



In Vitro* Antioxidant and Anti-Inflammatory Activities of pericarp of *Terminalia catappa

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ABSTRACT

The present study was designed to evaluate the *in vitro* antioxidant and anti-inflammatory activity of aqueous leaf extract of *Terminalia catappa* (ALTC). Antioxidant activity of the ALTC was determined by following some well established methods for free radical scavenging such as superoxide, hydroxyl, hydrogen peroxide, nitric oxide and DPPH radical scavenging activities using ascorbic acid as standard. The anti-inflammatory activity was evaluated by using HRBC membrane stabilization assay and albumin denaturation assay using diclofenac as standard. Treatment with ALTC showed dose dependent activity against superoxide, hydroxyl, hydrogen peroxide, nitric oxide and DPPH radicals. The ALTC showed significantly stabilization of membrane and denaturation of protein at a concentration range of 100-500µg/ml. The antioxidant and anti-inflammatory activity of *Terminalia catappa* might be due to the anthocyanins in pericarp.

Key words: *Terminalia catappa*, Antioxidant, Anti-inflammatory activity

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Received 08 August 2017, Accepted 26 August 2017

INTRODUCTION

Oxidative stress is defined as an imbalance between cellular production of reactive oxygen species (ROS) and antioxidant defense mechanisms. ROS such as superoxide anion, hydroxyl radical and hydrogen peroxide are physiological metabolites formed during aerobic life as a result of the metabolism of oxygen, which are capable of causing damage to DNA, proteins and lipids, have been associated with carcinogenesis, coronary heart disease, and many other health problems¹

Now a day researchers focused on natural antioxidants and their association with chronic disorders in order to avoid the adverse effects of orthodox drugs upon long term usage. The *Terminalia catappa* is belongs to family combretaceae. The Indian almond contains variety of phytochemicals includes tannins such as punicalagin, punicalin, terflavins A and B and chebulagic acid; flavanoids like isovitexin, vitexin, isoorientin, rutin and triterpinoids (ursolic acid)². Based on the phytochemical present in the *Terminalia catappa*, study was designed to evaluate the effect of aqueous leaf extract of *Terminalia catappa* (ALTC) for *in vitro* antioxidant activity and anti-inflammatory activity.

MATERIALS AND METHOD

Plant Material

The Indian almond (*Terminalia catappa*) was obtained from local market. The pericarps were manually separated and shade dried. The pericarps were powdered in a grinder to get 40-mesh size powder. The moisture content of the dried powder was found to be 13.5%. The extract was suspended in 2% gum acacia and used in the experimental studies.

Methods:

Superoxide radical scavenging activity

The riboflavin-light- NBT system contained 58 mM phosphate buffer, pH 7.6, 20µM riboflavin, 6mM EDTA, and 50µM NBT, final volume made up to 3 ml, added in that sequence. Reaction was started by illuminating 40 volts. Immediately after illumination, the absorbance was measured at 560 nm. The potency of ALAG measured in terms of NBT photo reduction capacity³

Hydroxyl radical scavenging activity:

The Fenton reaction (Fe³⁺-ascorbate-EDTA-H₂O₂ system) reaction mixture contained 2-deoxy-2-ribose (2.8 mM), phosphate buffer (0.1 mM, pH 7.4), ferric chloride (20 µM), EDTA (100 µM), hydrogen peroxide (500 µM), ascorbic acid (100 µM) and various concentrations of the

test sample in a final volume of 1 ml. The mixture was incubated for 1 h at 37 °C. After the incubation an aliquot of the reaction mixture (0.8 ml) was added to 2.8% TCA solution (1.5 ml), followed by TBA solution (1% in 50 mM sodium hydroxide, 1 ml) and sodium dodecyl sulphate (0.2ml). The mixture was then heated (20 min at 90 °C) to develop the colour. After cooling, the absorbance was measured at 532 nm against an appropriate blank solution. The percentage of inhibition of ALAG was expressed deoxyribose degradation capacity ⁴.

Hydrogen peroxide radical scavenging activity:

The hydrogen peroxide scavenging assay was carried out following the procedure of. The principle of this method is that there is a decrease in absorbance of H₂O₂ upon oxidation of H₂O₂. A solution of 43mM H₂O₂ was prepared in 0.1M phosphate buffer (pH 7.4). ALAG at concentration in 3.4mL phosphate buffer was added to 0.6mL of H₂O₂ solution (43mM) and absorbance of the reaction mixture was recorded at 230 nm. A blank solution contained the sodium phosphate buffer without H₂O₂ ⁵.

DPPH radical scavenging activity:

The potential AA and ALAG was determined on the basis of the scavenging activity of the stable 1,1 -diphenyl-2-picrylhydrazyl (DPPH) free radical. Aliquots of 1ml of a methanolic solution containing each pure compound were added to 3ml of 0.004% MeOH solution of DPPH. Absorbance at 517 nm, against a blank of methanol without DPPH, was determined after 30 min (UV, Perkin-Elmer-Lambda 11 spectrophotometer) and the percent inhibition activity was calculated. IC₅₀ values denote the concentration of sample required to scavenge 50% DPPH free radicals. All tests were run in triplicate and averaged ⁶.

Nitric oxide radical scavenging activity:

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions, which were measured by the Griess reaction. The reaction mixture (3 ml) containing sodium nitroprusside (10 mM) in phosphate buffered saline (PBS) and ALAG in different concentrations were incubated at 25°C for 150 min. After incubation 1.5ml of the Griess reagent (1% sulphanilamide, 0.1% naphthyl ethylene diamine dihydrochloride in 2% H₃PO₄) was added. The absorbance of the chromophore formed was measured at 546nm. The percentage inhibition of nitric oxide generated was measured by comparing the absorbance values of control and test samples ⁷.

HRBC membrane stabilization assay:

The HRBC membrane stabilization has been used as method to study the anti-inflammatory activity. Blood was collected from healthy volunteer and was mixed with equal volume of

sterilized Alsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% sodium chloride in water). The mixer was centrifuged at 3000 rpm and packed cell were washed with isosaline (0.85%.pH 7.2) and a 10 % (v/v) suspension was made with isosaline. The assay mixture contained the drug, 1 ml of phosphate buffer (0.15M, pH 7.4), 2 ml of hyposaline (0.36%) and 0.5ml of HRBC suspension. Diclofenac was used as reference drug. All the assay mixture were incubated at 37°C for 30 min and centrifuged. The hemoglobin content in the supernatant solution was estimated using spectrophotometer at 560 nm ⁸.

Albumin denaturation inhibition assay:

Different concentrations of ALAG (1mL) was mixed with 1 mL of 1 mM albumin solution in phosphate buffer and incubated at 27° ± 1° C in BOD incubator for 15 min. Denaturation was induced by keeping the reaction mixture at 60° ± 10° C in water bath for 10 min. After cooling, the turbidity was measured at 660 nm (UV-Visible Spectrophotometer SL-159, Elico India Ltd.). The diclofenac sodium was used as standard drug. Percentage of inhibition of denaturation was calculated from control where no drug was added. Each experiment was done in triplicate and average was taken ⁹.

RESULTS AND DISCUSSION:

Oxygen free radicals such as superoxide, hydrogen peroxide and hydroxyl radical are formed constantly in normal metabolic action of the human body. The antioxidant compounds and enzymes are act by delay or prevent the oxidation of cellular metabolic reactions. Many synthetic drugs are available in market to fight against free radicals with adverse side effects. An alternative solution to the problem is to consume natural antioxidants from food supplements and traditional medicines ¹⁰

Weak oxidant superoxide radical decomposes to form singlet oxygen and hydroxyl radicals as stronger reactive oxidative species and are involved in lipid peroxidation ¹¹. The ALTC showed notable scavenging activity against superoxide and hydroxyl radicals compared with ascorbic acid (figure 1 and figure 2). Hydrogen peroxide rapidly cross cell membranes and react with Fe²⁺ and Cu²⁺ ions to form highly reactive hydroxyl radicals ¹². The ALTC were shown similar activity like AA in scavenging the hydrogen peroxide radicals in a dose dependent manner (figure 3). Nitric oxide is an important chemical mediator generated by endothelial cells, macrophages, neurons, etc. and is involved in the regulation of various physiological processes ¹³. The ALTC shown to notable scavenging of nitric oxide free radical in a dose dependent manner (figure 4).

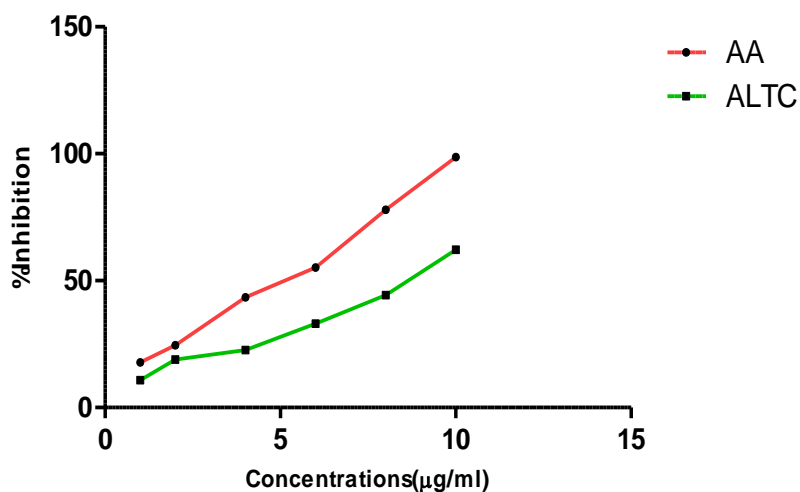


Figure 1: Effect of ALTC on superoxide radical scavenging activity

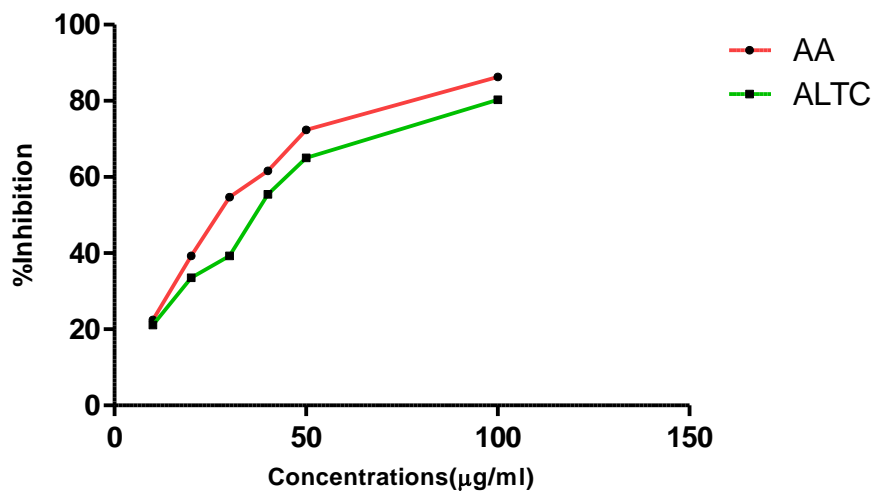


Figure 2: Effect of ALTC on hydroxyl radical scavenging activity

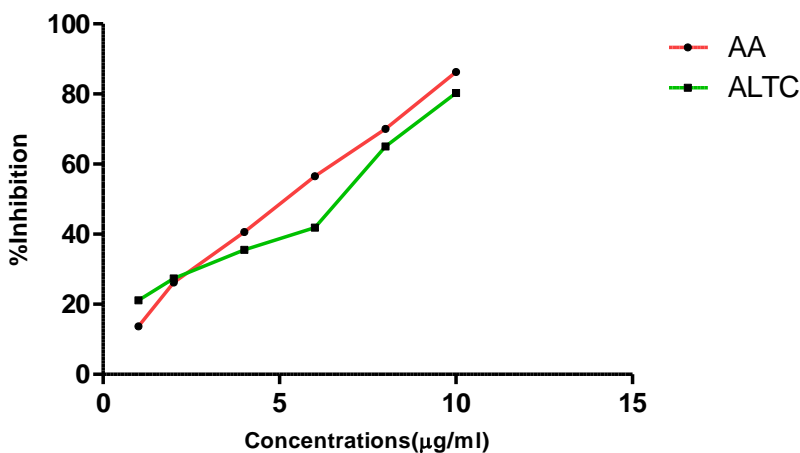


Figure 3: Effect of ALTC on hydrogen peroxide radical scavenging activity

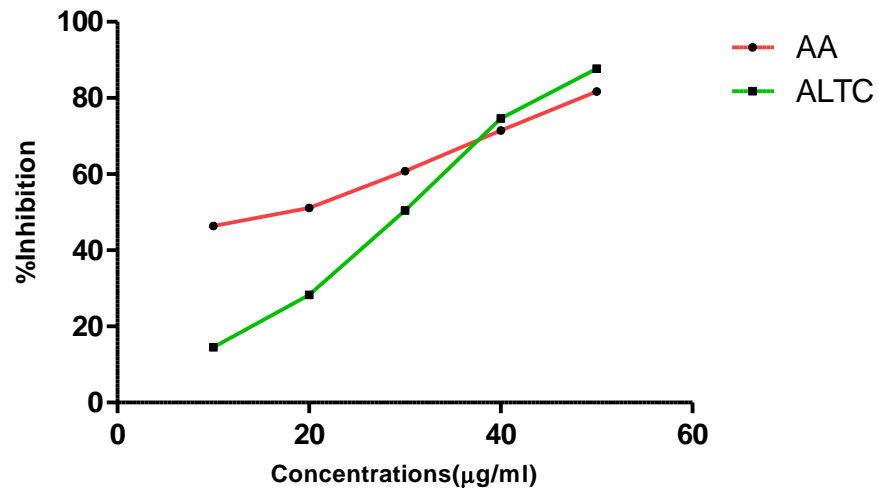


Figure 4: Effect of ALTC on nitric oxide radical scavenging activity

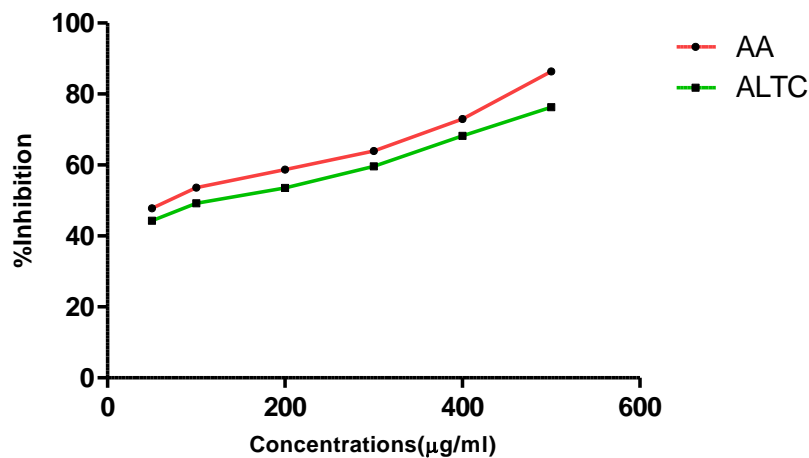


Figure 5: Effect of ALTC on DPPH radical scavenging activity

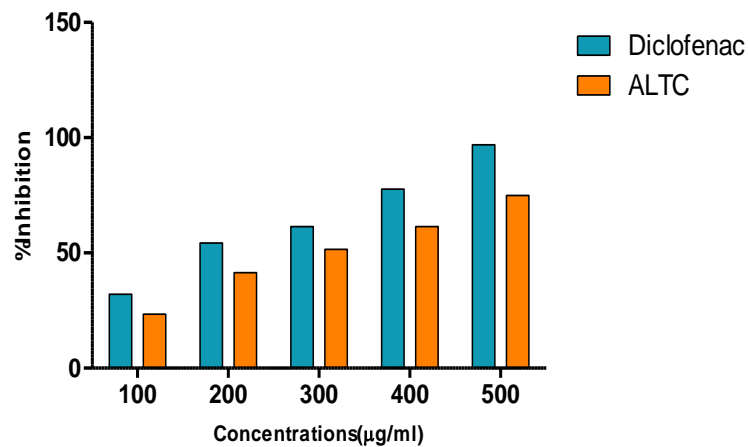


Figure 6: Effect of ALTC on HRBC membrane stabilization assay:

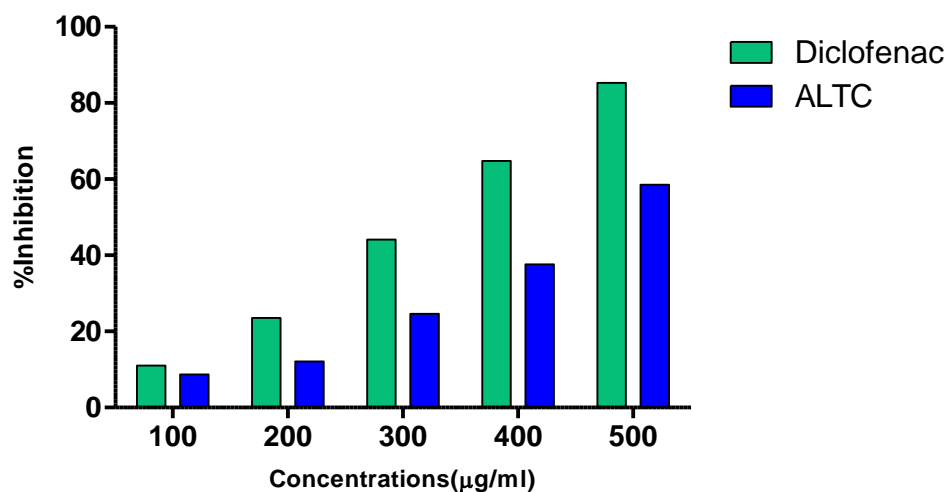


Figure 7: Effect of ALTC on albumin denaturation assay

The DPPH antioxidant assay is based on the ability antioxidant to decolorize DPPH, a stable free radical, When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively measured from the changes in absorbance. The ALTC showed a significant dose-dependent inhibition of DPPH radical scavenging activity (figure 5).

Acute and chronic inflammation characterized by release of lysosomal enzymes in many chronic disorders causes. The stabilization of lysosomal membrane is to control the release of these enzymes extracellularly is during inflammation¹⁴. The *in vitro* anti-inflammatory activity of ALTC and diclofenac was conducted by using HRBC membrane stabilization assay, because of HRBC or erythrocyte membrane has resembles to lysosomal membrane¹⁵. The ALTC showed significant membrane stabilization effect in a concentration dependent manner as showed with standard diclofenac (figure 6).

The denaturation of proteins is one of the causes of inflammation. The reason might be due to the production of auto antigens involved in denaturation of protein and membrane lysis action. The ALTC had better activity against albumin denaturation among all the selected plant extracts (fig 7).

CONCLUSION

The present study concluded that the ALTC showed antioxidant and anti-inflammatory activity. All the activities might be due to the presence of active constituents like anthocyanins, flavanoids and terpenoids in the pericarp of *Terminalia catappa*.

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