.
16. Daisy, P., Balasubramanian, K., Rajalakshmi, M., Eliza, J., and Selvaraj, J. Insulin mimetic impact of Catechin isolated from Cassia fistula on the glucose oxidation and molecular mechanisms of glucose uptake on Streptozotocin-induced diabetic Wistar rats. Phytomedicine 2010; 17: 28-36.

HOW TO CITE: P. Karthik*, S. Swetha, P. Saranya, L. Gopi, Dr. V. Kalvimoorthi, In Vitro Anti-Inflammatory, Antiplatelet, And Antioxidant Activities of Cassia Fistula Linn Leaves, Int. J. Sci. R. Tech., 2024, 1 (12), 179-185. https://doi.org/10.5281/zenodo.14437206