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ANTI DENATURATION AND ANTIOXIDANT ACTIVITIES OF ANNONA CHERIMOLA IN-VITRO

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ABSTRACT

Annona cherimola is believed indigenous to South Central America and also found in some parts of Asia. An attempt is made to evaluate the anti inflammatory and antioxidant activites of *Annona cherimola* leaf extract as the denaturation of proteins and free radical damage or oxidative stress are inter-linked and influence each other. *Invitro* anti inflammatory and antioxidant properties were tested on hexane and methanolic leaf extracts of *Annona cherimola* by means of anti denaturation of bovine serum albumin and reducing antioxidant power respectively. In anti denaturation study it was observed that methanolic extract showed greater percentage of inhibition of bovine serum albumin denaturation i.e 77.6% where as hexane extract showed 63.5% at the lowest concentration respectively. In case of antioxidant screening also, methanolic extract showed better reducing antioxidant power compared to hexane extract dose dependently. Thus the results indicate that methanolic extract of *Annona cherimola* was found to possess significantly good anti inflammatory and antioxidant activities and this can be attributed to the presence of phenolic compounds.

KEYWORDS

Annona cherimola, Anti denaturation, Reducing antioxidant power.

INTRODUCTION

Traditional medicinal plants are the best natural source of therapeutic remedies to treat various diseases. Most of the conventional drugs to treat inflammation and to relieve oxidative stress that are available in the market are potent enough but upon long term administration may lead to severe side effects. This is the major reason why the natural remedies are drawing great attention since they are almost free from side effects.

When cells in the body are damaged by microbes, physical agents or chemical agents, the injury is in the form stress. The response to the stress of tissue damage is called as **inflammation**. It is a defensive response that is characterized by redness, pain, heat, and swelling and **loss of function** in the injured area. Whether loss of function occurs depends on the site and extent of injury. Since inflammation is one of the body's nonspecific internal systems of defense, the response of a tissue to an accidental cut is similar to the response that results from other types of tissue damage, caused by **burns due to heat**, radiation, bacterial or viral invasion[1].

Protein Denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an oraganic solvent or **heat**. Most biological proteins lose their biological function when denatured. For example, enzymes lose their activity, because the substrates can no longer bind to the active site[2].

Oxidative stress is induced by wide range of environmental factors including UV stress, pathogen invasion (hypersensitive reaction), herbicide action and oxygen shortage. Oxygen deprivation stress in plant cells is distinguished by three physiologically different states: transient hypoxia, anoxia and reoxygenation. Generation of reactive oxygen species (ROS) is the characteristic feature for hypoxia and essentially for reoxygenation. Of the ROS, hydrogen peroxide (H_2O_2) and superoxide ($O_2^{\cdot-}$) are both produced in number of cell reactions, including iron catalysed Fenton-reaction and by various enzymes such as lipoxygenases, peroxidases, NADPH oxidase and xanthine oxidase. The main cellular components susceptible to damage by free radicals are lipids (peroxidation of unsaturated fatty acids in cell membrane), **proteins (denaturation)**, carbohydrates and nucleic acids[3].

Both inflammation and free radical damage are inter-related aspects that influence each other. As said above proteins are susceptible to undergo denaturation by formation of free radicals and the mechanism of inflammation injury is attributed, in part, to release of Reactive Oxygen species from activated neutrophil and macrophages. This over-production leads to tissue injury by damaging the macromolecule and lipid peroxidation of membranes. In addition, ROS propagate inflammation by stimulating the release of the cytokines such as interleukin- I, tumor necrosis factor-α, and interferon-γ, which stimulate recruitment of additional neutrophil and macrophages. Thus free radicals are important mediators that provoke or sustain inflammatory processes and consequently, their neutralization by antioxidants and radical scavengers can attenuate inflammation[4-6].

Thus our study aims to find a natural remedy that will be useful to treat both inflammation and free radical damage.

MATERIALS AND METHOD

(i) Collection and Authentication

Annona cherimola belonging to family Annonaceae has been selected for the study. The plant material was collected from Thirumala forest, Chittor district, Andhra Pradesh, India and authenticated by Dr. Madhava Chetty, botanist, Sri Venkateshwara University, Tirupathi. The leaves were shade dried and ground into coarse powder.

(ii) Chemicals

Bovine serum albumin (BSA), potassium ferricyanide and trichloro acetic acid were purchased from Hi Media and Diclofenac sodium from Cipla Pharmaceuticals. Solvents were purchased from Sd fine chem Ltd. And all other chemicals used were of analytical grade and purchased locally.

(iii) Preparation of Extract

500g of powdered leaves of *Annona cherimola* was extracted continuously using soxhlet apparatus with hexane and methanol successively (increasing order of polarity) for about 48 hours at 30°C. The extracts were concentrated under reduced pressure using Rotary vacuum flash evaporator to get a constant volume.

(iv) Preliminary Phytochemical Screening

The plant is a biosynthetic laboratory, not only for chemical compounds such as carbohydrates, proteins and lipids that are utilized as food by man, but also for a multitude of compounds like glycosides, alkaloids, gums, tannins, saponins etc. that exert physiological and therapeutic effect. The compounds that are responsible for medicinal property of the drug are usually secondary metabolites. A systematic study of a crude drug embraces, thorough consideration of primary and secondary metabolites derived as a result of plant metabolism[7]. The plant material is subjected to preliminary phytochemical screening for the detection of various plant constituents (Tab1)

Table 1

Preliminary Phytochemical Screenig for various phytoconstituents

S No	Test	Hexane Extract	Methanolic Extract
1	**Carbohydrates** (Benedict's test)	-	+
2	**Proteins** (Biuret test)	-	+
3	**Amino acids** (Ninhydrin test)	-	-
4	**Alkaloids** (Mayer's test)	+	+
5	**Steroids** (Salkowaski's Test)	+	+
6	**Phenolic compounds** (CH_3COOPb) ($FeCl_3$)	+	+
7	**Tannnins**	+	+
8	**Cardiac Glycosides** (Kellar killani Test)	-	-
9	**Saponins** (Foam Test)	-	-

(v) Anti-denaturation Activity

The method of Williams et al[8], was employed for antidenaturation assay. A solution of 0.2% W/V of BSA was prepared in Tris buffer saline and P^H was adjusted to 6.8 using glacial acetic acid. Stock solutions of 10,000µg/ml of all extracts were prepared by using methanol as a solvent. From these stock solutions 4 different concentrations of 1,100, 200 and 500 µg/ml were prepared by using methanol as a solvent. 50µl of each extract was transferred to Eppendorf tubes using 1ml micro pipette. 5ml of 0.2% W/V BSA was added to all the above Eppendorf tubes. The control consists of 5ml 0.2% W/V BSA solution with 50 µl methanol. The standard consists of 100 µg/ml Diclofenac Sodium in methanol with 5ml 0.2% W/V BSA solution. The test tubes were heated at 72° C for 5 minutes and then cooled for 10 minutes. The absorbance of these solutions was determined by using UV/Vis Double beam spectrophotometer (Elico SL-196) at a wavelength of 660nm. The % inhibition of precipitation (denaturation of the protein) was determined on a % basis relative to the control using the following formula.

$$\% \text{ inhibition of denaturation} = \frac{(\text{Abs of control} - \text{Abs of extract})}{\text{Abs of control}} \times 100$$

(vi) Reducing Antioxidant Activity

Different concentrations of plant extracts (10 – 500µg/ml) in 1 ml of distilled water were mixed with phosphate buffer (2.5 ml, 0.2 M, P^H 6.6) and potassium ferri-cyanide [$K_3Fe(CN)_6$] (2.5 ml,1%). The mixture was incubated at 50°C for 20 min. Then, 2.5 ml of trichloroacetic acid (10%) was added to mixture, which was then centrifuged for 10 min at 3000 rpm. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%). The absorbance was measured at 700 nm against a blank using UV-Vis spectrophotometer (Elico –SL 196). Increased absorbance of the reaction mixture indicates increase in reducing power[9].

RESULTS

Anti-denaturation Activity

Table2

Anti denaturation of BSA in presence of Annona cherimola extracts

SNo	Type of Sample	Concentration (µg/ml)	%Inhibition
1	Methanolic	1	77.6±0.61
2	Methanolic	100	49.8±0.32
3	Methanolic	200	33.0±0.57
4	Methanolic	500	22.1±0.28
5	Hexane	1	63.5±0.21
6	Hexane	100	44.2±0.53
7	Hexane	200	27.5±0.16
8	Hexane	500	11.4±0.65
9	Diclofenac sodium	100	88.9±0.46

Both methanolic and hexane extracts of *Annona cherimola* inhibited the denaturation of Bovine serum albumin (BSA). The degree of inhibition of BSA denaturation increased with the decrease in the concentration of both the extracts as stated by Williams et al., that the anti denaturation of the drug will be more at lower concentration. As shown in Tab(2) among the two extracts under the study Methanolic extract (from 22.1±0.28% to 77.6±0.61%) has shown better inhibition of BSA denaturation at any concentration compared to hexane extract (from 11.4±0.65% to 63.5±0.21%). The standard drug Diclofenac sodium showed 88.9±0.46% inhibition of denaturation at 100 µg/ml concentration

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Reducing Antioxidant Activity:

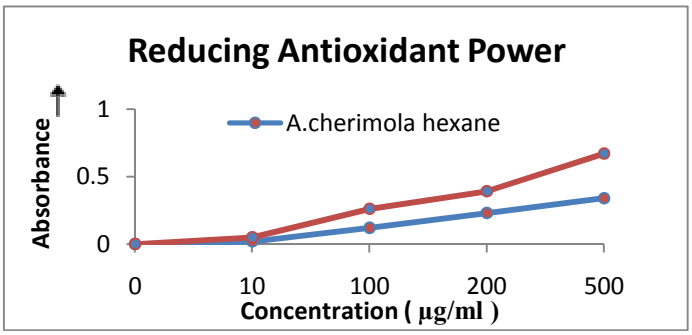

Fig 1
Reducing Antioxidant activity of different extracts of Annona cherimola

Methanolic and hexane extracts of *Annona cherimola* were tested for reducing antioxidant property (Fig 1). The absorbance of the two extracts increased with the rise in their concentrations since the absorbance is directly proportional to the reduction of ferric (Fe^{+3}) to ferrous ions (Fe^{+2}) in the reaction mixture. As shown in Fig 1 hexane extract (10-500 µg/ml) exhibited OD_{700} values ranging from 0.02 to 0.34 indicates mild reducing antioxidant power, where as methanolic extract (10-500 µg/ml) exhibited OD_{700} values from 0.05 to 0.67 indicates good reducing antioxidant power.

DISCUSSION

The method of Anti denaturation of BSA was chosen to evaluate anti inflammatory property of *Annona cherimola*. In antidenaturation assay the denaturation of BSA is induced by heat treatment. The denatured BSA expresses antigens associated to Type III hyper-sensitive reaction which are related to diseases such as serum sickness, glomerulo nephritis etc[10]. Heat-denatured proteins are as effetcive as native proteins in provoking delayed hypersensitivity[11]. Moreover it was already proved that Conventional NSAID's like phenylbutazone and indomethazine does not act only by the inhibition of endogenous prostaglandins production by blocking COX enzyme but also by prevention of denaturation of proteins[12]. Thus antidenaturation assay is the covenient method to check the anti inflammatory activity. In our results both the extracts has shown considerable anti inflammatory activity and methanolic extract was found to be more potent than hexane extract. The secondary metabolites like Phenolic compounds and tannins which were found in preliminary phytochemical screening might be responsible for this activity.

Reducing antioxidant screening method is the simple and foremost assay method to assess the antioxidant potency of natural products in the process of drug discovery. In this test the increase in the absorbance was due to reduction of Fe^{+3} to Fe^{+2} which indicates the reducing potential of the extracts. The results clearly indicate that methanolic extract of *Annona cherimola* was found to have more reducing antioxidant power than hexane extract. Since phenolic compunds (flavonoids, tannins, hydroxycinnamate esters and lignin) are abundant in plant tissues and possess ideal structural chemistry for free radical scavenging activity. Phenolic compounds are the ideal antioxidants and act by different mechanisms[3] . Thus the presence of antioxidant activity can be attributed to the presence of Phenolic compounds in *Annona cherimola* leaf extracts.

CONCLUSION

Our investigation clearly demonstrates that methanolic and hexane extracts of *Annona cherimola* leaves possess significant anti inflammatory and antioxidant properties. Among them methanolic extract was found to be more potent than the hexane extract. Futher studies are recommended to isolate the active principle responsible for these activities.

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