Anti-Inflammatory Activity of *Cassia auriculata* Flower Extract

Soorya Ganesh a, Lakshminarayanan Arivarasu b*, S. Rajeshkumar c# and Lakshmi Thangavelu b]+)/

a Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai 77, Tamil Nadu, India.
b Department of Pharmacology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai 77, Tamil Nadu, India.
c Nanomedicine Laboratory, Department of Pharmacology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Chennai-77, Tamil Nadu, India.

Authors’ contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i62A35614

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/77951

Received 24 October 2021
Accepted 27 December 2021
Published 28 December 2021

ABSTRACT

Introduction: Medicinal plants have provided recent medicines with numerous plant derived therapeutic agents. Avaram is extremely valued in Indian medicines for management of painful inflammation and diabetes. The target of this study was to measure the anti-inflammatory potential of *Cassia auriculata* flowers extract. The anti-inflammatory activity was evaluated using albumin denaturation assay, proteinase inhibitory activity and membrane stabilization at different concentrations. Aspirin and Voltaren were used as standard drugs. The share of inhibition was compared with those of normal drugs

Materials and Methods: BSA and EAA assay was used to test the anti-inflammatory activity of plant extract. *Cassia auriculata* were purchased commercially from an herbal health centre in Chennai. The obtained powder *Cassia auriculata* is stored in an airtight container. 5 gram of powder is mixed with 50 ml of ethanol and kept in the orbital shaker for 72 hours, after it has boiled in a heating mantle at 62- 70 degree c for 5-10 min. The extract is filtered using whatman filter paper 1. The filter extract again contracted using heating mantle.
Results: The extract shows very good antiinflammatory activity for the *Cassia auriculata* extract by using BSA and EAA Assay. 
Conclusion: The antiinflammatory activity of *Cassia auriculata* ethanolic extract preparation was effective.

Keywords: Cassia auriculata; anti-inflammatory; drugs; innovative technique.

1. INTRODUCTION

Inflammation (swelling), which is a component of the body’s natural healing system, helps fight injury and infection or in other words it can be defined as a localized reaction that produces redness, warmth, swelling, and pain as a result of infection, irritation, or injury. Inflammation may be external or internal *Cassia auriculata*. Linn (Caesalpinia) commonly mentioned as Tanner’s Cassia [Avaram] a shrub with large bright yellow flowers, growing wild in Central Provinces and Western peninsula parts of India. People use *C. auriculata* for diabetes, eye infections (conjunctivitis) joint and muscle pain (rheumatism), constipation, jaundice, disease and tract disorders. The flowers treat urinary discharges, nocturnal emissions, diabetes and throat irritation. Inflammation is that the reaction of living tissues to injury, infection or irritation. Lysosomal enzymes released during inflammation produce a range of disorders which ends up in the tissue injury by damaging the macromolecules and lipid peroxidation of membranes which are assumed to be responsible for certain pathological conditions as heart attacks, septic shocks and atrophic arthritis etc. The extracellular activity of these enzymes is alleged to be related to acute or chronic inflammation. Stabilization of lysosomal membrane is important in limiting the inflammatory response by inhibiting the discharge of lysosomal constituents of activated neutrophil like bacterial enzymes and proteases which cause further tissue inflammation and damage upon extracellular release or by stabilizing the lysosomal membrane [1].

Previous studies have proved that the chemical constituents such as flavonoids, alkaloids, tannins and terpenoids are promising agents in treatment of inflammation. Flavonoids such as hesperidin, apigenin, luteolin and quercetin are found to be a potent anti-inflammatory constituent. The previous studies had also explained about the Methanolic extract of avaram flowers (MECA) and leaves exhibited profound anti-inflammatory activity in both acute and chronic animal models it had been also seen that 50% acetone extract of the flower of *C. auriculata* showed marked anti-inflammatory activity in carrageen induced oedema in rats. The effect was thanks to the presence of the flavonol glycoside 5-O-methylquercetin 7-O-glucoside and tannin and steroid present within the flowers and therefore the leaves.

This research is needed to know the importance of *Cassia auriculata* in anti-inflammatory activity. The main deficiency it fulfill that the *Cassia auriculata* is related to histamine, kinn and prostaglandin inhibiting activity. Our team has extensive knowledge and research experience that has translate into high quality publications [2–12], [4,13-39]. The aim of this study is to determine anti-inflammatory activity of *cassia auriculata* flower extract.

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Plants

*Cassia auriculata* were purchased commercially from an herbal health centre in Chennai. The obtained powder avaram is stored in an airtight container. 5 gram of powder is mixed with 50 ml of ethanol and kept within the orbital shaker for 72 hours, after it's boiled during a heating mantle at 62- 70 degree c for 5-10 min. The extract is filtered using what man paper 1. The filter extract again contracted using heating mantle.

2.2 Inhibition of Albumin Denaturation Assay

Bovine albumin (BSA) was used as a reagent for the assay. Approximately 60% of all proteins in animal serum made up by BSA. It's commonly utilized in culture, particularly when protein supplementation is important and therefore the other components of serum are unwanted. BSA undergoes denaturation on heating and starts expressing antigens associated with Type III hypersensitivity reactions which are related to a disease like atrophic arthritis, glomerulonephritis, serum disease, and systemic LE. 2 ml of 1%
bovine albumin fraction was mixed with 400 μl of red sandal ethanolic extract in several concentration (10,20,30,40,50 μg/mL), and therefore the pH of reaction mixture was adjusted to six.8 using 1N HCl. The reaction mixture was incubated at temperature for 20 min then heated at 55°C for 20 min during a water bath. The mixture was cooled to temperature , and therefore the absorbance value was recorded at 660 nm. An equal amount of plant extract was replaced with dimethyl sulfoxide for control. Voltaren in several concentrations was used as standards.

\[
\% \text{ Anti-Denaturation Activity} = \frac{\text{Absorbance of control} - \text{Absorbance of sample} \times 100}{\text{Absorbance of control}}
\]

2.3 Egg Albumin Assay

The avaram of ethanolic extract was prepared in several test tubes 2.8 ml of the avaram of ethanolic solution was added to the concentration (10μL,20μL,30μL,40μL,50μL) and this was added to 0.45 mL egg albumin (1% solution) and therefore the pH of the mixture was acclimated to six .3 with 1N hydro- acid. The specimens were then incubated at room temperature for 20 minutes. The samples were then heated at 55 degrees during a water bath for half-hour. The samples were cooled following which the absorbance was measured spectrophotometrically at 660 nm. Voltaren was used as the standard group.

\[
\text{Protein denaturation} \ % \text{ of inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample} \times 100}{\text{Absorbance of control}}
\]

3. RESULTS

The results of anti-inflammatory activity and egg albumin assay were depicted in (Figs. 1-2). In the present study, the total anti-inflammatory of Cassia auriculata ethanolic extract (CAE) was determined using the egg albumin assay method.CAE Ext showed anti-inflammatory property in a concentration dependent manner. The result indicated that the CAE Ext significantly (<0.05) inhibited albumin Denaturation Assay method. Egg albumin assay is an easy, rapid and sensitive method for the anti-inflammatory screening of plant extracts. The present study investigated the anti-inflammatory activity of CAE Ext, and expressed the inhibition of albumin denaturation Assay using BSA as standard reference.

4. DISCUSSION

In this study, ethanolic crude extract of Cassia auriculata flowers showed potential anti-inflammatory activity when it was compared with the leaves of ethyl acetate extracts because the anti-inflammatory effect of Cassia auriculata has more significant activity in later phase of inflammation. In accordance with previous studies steroids, flavonoids, alkaloids, terpenoids and tannins are shown to possess anti-inflammatory activity. Thus the anti-inflammatory effect of ethanolic crude extract may be because of the presence of active constituent flavonoids. supporting the results described, it may be concluded that the ethanolic crude extract shown above the result could also be because of the absence of Flavonoids. The results strongly suggest anti-inflammatory effects by percentage of inhibitions. Medically, avaram is used as a remedy in various disorders which could be a result of its excellent anti-inflammatory potential. However, chemical constituents and mechanisms that are answerable for the pharmacological activities remain to be investigated [40].

As we compare with previous study of Pinheiro et al investigated that methanolic extract of the Couroupita guianensis flower shows maximum anti-inflammatory activity when compared with cassia auriculata flower extract by inhibiting the lysosomal enzyme whereas cassia auriculata inhibits the the release of histamine, kinn and prostaglandin inhibitory activity [41]. Most recently in a study by Silva et al conclude that Calendula officinalis L. Flower Extract shows no inhibitory activity upto 50% but in Overall, this study supports the usefulness of Calendula oil which shows the treatment of injured skin and for conditions or diseases which contributes to the pathophysiology, like irritative and allergic dermatitis, vitiligo, rosacea, melasma, psoriasis and cutaneous toxicities derived from cancer treatment whereas cassia auriculata shows the treatment for diabetes, rheumatism, pink eye, constipation and liver disease which does not have sufficient evidence about treatment of cassia auriculata [42].

Similarly, In the study by Prabhakaran D et al concluded that the solid powder obtained from the ethyl acetate fraction from the flower Opuntia stricta has significant anti-inflammatory activities [43]. A similar study was undertaken by Shahahi et al. Methanolic extract of Butea monosperma flowers was given at a similar doses was found...
to significantly inhibit granuloma tissue formation which also includes significant reduction in levels of serum lysosomal enzymes and lipid peroxides [44].

Fig. 1. Image showing the Synthesis *Cassia auriculata* ethanolic extract

![Image of Synthesis Cassia auriculata ethanolic extract]

Fig. 2. The above graph depicts the anti-inflammatory activity with an increased percentage of inhibition with a concentration in microlitres. X axis denotes concentration and Y axis denotes percentage of inhibition of *Cassia auriculata*

![Graph showing anti-inflammatory activity]

Fig. 3. The above graph depicts the anti-inflammatory activity of *Cassia auriculata* ethanolic extract on egg albumin assay increased percentage of inhibition with a concentration in microlitres. X axis denotes concentration and Y axis denotes percentage of inhibition of *Cassia auriculata*
The limitations of this study are that the anti-inflammatory activity of *Cassia auriculata* was only taken into consideration, like it was not done in rats, human blood cells and so on. Also, only the flower of the plant was studied, the other parts of the plants such as the stem, leaves and root in combination with ethanolic extract must be studied. In the future, the extract must be tested on various cell lines to check its effectiveness and compatibility, and active compounds which exhibit anti-inflammatory properties might be isolated and formulated with other herbal products.

5. CONCLUSION

Based on our observations, it had been confirmed that avaram (flower) showed strong in vitro anti-inflammatory effect within the cell free system. Phytochemical research is required to spot the active principles liable for this biological activity of this medicinal plant. Further studies are aimed toward the isolation and identification of bio-active molecules from the ethanolic extract of avaram. The increased demand has placed an excellent strain on the natural populations of avaram. Collectors of medicinal plants are resorting to unsustainable exploitation causing serious threat to the survival of the species. Thus, the species is reported to indicate a poor regeneration capacity. Cultivation on a substantially high scale is yet to be started. Therefore, there's a requirement to conserve the species for the advantage of mankind. More importantly, critical elements of effective conservation strategies ought to be discussed.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENT

The authors are thankful to Saveetha Institute of medical and technical sciences, Saveetha Dental College and Hospitals, Saveetha University for giving a platform to conduct the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Bandawane DD, Mali AA, Hivrale MG. Evaluation of Anti-inflammatory and Analgesic Activity of Methanolic Extract of *Cassia auriculata* Leaves [Internet]. Pharmacologia. 2013;4:117–25. Available: http://dx.doi.org/10.5567/pharmacologia.
2. Rajeshkumar S, Kumar SV, Ramaiah A, Agarwal H, Lakshmi T, Roopan SM. Biosynthesis of zinc oxide nanoparticles using *Mangifera indica* leaves and evaluation of their antioxidant and cytotoxic properties in lung cancer (A549) cells. Enzyme Microb Technol. 2018 117:91–5.
3. Nandhini NT, Rajeshkumar S, Mythili S. The possible mechanism of eco-friendly synthesized nanoparticles on hazardous dyes degradation. Biocatal Agric Biotechnol. 2019;19:101138.
4. Vairavel M, Devaraj E, Shanmugam R. An eco-friendly synthesis of *Enterococcus sp.*—mediated gold nanoparticle induces cytotoxicity in human colorectal cancer cells. Environ Sci Pollut Res. 2020; 27(8):8166–75.
5. Gomathi M, Prakasam A, Rajkumar PV, Rajeshkumar S, Chandrasekaran R, Anbarasan PM. Green synthesis of silver nanoparticles using *Gymnema sylvestre* leaf extract and evaluation of its antibacterial activity [Internet]. South African Journal of Chemical Engineering. 2020;32:1–4. Available: http://dx.doi.org/10.1016/j.sajce.2019.11.005
6. Rajasekaran S, Damodharan D, Gopal K, Rajesh Kumar B, De Pores MV. Collective influence of 1-decanol addition, injection pressure and EGR on diesel engine characteristics fueled with diesel/LDPE oil blends. Fuel. 2020; 277:118166.
7. Santhoshkumar J, Sowmya B, Venkat Kumar S, Rajeshkumar S. Toxicology evaluation and antidermatophytic activity of silver nanoparticles synthesized using leaf extract of *Passiflora caerulea*. S Afr J Chem Eng. 2019;29:17–23.
8. Raj RK, DE, SR. β-Sitosterol-assisted silver nanoparticles activates Nrf2 and triggers mitochondrial apoptosis via oxidative stress in human hepatocellular
cancer cell line. J Biomed Mater Res A. 2020;108(9):1899–908.

9. Saravanan M, Arokiyaraj S, Lakshmi T, Pugazhendhi A. Synthesis of silver nanoparticles from Phenerochaete chrysosporium (MTCC-787) and their antibacterial activity against human pathogenic bacteria. Microb Pathog. 2018;117:68–72.

10. Gheena S, Ezhilarasan D. Syringic acid triggers reactive oxygen species–mediated cytotoxicity in HepG2 cells. Hum Exp Toxicol. 2019;38(6):694–702.

11. Ezhilarasan D, Sokal E, Najmi M. Hepatic fibrosis: It is time to go with hepatic stellate cell-specific therapeutic targets. Hepatobiliary Pancreat Dis Int. 2018;17(3):192–7.

12. Ezhilarasan D. Oxidative stress is bane in chronic liver diseases: Clinical and experimental perspective. Arab J Gastroenterol. 2018;19(2):56–64.

13. Gomathi AC, Xavier Rajarathinam SR, Mohammed Sadiq A, Rajeshkumar S. Anticancer activity of silver nanoparticles synthesized using aqueous fruit shell extract of Tamarindus indica on MCF-7 human breast cancer cell line. J Drug Deliv Sci Technol. 2020;55:101376.

14. Dua K, Wadhwa R, Singhvi G, Rapalli V, Shukla SD, Shastri MD, et al. The potential of siRNA based drug delivery in respiratory disorders: Recent advances and progress. Drug Dev Res. 2019;80(6):714–30.

15. Ramesh A, Varghese S, Jayakumar ND, Malaiappan S. Comparative estimation of sulfiredoxin levels between chronic periodontitis and healthy patients: A case-control study. J Periodontol. 2018;89(10):1241–8.

16. Arumugam P, George R, Jayaseelan VP. Aberrations of m6A regulators are associated with tumorigenesis and metastasis in head and neck squamous cell carcinoma. Arch Oral Biol. 2021;122:105030.

17. Joseph B, Prasanth CS. Is photodynamic therapy a viable antiviral weapon against COVID-19 in dentistry? Oral Surg Oral Med Oral Pathol Oral Radiol. 2021;132(1):118–9.

18. Ezhilarasan D, Apoorva VS, Ashok Vardhan N. Syzygium cumini extract induced reactive oxygen species-mediated apoptosis in human oral squamous carcinoma cells. J Oral Pathol Med. 2019;48(2):115–21.

19. Duraisamy R, Krishnan CS, Ramasubramanian H, Sampathkumar J, Mariappan S, Navarasampatti Sivaprasakam A. Compatibility of Nonoriginal Abutments With Implants: Evaluation of Microgap at the Implant-Abutment Interface, With Original and Nonoriginal Abutments. Implant Dent. 2019;28(3):289–95.

20. Gnanavel V, Roopan SM, Rajeshkumar S. Aquaculture: An overview of chemical ecology of seaweeds (food species) in natural products. Aquaculture. 2019;507:1–6.

21. Markov A, Thangavelu L, Aravindhan S, Zekiy AO, Jarahian M, Chartrand MS, et al. Mesenchymal stem/stromal cells as a valuable source for the treatment of immune-mediated disorders. Stem Cell Res Ther. 2021;12(1):192.

22. Veerasamy R, Roy A, Karunakaran R, Rajak H. Structure–Activity Relationship Analysis of Benzimidazoles as Emerging Anti-Inflammatory Agents: An Overview. Pharmaceuticals. 2021;14(7):663.

23. Dhayanithi J, Rajeshkumar S, Roy A, Lakshmi T. Preparation and Evaluation of Anti fungal Activity of Arrow Root Mediated Selenium Nanoparticles Against Candida Albicans -. Journal of Complementary Medicine Research. 2020;11(5):83–8.

24. Blessy PS, Rajeshkumar S, Lakshmi T, Roy A. Enhanced Antibacterial Activity of Arrowroot Mediated Selenium Nanoparticles Against Streptococcus Mutans And Lactobacillus Species -. Journal of Complementary Medicine Research. 2020;11(5):17–23.

25. Lakshmi T, Roy A, Raghunandhakumar S, Merlin ARS. Invitro Cytotoxicity Assay of Acacia Catechu Ethanolic Seed Extract Using Brine Shrimp -. Journal of Complementary Medicine Research. 2020;11(5):89–92.

26. R. V Geetha TL. In vitro evaluation of antimicrobial activity and estimation of Epicatechin from the fruit extract of Prunus armeniaca L using HPTLC technique -. Journal of Complementary Medicine Research. 2020;11(5):113–22.

27. Assessment of Oxidative Stress and Antioxidant Levels in Chronic Periodontitis Patients [Internet]. [cited 2021 Aug 31]. Available: http://alinteridergisi.com/article/assessment-of-oxidative-stress-and-
antioxidant-levels-in-chronic-periodontitis-patients/

28. Dharaahasa C, Lakshmi T, Roy A, Raghunandhakumar S. Genotoxicity potentials of methanolic extracts of Mimosa pudica against oral cancer cells. Journal of Complementary Medicine Research. 2020;11(5):24–9.

29. Lakshmi T, Roy A, George RS, Raghunandhakumar S. Antibacterial Activity of Acacia Catechu Seed Against Urinary Tract Pathogens. Journal of Complementary Medicine Research. 2020;11(5):123–7.

30. Jai Rexlin PE, Roy A, Rajeshkumar S, Lakshmi T. Antimicrobial Activity of Coriander Oleoresin Mediated Selenium Nanoparticles Against Oral Pathogens. -. Journal of Complementary Medicine Research. 2020;11(5):35–40.

31. Lakshmi T, Ramasamy R, Thirumalaikumaran R. Preliminary Phytochemical analysis and In vitro Antioxidant, FTIR Spectroscopy, Anti-diabetic activity of Acacia catechu ethanolic seed extract. 2015 [cited 2021 Aug 31]; Available:https://pdfs.semanticscholar.org/983d/dacc94d0aa8287a779084d6b26b975bd7bea.pdf

32. Ganapathy, Dhanraj, Shanmugam, Rajeshkumar, Thangavelu, Lakshmi. Nanobiotechnology in combating CoVid-19. Bioinformation. 2020;828–828.

33. Murali N, Lakshmi T, RajeshKumar S, Roy A, Geetha RV. Characterization of Silver nanoparticles synthesized from Curculigo orchioides extract using UV vis spectroscopy -. Journal of Complementary Medicine Research. 2020;11(5):68–74.

34. Ahamad ST, Tanish Ahamad S, Lakshmi T, Rajeshkumar S, Roy A, Gurunadhan D, et al. Antibacterial Activity of Taxifolin Isolated from Acacia Catechu Leaf Extract-An Invitro Study [Internet]. Indian Journal of Public Health Research & Development. 2019;10:3540. Available: http://dx.doi.org/10.5958/0976-5506.2019.04135.4

35. Ezhillarasan D, Lakshmi T, Subha M, Deepak NV, Raghunandhakumar S. The ambiguous role of sirtuins in head and neck squamous cell carcinoma. Oral Dis [Internet]. 2021 [cited 2021 Aug 31] Available:https://pubmed.ncbi.nlm.nih.gov/33570800/

36. Thakur M, Guttikonda VR. Estimation of hemoglobin, serum iron, total iron-binding capacity and serum ferritin levels in oral submucous fibrosis: A clinicopathological study. J Oral Maxillofac Pathol [Internet]. 2017 [cited 2021 Aug 31];21(1). Available:https://pubmed.ncbi.nlm.nih.gov/28479683/

37. Lakshmi T, Ezhillarasan D, Vijayaragavan R, Bhullar SK, Rajendran R. Acacia catechu ethanolic bark extract induces apoptosis in human oral squamous carcinoma cells. J Adv Pharm Technol Res [Internet]. 2017 [cited 2021 Aug 31];8(4). Available:https://pubmed.ncbi.nlm.nih.gov/29184846/

38. Role of Nanomedicine in Novel Corona Virus Pandemic: A perspective [Internet]. 2020 [cited 2021 Aug 31]. Available: http://bbrc.in/bbrc/role-of-nanomedicine-in-novel-corona-virus-pandemic-a-perspective/

39. Anitha R, Prathoshni S, Lakshmi T. The effect of capsicum oleoresin on nitric oxide production and nitric oxide synthase gene expression in macrophage cell line [Internet]. Pharmacognosy Research. 2018;10:343. Available:http://dx.doi.org/10.4103/pr.pr_4_6_18

40. Muruganantham N, Soloman S, Senthamilselvi M. Anti-oxidant and Anti-inflammatory Activity of Cassia auriculata (Flowers). J Pharm Health Serv Res. 2015;8(1):1–9.

41. Pinheiro MMG, Fernandes SBO, Fingolo CE, Boylan F, Fernandes PD. Anti-inflammatory activity of ethanol extract and fractions from Couroupita guianensis Aublet leaves [Internet]. Journal of Ethnopharmacology. 2013;146: 324–30. Available:http://dx.doi.org/10.1016/j.jep.2012.12.053

42. Silva D, Ferreira MS, Sousa-Lobo JM, Cruz MT, Almeida IF. Anti-Inflammatory Activity of Calendula officinalis L. Flower Extract [Internet]. Cosmetics. 2021;8:31. Available:http://dx.doi.org/10.3390/cosmetics8020031

43. DP, Prabhakaran D, Rajeshkanna A, Mm S. In vitro antioxidant and anti-inflammatory activity of the flower extracts of opuntia stricta [Internet]. Asian Journal of Pharmaceutical and Clinical Research. 2019;208–12.
