Screening of Tropical Fruits for Anti-Inflammation Activity in Vitro in South Kalimantan Indonesia

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***Abstract*—In recent years many studies revealed that fruits contain high antioxidant and anti-inflammatory activity. South Kalimantan Indonesia is a tropical country that has a variety of fruits that are consumed for food and health. The present study was aimed to evaluate the *in vitro* anti inflammation activity of *Annona muricata, Averrhoa carambola* and *Ananas comosus merr*. The anti-inflammatory activities was determined by inhibition protein denaturation method. Result of this study revealed there is inhibitory action on protein denaturation. *Averrhoa carambola* extract has shown better inhibition of BSA denaturation at any concentration compared to *Annona muricata and Ananas comosus merr* extract. Our investigation suggest that the *Annona muricata, Averrhoa carambola,* and *Ananas comosus merr* extracts possess significant anti-inflammatory activity. Among them *Averrhoa carambola* extract was found to be more potent than the *Anona muricata* and *Ananas comosus merr* extract.**

***Index Terms*—ananas comosus merr, annona muricata, anti-inflammation activity, averrhoa carambola**

I. Introduction

Inflammation is the complex biological response of vascular tissues to harmful stimuli including pathogens, irritants, or damaged cells. It is a protective mechanism by the organism to remove the injurious stimuli as well as initiate the healing process for the tissue. The process of inflammation is required in healing of wounds. However, if inflammation is unchecked, leads to onset of diseases like rhinnorhoea, rheumatoid arthritis and atherosclerosis. Acute inflammation is characterized by classical signs like edema, erythema, pain, heat, and primarily loss of function [1].

Early inflammatory changes in damaged tissues are known to release various biologically active materials from polymorph nuclear leukocytes, lysosomal enzymes and others. The vascular effects are mediated by prostaglandins, kinins and vasoactive amines (histamine) released by mast cells. Acute models are designed to test drugs that modulate erythema, measurement of local pain, antipyretic activity, local analgesic action and rat paw edema, changes in vascular permeability, leukocyte migration and chemotaxis, phagocytosis-polymorphonuclear leucocytes and other phagocytic cells [2].

All the steroidal and non-steroidal anti-inflammatory drugs (NSAIDs) although effective, cause undesirable and side effects. Thus, the development of a safer and efficacious alternative is needed. Natural products serve as an important source of therapeutically effective medicines [1].

In recent years many studies revealed that fruits contain high antioxidant and anti-inflammatory activity. [3]. Medicinal plants, which form the backbone of traditional medicine, have in the last few decades been the subject of very intense pharmacological studies. Plant derived drugs serve as a prototype to develop more effective and less toxic medicines [4]. The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, phenols, saponins, sterols etc [5]. There is a growing attention in correlating the phytochemicals of a medicinal plant with its pharmacological activity [6].

South Kalimantan Indonesia is a tropical country that has a variety of fruits that are consumed for food and health. For example *Sirsak* (*Annona muricata*). *Annona muricata* is a member of the family of custard apple tree called annonaceae and a species of the genus Annona known mostly for its edible fruits annona [7]. *Annona muricata* produces fruits that are usually called sour sop due to its slightly acidic taste when ripe. The creamy and delectable flesh of the fruit consist of 80% water, 1% protein, 18% carbohydrates and fair amount of vitamins B, B2 and C, potassium and dietary fiber [8], [9]. *A. muricata* ethnomedicinal use, especially for inflammation, rheumatism and neuralgy [9].

Besides *Sirsak*, there is *Belimbing* (*Averrhoa carambola*) and *Nenas* (*Ananas comosus merr*). *Averrhoa carambola L.* (Oxalidaceae) is an Asian tree. This tree is also known as the star fruit tree and is commonly used to treat headaches, vomiting, coughing and hangovers. Furthermore, it is used as an appetite stimulant, a diuretic, and as an antidiarrheal and febrifugal agent [10].

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Sripanidkulchai B. *et al* (2002) reported that *Averrhoa carambola* inhibited carrageenan-induced rat paw inflammation In another investigation, Cabrini DA. *et al* (2010) reported that upon topical application of the ethanolic extract of *Averrhoa carambola* leaf and its butanol, ethyl acetate and hexane fractions, croton oil-induced ear edema and cellular migration in mice were both reduced effectively [11].

Ananas comosus (L.) Merrill is belonging to the family Bromeliaceae and it is an important tropical and subtropical plant widely cultivated in the tropical areas of the world. Its fruit is consumed fresh or canned as a commercial product in many countries. Pineapple has also been known for a number of beneficial biological activities such as antioxidative, anti-browning, anti-inflammatory and anti-platelet activities. The enzyme complex of *A. comosus* called bromelain is known for its clinical applications particularly modulation of tumor growth, blood coagulation and ant inflammatory effect. Pineapple has been extensively used in foods or for health benefits[12].

The present study was aimed to evaluate the *in vitro* anti-inflammation activity of *Annona muricata*, *Averrhoa carambola* and *Ananas comosus merr*.

II. Material and Methods

A. Plant Materials and Preparation of Fruits Extracts

Fruit of *Annona muricata*, *Averrhoa carambola*, and *Ananas comosus merr* were obtained from Banjarbaru Traditional Market, South Kalimantan, Indonesia. The fruit with seed were separated. Fruits were washed with distilled water. The collected fruits were cut into small pieces and blended by juicer.

B. In Vitro Anti-Inflammation Activity

Test solution (0,5 ml) consist of 0,45 ml of Bovine Serum Albumin (BSA) (5% w/v aqueous solution) and 0,05 ml of test solution (250 μg/L).

Test control solution (0,5 mL) consists of 0,45 ml of BSA (5% w/v aqueous solution) and 0,05 ml of distilled water.

Product control solution (0,5 ml) consists of 0,45 ml of distilled water and 0,05 ml of test solution (250 μg/ml).

Standard solution (0,5 ml) consists of 045 ml of BSA (5% w/v aqueous solution) and 0,05 ml diclofenac sodium (250 μg/ml).

All the above solution were adjusted to pH 6,3 using 1 N hydrochloric acid. The samples were incubated at 37^{O}C for 20 min and the temperature was increased to keep the samples at 57^{O}C for 3 min. After cooling 2,5 ml of phosphate buffer saline was added to the above solutions. The absorbance was measured using UV visible spectrophotometer at 416 nm. The percentage inhibition of protein denaturation was calculated as, [13]

$$\% \text{ Inhibition} = \frac{100 - 0.D \text{ test solution} - 0.D \text{ of product solution}b}{0,D \text{ of test control}} \quad (1)$$

The control represents 100% protein denaturation. The results were compared with diclofenac sodium (250μg/ml).

C. Statistical Analysis

For the anti-inflammation activity, Inhibitory Concentration 50% (IC_{50}) was measured. The IC_{50} was calculated from the linear curve using microsoft excell 2007 and obtained by plotting the percentage of inhibition versus the concentrations.

III. Results

Anti-inflammation activity of three fruits extracts was measured by protein denaturation inhibition method. The results are showed in Fig. 1-Fig. 3 and Table I.

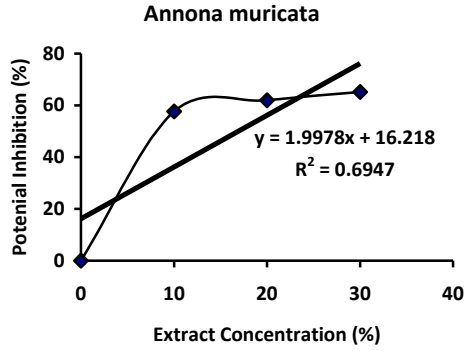

Figure 1. Anti-Inflammation Activity of *Annona muricata*

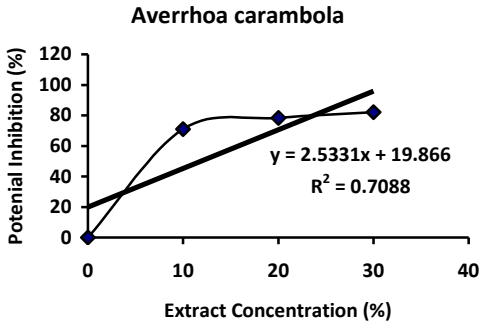

Figure 2. Anti-Inflammation Activity of *Averrhoa carambola*

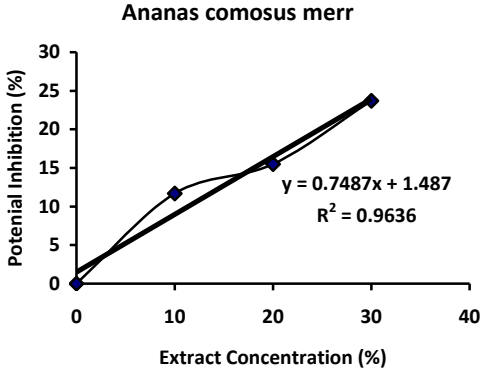

Figure 3. Anti-Inflammation Activity of *Ananas comosus merr*

The three fruits extracts inhibited the denaturation of Bovine Serum Albumin (BSA). The degree of inhibition of BSA denaturation increased with the increasing in the concentration of extracts. As shown in Fig. 1-Fig. 3 and Table I among the three extracts under the study *Averrhoa carambola* extract (from 71,07% to 82,00%) has shown better inhibition of BSA denaturation at any concentration compared to *Annona muricata and Ananas comosus merr* extract. The standard drug Diclofenac sodium showed 85,65% maximum inhibition of denaturation at 30 μg/ml concentration.

TABLE I. EFFECT OF SELECTED FRUITS ON PROTEIN DENATURATION

Sample	Concentration (%)	% Inhibition (%)	IC 50
Annona muricata	0 10 20 30	0,00 57,63 61,96 65,15	16,19
Averrhoa carambola	0 10 20 30	0,00 71,07 78,38 82,00	11,81
Ananas comosus merr	0 10 20 30	0,00 11,69 15,49 23,69	64,71
Natrium Diclofenac	0 10 20 30	0,000 70,16 77,22 85,65	11,87

Result of this study also revealed he concentration required for 50% inhibition (IC50). IC50 was found to be 16,19 for *Annona muricata*, 11,81 for *Acerrhoa carambola*, and 64,71 for *Ananas comosus merr* extracts (Table I). Meanwhile, IC50 for sodium diclofenac is 11,87. The results showed that, the closest IC50 values IC50 values of diclofenac sodium is *Averrhoa carambola* extracs.

IV. DISCUSSION

There are certain problems in using animals in experimental pharmacological research, such as ethical issues and the lack of rationale for their use when other suitable methods are available or could be investigated. Hence, in the present study the protein denaturation bioassay was selected for in vitro assessment of antiinflammatory property of three selected tropical fruits extract [14].

Denaturation of tissue proteins is one of the well-documented causes of inflammatory and arthritic diseases. Production of auto antigens in certain arthritic diseases may be due to denaturation of proteins in vivo[15]. Agents that can prevent protein denaturation therefore, would be worthwhile for anti-inflammatory drug development. The increments in absorbances of test samples with respect to control indicated stabilization of protein i.e. inhibition of protein (albumin) denaturation by extracts and reference drug diclofenac sodium [16]. From the IC50 values it becomes evident that one of the extracts (*averrhoa carambola*) was more active than diclofenac sodium, being effective in lower concentrations.

Inflammation is natural response of the mammalian body to a variety of hostile agents including parasites, pathogenic microorganism, toxic chemical substances and physical damage to tissue. The process associated with the inflammatory response are complex but important aspects which have been exploited for screening of anti inflammatory compound [17]. Although steroidal anti-inflammatory drugs and NSAIDs are currently used to treat acute inflammation, these drugs have not been entirely successful in curing chronic inflammatory disorders while such compounds are accompanied by unexpected side effects. Therefore, there is an urgent need to find safer anti-inflammatory compounds [18]. Traditional medicine has used extracts of different plants for the treatment of a wide variety of disorders including acute and chronic inflammation [19].

It is commonly accepted that in a situation of oxidative stress, reactive oxygen species (ROS) such as superoxide, hydroxyl and peroxyl radicals are generated. The ROS play an important role in the pathogenesis of various serious diseases, such as neurodegenerative disorders, cancer, cardiovascular diseases, atherosclerosis, cataracts, and inflammation. The mechanism of inflammation injury is attributed, in part, to release of reactive oxygen species from activated neutrophils and macrophages [20]. This over production leads to tissue injury by damaging macromolecules [20]. The main cellular components susceptible to damage by ROSare lipids (peroxidation of unsaturated fatty acids in cell membrane), proteins (denaturation), carbohydrates and nucleic acids [14].

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 ROS from activated 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 [14].

Fruits and vegetables contain significant levels of biologically active components that impart health benefits beyond basic nutrition. They are a major source of dietary antioxidants that increase the plasma antioxidant capacity. Several classes of antioxidant dietary compounds have been suggested to present health benefits, and there are evidences that consumption of these products leadsto a reduction of the expression of various inflammation reaction [21].

Annona muricata is one of the tropical fruits that demonstrate antioxidant properties [22]. *Annona muricata* of the family Annonaceae, commonly known as *Sirsak*, is a well known medicinal tree with anti-bacterial, antiviral, molluscicidal, anti-oxidative stress and diuretic properties [23].

Fruits of the genus Annona contain a considerable amount of polyphenolic compounds. These compounds are antioxidants and help to prevent diseases associated

with oxidative stress, such as cancer, atherosclerosis and neurodegener ative diseases. A mechanism for antioxidant activity of natural compounds is the inhibition and suppression of the formation of reactive species either by inhibiting enzymes or by chelation of the trace elements involved in the formation of free radicals. Antioxidants also participate as free radical scavengers by regulating or protecting the endogenous antioxidant defense [24, [25].

Averrhoa carambola is a small, attractive, multistemmed, slow growing evergreen tree with a short trunk or a shrub, 5-7m of height or rarely, 10m high, spreading 20-25 ft in diameter The fruits are green when small and unripe but turn to yellow or orange when matured and ripe [11]. Phytochemistry studies have shown that the fruit of *A. carambola* is rich in antioxidants, especially polyphenolic compounds, which act against reactive oxygen species [26]. Besides polyphenolic, the fruit of *A. Carambola* contain saponin, alkaloid, flavonoid, and tannin. Sripanidkulchai B. et al (2002) reported that *Averrhoa carambola* inhibited carrageenan-induced rat paw inflammation [11].

Pineapple (*Ananas comosus Merr*) is an important tropical and subtropical plant widely cultivated in the tropical areas of the world. Its fruit is consumed fresh or canned as a commercial product in many countries. Pineapple has also been known for a number of beneficial biological activities such as antioxidative, anti-browning, anti-inflammatory and anti-platelet activities [27]. Pineapple fruit is considered a highly nutritious fruit because it contains a high level of vitamin C, a natural antioxidant which may inhibit the development of major clinical conditions including heart disease and certain cancers. The fruit also contains phenolic compounds and β-carotene, which constitute natural sources of antioxidants [28].

As said above neutralization by antioxidants and radical scavengers can attenuate inflammation. Thus the presence of anti-inflammatory activity can be attributed to the phytochemical compounds in three selected fruit extracts which acts as an antioxidant or radical scavenger. Further definitive studies are necessary to ascertain the mechanism and costituents behind its anti-inflammatory actions.

V. Conclusion

Our investigation clearly demonstrates that the *Annona muricata*, *Averrhoa carambola*, and *Ananas comosus merr* extracts possess significant anti-inflammatory activity. Among them *Averrhoa carambola* extract was found to be more potent than the *Anona muricata* and *Ananas comosus merr* extract. Futher studies are recommended to isolate the active principle responsible for these activities.

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