



**IN VITRO ALPHA AMYLASE INHIBITORY EFFECT AND
ANTIOXIDANT ACTIVITY BY PEEL AND SEED EXTRACTS OF
Persea americana.**

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ABSTRACT

Over the last century human life style and food habits have drastically changed which has lead to various chronic diseases. Diabetes milletus is one such disease which is causing serious problems to human health. It is a metabolic disorder characterized by hyperglycemia, due to defect in insulin secretion, insulin action or both The inhibition of carbohydrates hydrolysing enzymes such as alpha amylase can be

important strategy in the postprandial blood glucose level in patients with type II diabetes & Medicinal plants play an important role in management of diabetes milletus. The avocado (*Persea americana*) belongs to the family Lauraceae is used as herbal medicine from ancient time, the methanolic Peel & Seed extracts of *Persea americana* were tested for Total phenolic content, DPPH assay & alpha amylase inhibition. Appreciable alpha amylase inhibition was seen in both the extracts with an IC₅₀ values of 36.02±0.23µg/ml & 59.34±1.01 µg/ml respectively when compared with acarbose 82.42±0.33µg/ml, the extracts possessed phenolic content of 0.533mg GAE/mg of sample for seed and 0.600mg GAE/mg of sample for peel, The free radical scavenging activity of methanolic seed and peel extract of *Persea americana* was found to increase with increase in concentration as 96.87% and 100% at 200µl concentration. This study supports that Methanolic Seed & Peel Extracts of *Persea americana* exhibits estimable antioxidant activity & has the potency to inhibit alpha amylase and thus suggesting the successful use of plant chemicals as “Drug Targets” in management of Diabetes Milletus.

KEYWORDS: *Persea americana*, free radical scavenging, alpha amylase inhibition, diabetes milletus.

INTRODUCTION

Medicinal plants would be the best source to obtain a variety of newer herbal drugs. For centuries plants have provided mankind with useful, sometimes life saving drugs. Modern pharmaceutical in cases where correlation between chemical structure and biological activities were noted, empirical science began to give way to rational drug design. This emerging approach to identify and develop potential new drug is largely successful, due to the intellectual cooperation of chemistry (medicinal). Therefore such plants should be investigated to better understand their properties, safety and efficacy. The use of drugs derived from plants has been in practice for a very long time. Using plants for medicinal purpose is an important part of the culture.

Diabetes mellitus a metabolic chaos of numerous etiologies is characterized by chronic hyperglycemia with strive of carbohydrates, fat and protein metabolism their consequences from imperfection in insulin secretions, insulin action or both. Globally type 2 diabetes afflicts 90% of all diabetes. One of the most beneficial therapies for type 2 diabetes is said to be control of post prandial hyperglycemia after a meal, and the best approach is to retard the absorption of glucose to inhibition carbohydrate hydrolyzing enzyme in the digestive organ.

Alpha amylase is one of the enzyme that catalyzes the breakdown of starch to maltose and finally to glucose which is the only sugar that can be utilized by the whole. The inhibition of these enzyme leads to a decrease in blood glucose level, since monosaccharides are a form of carbohydrates which are absorbed through the small intestine. There are several reports established for screening and developed in enzyme inhibitors and its effect on blood glucose level after food uptake. Modern medicines such Biguanides, sulfonylurease & Thizoliginediones are available for the treatment of diabetes. However they also have undesirable effect associated with their uses. Alternative medicines predominantly Herbal drugs are available for treatment of diabetes, advantage of herbal drug are effectiveness, safety and acceptability. Based on the existing studies the polyphenols and flavanoids are among the natural active anti diabetic agents. These compounds exhibit various biological efforts including carbohydrate hydrolyzing enzyme inhibition and antioxidant activity. Polyphenolic compounds are able to inhibit the activities of digestive system due to their

ability to bind proteins, thus contribute to lower post prandial hyperglycemia in management of diabetes.

Persea americana of west Indian race have been advocated in traditional medicine for lower glycemic effect, commonly known as avocado of west Indian race. The cultivars of west Indian race are localized pockets in Maharashtra, Tamil nadu and Karnataka. They are well known among the people and widely used for nutritive and medicinal properties. *Persea americana* are a rich source of polyphenolic compounds and phenolic acids. The aim of the present study is to evaluate antioxidant properties & alpha amylase inhibitory potential of seed and peel extracts of *Persea americana*.

MATERIALS AND METHODS

Material

Folin-ciocalteau reagent, α,α -diphenyl- β -picrlhadrazyl(DPPH), Porcine Pancreatic amylase, Gallic acid were obtained from sigma chemical co.,. All solvents/chemicals used were of analytical grade and obtained from himedia. Visible spectra measurements were done using UV spectrophotometer by shimadzu.

Plant Material

The fresh *Persea americana* fruits were obtained from the local markets of wayanad district, Kerala, India which is a variety of west Indian race, washed properly peel and seed samples are sliced shade dried. The dried samples are then powdered and kept in moisture free container and used for further analysis.

Preparation of extract

Seven solvents are selected for extraction purpose based on their polarity. They are *hexane, ethyl acetate, water, methanol, acetone, chloroform & ethanol* .35.27% & 35 % extractable compound of Peel and seed samples of *Persea americana* was extracted in Soxhlet apparatus in 250 ml of each solvent separately. The extractions performed for 48 hrs and were concentrated by slow evaporation process. The obtained extracts kept in moisture free container and used for total phenolic content, DPPH assay & alpha amylase inhibitory activities.

Determination of total phenolic content

Total polyphenols are determined by folin-ciocalteau(FC) procedure. This assay is based on the principle that the polyphenols react with FC reagent that gives a blue colour in alkaline

medium, which is measured at absorbance of 760nm and the concentration of polyphenols samples were calculated using the standard curve prepared with gallic acid. The total phenolic content was determined by the spectrophotometric method. In brief, a 1ml of sample (1mg/ml) was mixed with 1ml of folin-ciocalteu's phenol reagent. After 5min. 10ml of a 7% sodium carbonate solution was added to the mixture followed by the addition of 13ml of deionized distilled water and mixed thoroughly the mixture was kept in the dark for 90 min at 23°C, after which the absorbance was read at 750nm. The TPC was determined from extrapolation of calibration curve which was made by preparing gallic acid solution. The estimation of the phenolic compounds was carried out in triplicate. The TPC was expressed as milligrams of gallic acid equivalents (GAE) per g of dried sample.

Free Radical Scavenging Activity: DPPH Assay

DPPH is a stable free radical which has maximum optical absorbance at 517nm. The reaction of DPPH with free radical scavenger causes a decline in the absorbance value. Free radical scavenging potential of extracts was tested against a methanolic solution of α , α -diphenyl- β -picrylhydrazyl (DPPH). Antioxidants react with DPPH and convert it to α , α -diphenyl- β -picrylhydrazine. The degree of discoloration indicates the scavenging potential of the antioxidant extracts. The change in absorbance at 517nm has been used as a measure of free radical scavenging activity. The antioxidant activity of the plant extracts and the standard was assessed on the basis of the radical scavenging effect of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH)-free radical activity by modified method. The diluted working solutions of the test extracts were prepared in methanol. Ascorbic acid was used as standard in 1-100 μ g/ml solution. 0.002% of DPPH was prepared in methanol and 1 ml of this solution was mixed with 1 ml of sample solution and standard solution separately. These solution mixtures were kept in dark for 30 min and optical density was measured at 517 nm using Cecil-Elect Spectrophotometer. Methanol (1 ml) with DPPH solution (0.002%, 1 ml) was used as blank.

The optical density was recorded and % inhibition was calculated using the formula given below:

$$\text{Percent (\%)} \text{ inhibition of DPPH activity} = \frac{A-B}{A} \times 100$$

Where A = optical density of the blank and B = optical density of the sample.

α - AMYLASE INHIBITORY ACTIVITY

α -Amylase is an enzyme EC 3.2.1.1 that hydrolyses alpha bonds of large, alpha-linked polysaccharides, such as starch and glycogen, yielding glucose and maltose. Pancreatic α -amylase inhibitors offer an effective strategy to lower the levels of post-prandial hyperglycemia via control of starch breakdown. α –amylase inhibitory activity of each extract was analyzed by the method of Bernfeld with a little modification as described below. In brief, 100 μ l of the test extract was allowed to react with 200 μ l of the porcine pancreatic α –amylase enzyme (sigma-aldrich-3176) and 100 μ l of 2 mM of phosphate buffer (ph 6.9). After 20 min incubation, 100 μ l of 1% starch solution was added. The same was performed for the control where 200 μ l of the enzyme was replaced by buffer. Enzyme working standard was prepared by dissolving 1mg porcine pancreatic amylase in 10ml of phosphate buffer (ph 6.9). After incubation for 5 min, 500 μ l of dinitrosalicylic acid reagent was added to both control and tests. They were kept in boiling water bath for 5 min. the absorbance was recorded at 540nm using a spectrophotometer and the percentage inhibition of α –amylase enzyme was calculated using this formula:

$$\text{Inhibition (\%)} = 100(\text{control-test}/\text{control})$$

Suitable reagent blank and inhibitor controls were simultaneously carried out and subtracted. Dose dependent variation in the α –amylase inhibitory activity was measured using 25 to 125 μ l of extract the triplicates were carried out, the concentration of acarbose and peel & seed extract required to inhibit 50% of alpha amylase activity was defined as IC 50 Value, was determined and calculated.

Statistical analysis

All assays were performed at least in triplicate and the results were expressed as mean \pm standard deviation (SD). Differences were evaluated by one-way analysis of variance (ANOVA) test, differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Through the Previous work peel & seed *Persea Americana* sample were extracted sequentially with *hexane, ethyl acetate, water, methanol, acetone, chloroform & ethanol* .All the extracts were subjected to qualitative phytochemical screening which provides the essential information regarding the chemical constituents, as methanolic extracts showed the presence of phytochemical constituents, thus used for estimation for total phenolic contents

& DPPH assay further evaluated for alpha amylase inhibitory. Inhibition of α - amylase activity and the objectives are carried out in invitro system.

Determination of total phenolic content

The total phenolic content was estimated using FC reagent. The results are expressed in the form of GAE/mg of sample, and it was found to be 0.533mg GAE/mg of sample for seed and 0.600mg GAE/mg of sample for peel.

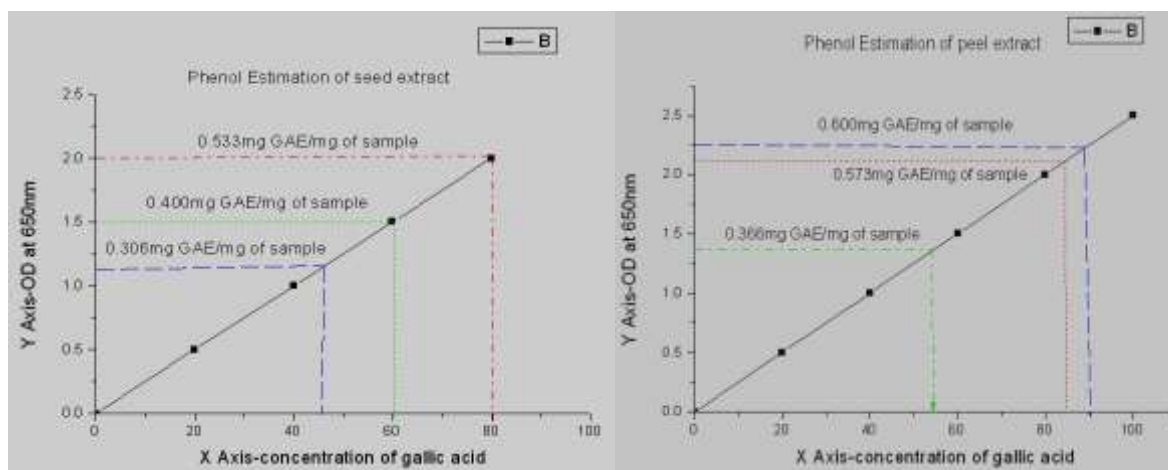


Fig 1) Phenol estimation of Seed extract & Peel Extract of Persea Americana.

Free Radical Scavenging Activity: DPPH Assay

DPPH has been widely used for radical scavenging assessment due to its ease and convenience. The free radical scavenging activity of both the extract was found to increase with increase in concentration. The essence of DPPH method is that the antioxidants react with the stable free radical i.e. α, α -diphenyl- β -picrylhydrazyl (deep violet color) and convert it to a α, α -diphenyl- β -picrylhydrazine with discoloration. The degree of discoloration indicates the scavenging potential of the sample antioxidant.

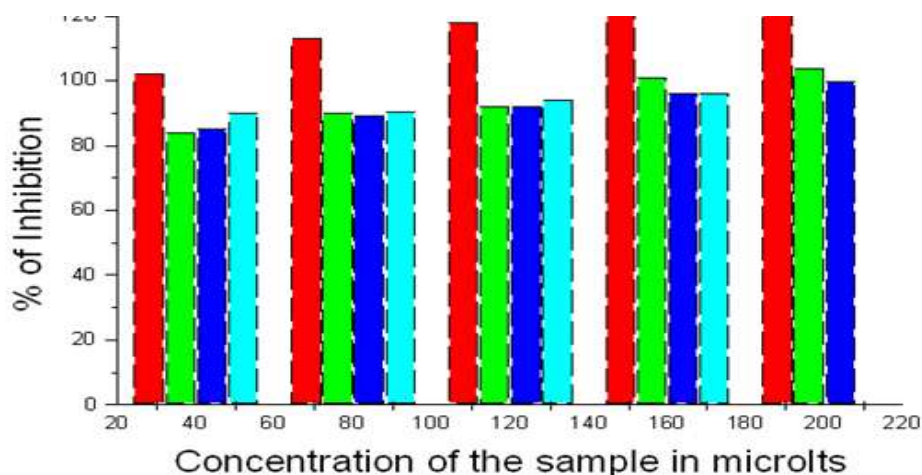


Fig 2: 1) std BHA, 2) Vit c, 3) Peel Methanolic extract & 4) Seed Methanolic extract

The scavenging activity of methanolic seed and peel extract of *Persea americana* was 96.87% (SM: 160 μ l) and 100% at 200 μ l (PM) concentration. The BHA and vit C standards were also carried out which showed 104% and 122% at 200 μ l concentration respectively. Thus these results indicate the outstanding scavenging effects on DPPH.

A- Amylase Inhibitory Activity

The α - amylase inhibition assay was carried out to check the α -amylase inhibitory potential of crude methanolic peel and seed extract of *Persea americana*. The % inhibition was tested with different concentration (25-125 μ l).

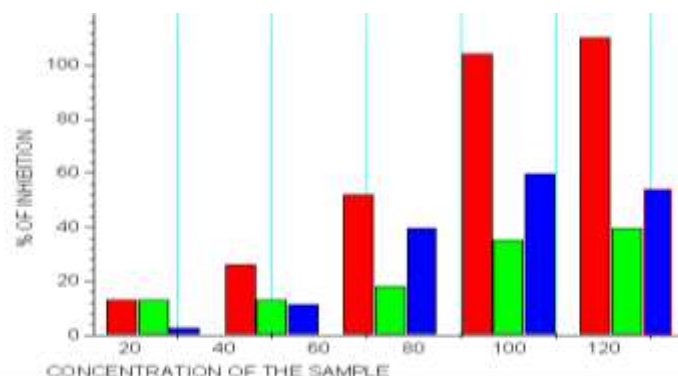


Fig 3: 1)Std acarbose ,2)Peel Methanolic extract &3) Seed Methanolic extract

The results indicate maximum inhibition of 35.7% and 60% by methanolic extracts of peel and seed at 100 μ l concentration when compared with std acarbose with 83.33%, with an IC_{50} values of $36.02 \pm 0.23 \mu\text{g/ml}$ & $59.34 \pm 1.01 \mu\text{g/ml}$ respectively when compared with acarbose $82.42 \pm 0.33 \mu\text{g/ml}$, thus indicating the efficacy of the seed and peel samples on alpha amylase inhibition. The assay performed shows a significant difference at $p < 0.05$ and is considered.

CONCLUSION

The traditional healthcare system in India, ayurveda offers attractive and holistic strategies for treatment of many diseases including diabetes. India has an exemplary source of medicinal plants with high therapeutic values. Diabetes mellitus, chronic disease that is developing along with increase in both obesity and ageing in general population. One of the therapeutic approaches for decreasing post-prandial hyperglycemia is to retard absorption of glucose by the inhibition of carbohydrates hydrolyzing enzymes, for example alpha amylase in digestive organ.

Therefore work was carried out with such interest in exploring some traditional fruits used for the amylase inhibitory potential in vitro. *Persea americana* is used locally as a nutritive fruit

and also are known for processing many medicinal values. They are known for their medicinal values but have not been significantly proven for their α -amylase inhibitory potential.

The research accomplished indicates the crude methanolic peel and seed samples have a potential source of natural substance with antioxidant properties and alpha amylase inhibitory potentials, However further purification of the extract and characterization would confirm the mode of action of peel and seed extracts of *Persea americana* and its impact on alpha amylase inhibition

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