ANTI-INFLAMMATORY ACTIVITY OF *CASSIA AURICULATA* FLOWER EXTRACT

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 antiinflammatory 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

Comment [O1]: Remove Recent

Comment [O2]: Traditional Therapeutics

Comment [O3]: These have inhibitory potential similar to standard anti inflammatory drugs

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 (Caesalpiniaceae) 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.T he 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(Bandawane, Mali and Hivrale, 2013).

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 seenthat 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(Rajeshkumar *et al.*, 2018; Nandhini, Rajeshkumar and Mythili, 2019; M. Gomathi *et al.*, 2020; Rajasekaran *et al.*, 2020; Vairavel, Devaraj and Shanmugam, 2020),(Santhoshkumar *et al.*, 2019),(Raj R, D and S, 2020),(Saravanan *et al.*, 2018),(Gheena and Ezhilarasan, 2019),(Ezhilarasan, Sokal and Najimi, 2018),(Ezhilarasan, 2018),((Dua *et al.*, 2019; A. C. Gomathi *et al.*, 2020; Vairavel, Devaraj and Shanmugam, 2020),(Ramesh *et al.*, 2018; Duraisamy *et al.*, 2019; Ezhilarasan, Apoorva and Ashok Vardhan, 2019; Arumugam, George and Jayaseelan, 2021; Joseph and Prasanth, 2021) .,(Gnanavel, Roopan and Rajeshkumar, 2019),(Markov *et al.*, 2021)(Veerasamy *et al.*, 2021)(Dhayanithi *et al.*, 2020)(Blessy *et al.*, 2020)(Lakshmi, Roy, Raghunandhakumar, *et al.*, 2020)(R. V Geetha, 2020)(*Assessment of Oxidative Stress and Antioxidant Levels in Chronic Periodontitis Patients*, no date)(Dharahaas *et al.*, 2020)(Lakshmi, Roy, George, *et al.*, 2020)(Jai Rexlin *et al.*, 2020)(Lakshmi, Ramasamy and Thirumalaikumaran, 2015)(Ganapathy *et al.*, 2020)(Murali *et al.*, 2020)(Ahamad *et al.*, 2019)(Ahamad *et al.*, 2019; Ezhilarasan *et al.*, 2021)(Thakur and Guttikonda, 2017)(Lakshmi *et al.*, 2017; Thakur and Guttikonda, 2017)(*Role of Nanomedicine in Novel Corona Virus Pandemic: A perspective*, 2020)(Anitha, Prathoshni and Lakshmi, 2018).The aim of this study is to determine anti-inflammatory activity of cassia auriculata flower extract.

Comment [O4]: All marked should be put into two lines

MATERIALS AND METHODS:

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 whatman paper 1.The filter extract again contracted using heating mantle.

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.

% Anti-Denaturation Activity = Absorbance of control – Absorbance of sample x100
Absorbance of control

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 follow- ing which the absorbance was measured spectrophotometrically at 660 nm. Voltaren was used as the standard group.

Protein denaturation % of inhibition = Absorbance of control - Absorbance of sample * 100/Absorbance of control

RESULTS:

Figure 1: Image showing the Synthesis *Cassia auriculata* ethanolic extract

The results of anti-inflammatory activity and egg albumin assay were depicted in (Figures 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.

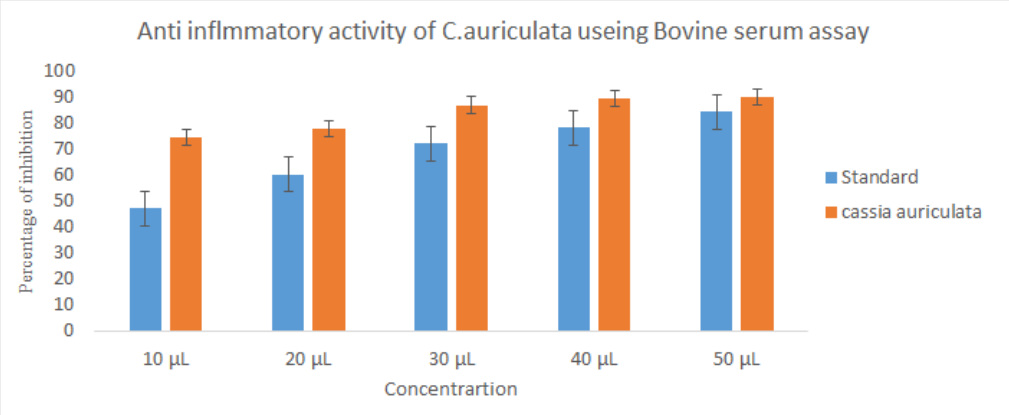

Figure 2: The above graph depicts the anti -inflammatory activity with an increased percentage of inhibition with a concentration in microlitres. X axis denotes concentration and Y axis denotes percentage of inhibition of *Cassia auriculata*.

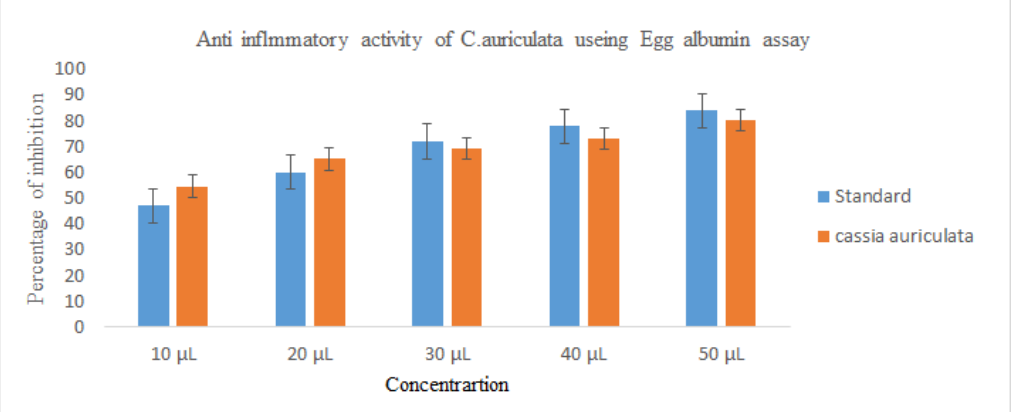

Figure 3: The above graph depicts the anti-inflammatory activity of *Cassia auriculata* ethanolic extract on egg albumin assay increased percentage of inhibition with a concentration in microlitres. X axis denotes concentration and Y axis denotes percentage of inhibition of *Cassia auriculata*.

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.(Muruganantham, Solomon and Senthamilselvi, 2015)

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(Pinheiro *et al.*, 2013).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.(Silva *et al.*, 2021). 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(D *et al.*, 2019).A similar study was undertaken by Shahavi 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(Shahavi and Desai, 2008).

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.

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.

Comment [O5]: Not essential

Comment [O6]: References should come in text as sequence and they should have linkage

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