In Vitro Anti-inflammatory Activity of Some Wild Fruits of Karnataka.

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***Abstract*—** The present study aimed to evaluate Anti-inflamatory activity of different fruits viz *Phyllanthus emblica, Limonia acidissima, Syzygium cumini, Artocarpus hirsutus, Carissa congesta, Anacardium occidentale*. Anti-inflammatory activity was evaluated using Albumin denaturation assay and Proteinase inhibitory activity at different concentrations. Diclofenac sodium was used as a reference drugs for the study of Anti- inflammatory activity. Among six fruits, *Phyllanthus emblica* showed highest inhibition in both Albumin denaturation assay and Proteinase inhibitory activity with IC_{50} values 85.93µg/ml and 97.24µg/ml respectively. Fruit extract of *Artocarpus hirsutus* was showed lowest inhibition with IC_{50} values 284.48µg/ml and 297.60µg/ml for Albumin denaturation and Proteinase inhibitory activity respectively. In Albumin denaturation assay, Diclofenac sodium showed highest inhibition at 200µg/ml (92.64±2.30%) and percentage inhibition at 200µg/ml (90.08±2.56%) for Proteinase inhibition activity.

***Keywords*—** Albumin denaturation, Anti-inflammatory activity, Diclofenac sodium, Proteinase inhibition.

I. Introduction

INFLAMMATION is a process by which the body's white blood cells and substances they produce protect us from infection with foreign organisms, such as bacteria and viruses. When cells in the body are damaged by microbes, physical agents or chemical agents, the injury is in the form of stress. Symptoms of inflammation include redness, swelling, and pain, joint stiffness, loss of joint function as a result of infection, irritation, or injury. Inflammation can be external or internal.

Plants may become the base for the development of a new medicine or they may be used as phytomedicine for the treatment of disease [1]. In this growing interest, many of the phytochemical bioactive compounds from medicinal plants have shown many pharmacological activities [2], [3]. Most of the Anti-inflammatory drugs available in the market, having a wide range of problems such as efficacy and undesired effects including gastrointestinal tract disorders and other unwanted effects, gastrointestinal disturbances, renal damages, respiratory depression [4], [5]. This situation highlights the need for advent of safe, novel and effective analgesic and anti-inflammatory compounds [6], [7].

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Phyllanthus emblica has been used to reduce pain [8] and fever treatments by rural populations in its growing areas [9]. *Limonia acidissima*, considered to be a hepatoprotectant, possess different biological activities namely adaptogenic activity against blood impurities, leucorrhoea, dyspepsia and jaundice. Traditionally, all parts of the plants are given as natural medicine as a cure for various ailments [10]. Although *Phyllanthus emblica, Limonia acidissima, Syzygium cumini, Artocarpus hirsutus, Carissa congesta, Anacardium occidentale* were widely used in ethnomedicine for the treatment of inflammatory and related disorders, their anti-inflammatory properties have not yet been pharmacologically evaluated. Hence, the present study was undertaken to evaluate anti-inflammatory activity of methanol fruit extracts by in-vitro methods.

II. Materials And Methods

A. Plant Materials

Samples of fresh ripe fruits were collected from the local forest of Udupi, and Kanakapura town of Ramanagar district, Karnataka. The fruits comprised of *Phyllanthus emblica, Limonia acidissima, Syzygium cumini, Artocarpus hirsutus, Carissa congesta, Anacardium occidentale.* The fruit samples were authenticated by the taxonomist, Dept. of Botany, Poornaprajna College, Udupi, Karnataka.

B. Extraction Procedure

Each Sample of fresh fruit was washed under running tap water followed by washing with distilled water to remove the surface debris. 100g of edible portions of the fruit were weighed and minced using a kitchen blender. After homogenization, it was extracted in methanol for 72 hours in dark at 37^{o}C incubator shaker. After 3 days, the whole extracts are filtered and then centrifuged to obtain clear extract. The filtrate was concentrated under Rotary vacuum evaporator. The resultant extract was lyophilized to obtain dry powder. The yield of crude extracts were noted and later preserved in a deep freezer (-20° C) for further use.

C. *Evaluation of In-vitro Anti-inflammatory Activity*

1. Inhibition of Albumin Denaturation

Inhibition of albumin denaturation was determined by the method described by Mizushima et al [11] with slight modifications. The reaction mixture (2ml) was consisting of test extract at different concentrations and aqueous solution of bovine serum albumin fraction. pH of the reaction mixture

(6.3) was adjusted using 1N HCl. The samples were incubated at 37°C for 20 min and then heated at 57°C for 30 min. After cooling the samples, 1ml of Phosphate buffer saline (P^H 6.3) was added to each sample tubes. The turbidity was measured spectrophotometrically at 660 nm against blank. The experiment was performed in triplicate. Percent inhibition of protein denaturation was calculated as follows:

Percentage inhibition (%) = (O.D control – O.D sample) X 100/ O.D control

2. Proteinase Inhibitory Activity

Proteinase inhibitory activity was determined by the method described by Oyedepo et al [12] with slight modifications. The reaction mixture (2ml) was consisting of test extract at different concentrations, 80µg trypsin and 20 mM Tris HCl buffer (pH 7.4). The mixture was incubated at 37°C for 5 min and then 0.5 ml of 1% casein was added. The mixture was incubated for an additional 15 min. 1 ml of 70% perchloric acid was added to terminate the reaction. The reaction mixture was centrifuged and the absorbance of the supernatant was read at 210 nm against buffer as blank. The experiment was performed in triplicate. The percentage inhibition of proteinase inhibitory activity was calculated.

Percentage inhibition (%) = (O.D control – O.D sample) X 100/ O.D control

D. *Statistical Analysis*

The results are expressed as the mean±SD for three replicates. Linear regression analysis was used to calculate IC_{50} value.

III. Results And Discussion

The results of albumin denaturation of the six fruit extracts were displayed in Fig.1. Albumin denaturations of six fruit extracts were analyzed. All fruit extracts exhibited albumin denaturation with percentage inhibition values between 79% to 28% at concentration of 200µg/ml. Inhibition of albumin denaturation at 200µg/ml was found to be highest in *Phyllanthus emblica followed by Limonia acidissima, Syzygium cumini, Anacardium occidentale, Carissa congesta, Artocarpus hirsutus* and the values were 79.01±3.07%, 65.75±2.53%, 51.57±2.83%, 33.71±1.10%, 28.73±2.53% and 28.00±1.94% respectively. Percentage inhibition value for the standard diclofenac sodium was found to be 92.64±2.30%. Denaturation of bovine serum albumin is a well documented cause of inflammation. Anti-inflammatory drugs like salicylic acid, flufenamic acid, Phenylbutazone etc, have shown dose dependent ability to thermally induced protein denaturation [13]. It is previously reported that many flavonoids and related polyphenols contribute significantly to the antioxidant and anti-inflammatory activity of many plants [14], [15].

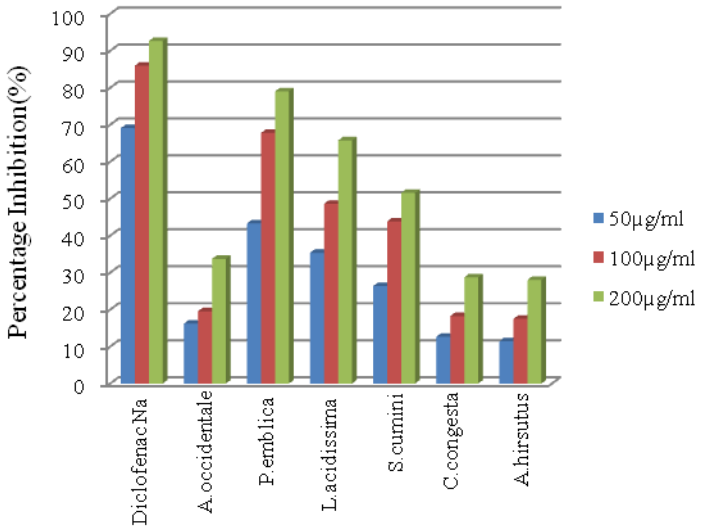

Fig. 1 Inhibition of Albumin denaturation of six fruit extracts

In Inhibition of albumin denaturation, the IC_{50} values for six fruit extracts were found to be highest in Phyllanthus *emblica followed by Limonia acidissima, Syzygium cumini, Anacardium occidentale, Carissa congesta, Artocarpus hirsutus* and values were 85.93µg/ml, 108.56 µg/ml, 133.92 µg/ml, 233.89 µg/ml, 269.07 µg/ml and 284.48 µg/ml respectively. IC_{50} value for the standard diclofenac sodium was found to be 67.44ug/ml. The results were displayed in (Table.1).

The Proteinase inhibitory activity at 200µg/ml was found to be highest in *Phyllanthus emblica* followed by *Limonia acidissima, Syzygium cumini, Artocarpus hirsutus, Carissa congesta, Anacardium occidentale* and the values were 70.49±1.59%, 61.11±1.82%, 48.09±1.59%, 27.08±1.56%, 25.35±0.79% and 23.96±1.56% respectively. The results are displayed in the Fig.2. Percentage inhibition value for the standard diclofenac sodium was found to be 90.08±2.56%.

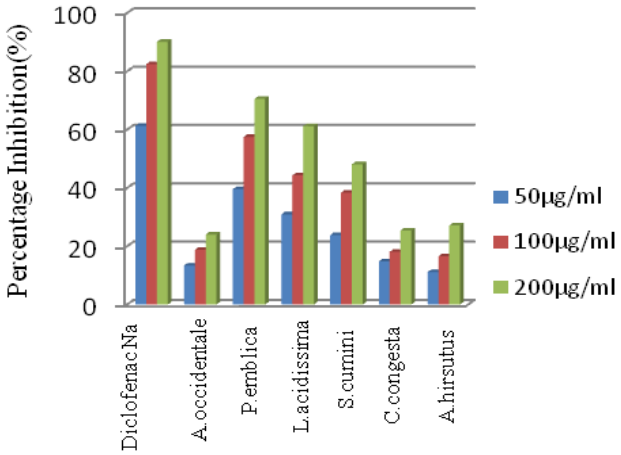

Fig. 2 Proteinase inhibitory activity of six fruit extracts

In Proteinase inhibitory activity, the IC_{50} values were found to be highest in Phyllanthus *emblica followed by Limonia*

acidissima, Syzygium cumini, Carissa congesta, Anacardium occidentale, Artocarpus hirsutus and values were 97.24 µg/ml, 119.01 µg/ml, 146.65µg/ml, 278.24 µg/ml, 289.60 µg/ml, and 297.60 µg/ml respectively. IC_{50} value for the standard diclofenac sodium was found to be 70.95 µg/ml. The results were displayed in (Table.1). It is previously proved that proteinases of leukocytes play an important role in the development of tissue damage during inflammatory reactions and significant level of protection was provided by proteinase inhibitors [16].

TABLE 1
IC_{50} VALUES OF SIX METHANOL FRUIT EXTRACTS

Fruit extrats	Albumin Denaturation (IC_{50} µg/ml)	Proteinase Inhibition (IC_{50} µg/ml)
P.emblica	85.93	97.24
L.acidissima	108.56	119.01
S.cumini	133.91	146.65
A.occidentale	233.89	289.60
C.congesta	269.07	278.24
A.hirsutus	284.48	297.60
Diclofenac sodium	67.44	70.95

Linear regression analysis was used to calculate IC_{50} value.

IV. CONCLUSION

In Inhibition of albumin denaturation activity, the IC_{50} values were found to be highest in *Phyllanthus emblica* followed by *Limonia acidissima, Syzygium cumini, Anacardium occidentale, Carissa congesta* and *Artocarpus hirsutus*. The same trend was seen in proteinase inhibitory activity of three methanolic fruit extracts except, *Anacardium occidentale, and Carissa congesta.* The results obtained in the present investigation indicate that *Phyllanthus emblica, Limonia acidissima and Syzygium cumini* fruits were a potential source Anti- inflammatory agents compared to other fruits of the study.

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