

## IN VITRO ANTI-INFLAMMATORY AND ANTI-ARTHRITIC ACTIVITY IN METHANOLIC PEEL EXTRACTS OF *Persea americana*

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Article Received on 16/04/2017

Article Revised on 05/05/2017

Article Accepted on 26/05/2017

### ABSTRACT

Herbs are staging a comeback and herbal “renaissance” is happening all over the globe. The herbal products today symbolise safety in contrast to the synthetics that are regarded as unsafe to human and environment. The present study deals with the *in vitro* anti-inflammatory and anti-arthritis activity in methanolic peel extracts of *Persea americana*. The previous phytochemical analysis of methanolic peel extract of *Persea americana* has indicated the presence of several physiologically active phytochemicals such as phenols, flavonoids, triterpenoids, steroids, alkaloids etc. Since these compounds are of pharmacological interest, coupled with the use of this plant in traditional medicine, prompted us to check peel extract of *Persea americana* for *in vitro* anti-inflammatory activity by Human Red Blood Cell (HRBC) membrane stabilization method and anti-arthritis activity by the inhibition of protein denaturation method. The peel methanolic extracts of *Persea americana* exhibited notable anti-inflammatory activity and remarkable anti-arthritis action. The membrane stabilization was found to be 95.719% (HRBC), 96.719 % (EYMS) at a dose of 1000µg/ml, and that of protein denaturation was also found to be at a 97.47% at a dose of 250µg/ml) which was comparable to the standard drug (Diclofenac sodium). Therefore, our studies support the isolation and the use of active constituents from methanolic peel extract of *Persea americana* in treating inflammations and Associated Rheumatism.

**KEYWORDS:** anti-inflammatory activity, diclofenac sodium, anti arthritic activity, *Persea americana*.

### INTRODUCTION

Plants have played a significant role in maintaining human health and improving quality of life for thousands of years. In particular, herbs have been used as food and for medicinal purposes for centuries. In herbal medicine, the term refers not only to seed-producing plants but also bark, roots, leaves, seeds, flowers, and the fruit of trees. According to the World Health Organization, about three-quarters of the world’s population relies on traditional medicine for primary healthcare needs and most of this treatment involves use of plant extracts or their active components (Egan, 2002). Arthritic conditions are treated using traditional medicine with considerable success. Although various modern drugs are used to treat these type of disorders their prolonged usage may cause severe side effects .So there is an urge to develop new therapeutic agents with minimum side effects. *Persea americana* commonly known as Avocado is a plant species belonging to Lauraceae family .The cultivators of West Indian race are localized folks in Maharashtra, Tamil nadu and Karnataka They are well known among the people and widely used for their nutritive and medicinal properties. Even though the

research has been extensively done and documented on the whole fruit, seed, leaf, pulp of *Persea americana*, so far no scientific evaluation has been made on “PEEL” which is usually discarded as waste. Our previous work on preliminary phytochemical screening indicated the presence of Alkaloids, carbohydrates, Proteins, flavonoids, terpenoids, glycosides, tannin, & Steroids etc., (Smitha et al., 2015) *In vitro* antioxidant activity has been performed, quantification of phytoconstituents has been carried out (Smitha et al., 2016), Since, so far no scientific evaluation has been made to carry out *in vitro* anti-inflammatory and anti-arthritis activity The current study is focused to determine the *in vitro* activities in selected medicinal plant.

### MATERIAL AND METHODS

The fresh *persea Americana* fruits were obtained from the local markets of wayanad district, Kerala, which is a West Indian race.

#### Chemicals

Folin-ciocalteau reagent, gallic acid, Bovine Serum Albumin (BSA), were obtained from Sigma chemical

co., Diclofenac sodium drug (nicip) obtained from cipla company. All the solvents/chemicals used were analytical grade and were obtained by Hi media co., visible spectra measurements were done using uv spectrophotometry by shimadzu, the quick evaporation of the Methanolic extracts were done by Rotatory evaporator by Butchi, the documentation of several analytical gels were provided by gel documentation system by Biorad.

#### Preparation of peel powder

23.25 grams of peel were scraped of fresh *persea americana* fruit and were dried in hot air oven for 2 days making sure the temperature not exceeding 40°C, and then the samples were grounded to obtain powder.

#### Preparation of extract

The sequential Extraction of *persea Americana* was done at room temperature starting with non polar solvents to polar solvents (hexane, chloroform-ethyl acetate, methanol, water) respectively. About 2g of dried sample was soaked in respective solvents overnight on magnetic stirrer. Subsequently using the solvent methanol, the solvent extraction is done by soxlet apparatus, the final extract were concentrated on vacuum and stored at 4°C for further assay.

#### In vitro anti inflammatory assay by HRBC stabilization method

The principle involved here is stabilization of human red blood cell membrane by hypo tonicity induced membrane lysis. The assay mixture contains 1ml phosphate buffer [pH 7.4, 0.15 M], 2 ml hypo saline [0.36 %], 0.5 ml HRBC suspension [10 % v/v] with 0.5 ml of plant extracts of various concentrations (125, 250, 500, 1000mcg/ml), standard drug diclofenac sodium (125, 250, 500, 1000mcg/ml) and control [distilled water instead of hypo saline to produce 100 % hemolysis] were incubated at 37°C for 30 min and centrifuged respectively. The hemoglobin content in the suspension was estimated using spectrophotometer at 560 nm.

Percentage of HRBC membrane stabilization or protection was calculated using the formula,  
 Percentage stabilization =  $100 - \frac{(\text{optical density of drug})}{(\text{optical density of control})}$

The percentage hemolysis produced in the presence of distilled water was taken as 100 %

#### Anti inflammatory assay by membrane stabilization method (Egg albumin)

The in vitro anti-inflammatory effect of methanol extract of *persea americana* was evaluated against the inhibition of the denaturation of protein egg albumin The reaction mixture (5ml) consisted of 0.2 ml of egg (from fresh hen's egg), 2.8 ml of phosphate buffer saline (PBS, pH - 6.4) and 2ml of varying concentrations of the test extract (methanol extract of *persea Americana* (125, 250, 500, 1000mcg/ml). Similar volume of double distilled water served as control. Then the mixtures were incubated at

37±2°C in a BOD incubator for 15 mins and then heated at 70°C for 5 mins. After cooling, their absorbance was measured at 660nm using UV-visible spectrophotometry by using PBS as blank. Diclofenac sodium of different concentrations of (125, 250, 500, 1000mcg/ml) was used as reference drug and treated similarly for determination of absorbance.

The percentage inhibition of protein denaturation was calculated by using the following formula.

$$\% \text{ of inhibition} = 100 \times \left[ \frac{V_t}{V_c} - 1 \right]$$

Where,  $V_t$  = absorbance of test sample,  $V_c$  = absorbance of control.

The extract or drug concentration for 50% inhibition (IC50) was determined from the dose response curve by plotting percentage inhibition with respect to control against treatment concentration.

#### In vitro anti arthritic activity by protein denaturation method

The arthritis is an auto immune disease, being chronic it is linked with the inflammatory responses, in few cases of arthritis diseases, the production of auto antigen, As a result auto antigen will acts upon own defense system, paving a way to Rheumatic arthritis, in the present studies, there is a scope for attempt and capability of methanolic extracts of *persea americana* in controlling the production of auto antigens by inhibiting the protein denaturation. Diclofenac Sodium is taken as a standard drug, against the denaturation of Bovine Serum Albumin (BSA). The procedure to analyze the percentage of protein denaturation inhibition using different concentrations of the sample/ml(125, 250, 500, 1000mcg/ml) and by taking different concentrations of diclofenac sodium(125, 250, 500, 1000) as standard, is done accordingly below:

1. The test solution of 0.5ml consists of 0.45ml Bovine Serum Albumin (5% w/v methanolic solution) and 0.05 ml of test solution(250mcg/ml)
2. The Test control solution of 0.5 ml consists of 0.45ml of Bovine Serum Albumin(5% w/v Methanolic solution) and 0.05 ml distilled water(250mcg/ml)
3. The Product control solution of 0.5ml consists of 0.45ml of distilled water and 0.05ml of test solution(250mcg/ml)
4. The standard solution of 0.5ml consists of 0.45ml of Bovine Serum Albumin(5% w/v ,methanolic solution) and 0.05ml of Diclofenac sodium (250mcg/ml)

All the above solutions were adjusted to pH 6.3 using 1N HCL. The samples were incubated at 37°C for 20 minutes and the temperature was increased to keep the samples at 57°C for 3 minutes. After cooling, add 2.5ml of phosphate buffer to the above solutions. The absorbance was measured at 416nm using uv-visible spectrophotometer

The percentage inhibition of protein can be calculated as. Percentage inhibition=  $[100 - (\text{optical density of test solution} - \text{optical density of product control}) \div (\text{optical density of test control}) \times 100]$

The control represents the 100% protein denaturation. The results were compared with Diclofenac sodium (250mcg/ml)

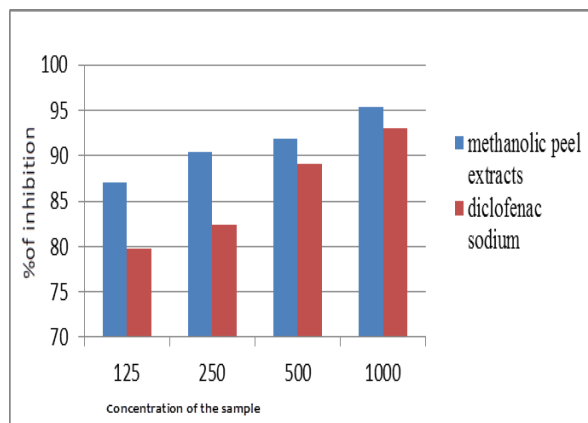
## RESULTS AND DISCUSSIONS

Inflammation is a common phenomenon and it is a reaction of living issue towards injury. Previous work on methanolic peel of *Persea americana* extract especially on their Phytochemical analysis, Antioxidant Properties indicated the presence of steroid, flavonoids and terpenoids as well has provided promising results on their antioxidant property. Since these compounds are of pharmacological interest, coupled with the use of this plant in traditional medicine, prompted us to check Methanolic peel extracts of *Persea americana* in this current research work for possible anti-inflammatory activity by HRBC (Human Red Blood Cell) membrane stabilization method, anti-arthritic activity by the inhibition of protein denaturation method. HRBC is similar to lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membrane.

### In Vitro anti inflammatory activity by stabilization of HRBC membrane

The mechanism of inflammation injury is attributed, in part, to release of Reactive Oxygen species from activated neutrophils and macrophages. This over production leads to tissue injury by damaging the macromolecule and lipid per oxidation of membranes. In addition, ROS propagate inflammation by stimulating the release of the cytokines such as interleukin- I, tumor necrosis factor- $\alpha$ , and interferon- $\gamma$ , which stimulate recruitment of additional neutrophils 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. The investigation is based on the quest for newer anti inflammatory drugs from the natural sources with potent activity and lesser side effects as substitutes for chemical drugs. The results were compared with diclofenac sodium. The percentages of inhibition are tabulated below.

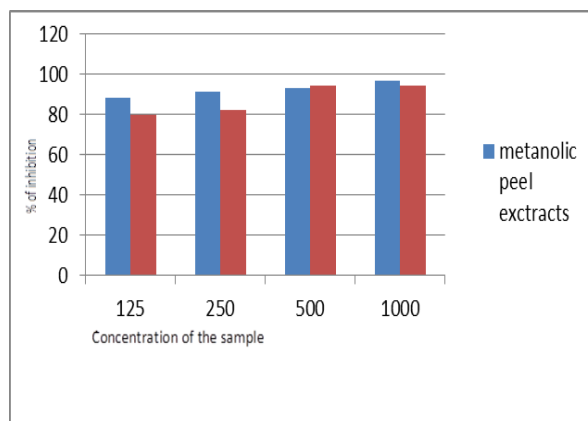
Concentration (mcg/1ml)	Methanolic Peel extracts of <i>Persea americana</i>	Diclofenac sodium
125	87.08	79.75
250	90.37	82.39
500	91.81	89.10
1000	95.719	93.10



### Anti inflammatory investigation by membrane stabilization method (Egg Yolk).

The purpose of this study is to find a potential, yet void of side effects as that of chemical drugs. The principle of this assay is similar to the previous one except the use of egg yolk membrane in place of HRBC. The results are tabulated in the table below.

Concentration (mcg/1ml)	Methanolic Peel extracts of <i>Persea americana</i>	Diclofenac sodium
125	88.08	79.85
250	91.37	81.99
500	93.81	89.80
1000	96.719	94.10



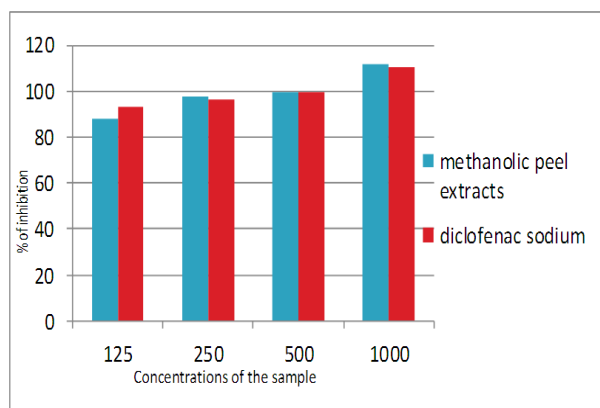
The percentage protection of methanolic extracts was 95.719% (HRBC), 96.719 % (EYMS) at 1000 $\mu$ g/ml. It possesses significant activity comparable with that of the standard Diclofenac sodium. *P.americana* methanolic peel extract has significant anti-inflammatory activity which may be due to presence of chemical profile such Terpenoids, Flavanoids and Phenols.

### In Vitro Anti arthritic activity by Protein denaturation method.

The methanolic extracts of *Persea Americana* show a very good anti-arthritic activity. The production of auto antigen in certain arthritic diseases may be due to the denaturation of protein. From the results of present study

it can be interpreted that methanolic extracts of *Persea americana* have the capability to control the production of auto antigens by inhibiting the protein denaturation, the results were tabulated below.

Concentration (mcg/1ml)	Methanolic Peel extracts of <i>Persea americana</i>	Diclofenac sodium
125	88.08	92.85
250	97.37	95.99
500	99.81	99.80
1000	111.719	110.10



The methanolic extract fabricates significant activity at 97.47% at 250µg/ml by inhibition of protein denaturation and its effect was compared with the standard drug Diclofenac sodium. The of auto antigen in certain arthritic disease may be due to denaturation of protein.<sup>[17-19]</sup> From the results of present study it can be stated that methanolic extract is capable of controlling the production of auto antigen and inhibits denaturation of protein in rheumatic disease.

## CONCLUSION

The Indian traditional systems of medicine and foods containing nutraceuticals and therapeutic principles are widely being appreciated for human well being across the globe. The Peel methanolic extracts of *Persea americana*, is a potential source of polyphenols, minerals, vitamin C and other nutraceuticals.

This is the first *in vitro* study on anti-inflammatory and anti-arthritic activities methanolic peel extracts of *Persea americana*. Scientific validation carried out on anti-inflammatory, anti-arthritic Activities shows comprehensive details on potentiality of the Methanolic Peel extracts of *Persea americana* as it contains many secondary metabolites e.g. flavonoids, steroids, alkaloids, tri-terpenoids and phenolics. These finding motivates and provokes us with an optimistic approach to perform more investigations to identify and characterize this novel compound and as a base for the development of novel potent drugs against inflammations and arthritis.

## ACKNOWLEDGEMENT

The author's thank Director, Pooja Bhagavat Memorial Mahajana Education Centre Post Graduate wing of SBRR Mahajana First Grade College, Mysore and Mahajana Education Society, Mysore for their constant encouragement and support.

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