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# Biochemical Screening of *persea macrantha* (NEES) Leaf extracts for its Antioxidant and Cytoprotective Properties

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**Abstract:** ***Plants are prosperous source of antioxidants. In the current investigation aqueous and methanolic extracts of persea macrantha were tested for total phenolics, radical scavenging activity, reducing power, inhibition of lipid per oxidation, DNA protection and cytoprotective effects. The aqueous and methanolic extracts exhibited 5.57mg and 8.80mg of total phenols/g of leaf. In addition to this 60% and 72% of free radical scavenging activity were seen /100µl of the extracts. Methanolic extracts exhibited extra reducing power activity as well as DNA fortification property when compared to aqueous extract. In RBC cytoprotection studies aqueous and methanolic extract showed $IC_{50}$ value of 1.57 and 1.05 mg of phenols/ml. In case of lipid peroxidation inhibition, methanolic extract exhibited $IC_{50}$ value 8.88 µg of phenol/ml and aqueous extract exhibited 17.89 $IC_{50}$ value.***

**Keywords:** Persea macrantha, Phenolics, Antioxidant, Lipid peroxidation, Cytoprotection

## 1. Introduction

It is well known that the free radicals are responsible for various types of diseases. In human ROS, such as superoxide anions, nitric oxide, hydrogen peroxide, peroxy nitrite, hydroxyl radicals play a vital role in various diseases. The severities of free radical damages include oxidative stress, lipid peroxidation, DNA damage, cell communication inhibition, ischemia, reperfusion injury of many tissues, atherosclerosis, arthritis, cancer, gastritis, diabetes mellitus and injury of nervous system etc. [1], [2]. In order to prevent free radical damage living organisms possess two main antioxidant mechanisms: one with enzymatic antioxidant mechanism which includes superoxide dismutase (SOD), catalases (CAT) and various peroxidases. Another one is with non enzymatic antioxidant mechanisms which involves polyphenols, vitamin-C, vitamin-E and carotenoids. [3]. Hence there is huge demand for antioxidant molecules.

The synthetic antioxidant molecules such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and tert-butylhydroquinone (TBHQ) are extensively used in food. But these are associated with toxic and carcinogenic effects. Therefore the demand for natural, side effect free antioxidants rose.[4] However, many plants and its sources such as vegetables, fruits, grain, cereals, legumes and nuts are rich source of antioxidants. Amusingly, many plants are rich source of phenolic antioxidants, such as phenolic acids, flavonoids and catechins. [5] Some phenolic compounds said to have potential to protect DNA from oxidative damage, inhibition of lipid peroxidation and radical scavenging activity of ROS, such as superoxide, hydrogen peroxide and hydroxyl radicals [6], [7]. Antioxidants are miraculous molecules capable of preventing or slowing down the oxidation of other molecules. They can terminate chain reactions by removing free radical intermediates, and can inhibit reactions by oxidizing themselves.

In the current investigation different extracts of *persea macrantha* leaves examined for phenolics content and various properties such as, DNA protection, lipid peroxidation and cytoprotective effects.

## 2. Materials and Methods

### 2.1 Collection of Plant material

*Persea macrantha* belonging to the family Lauraceae were used in this study. The leaves were obtained from the forest areas around Mysore, Karnataka (India) and used for preparation of extract.

### 2.2 Preparation of extracts

1g of shade dried leaves of *persea macrantha* was weighed and homogenized in pestle and mortar with little water/ methanol to the homogenized sample. The homogenate was centrifuged for 5 minutes at 1500 g. The pellet was discarded and supernatant was stored. The extracts were prepared separately and named as PMAE and PMME respectively.

#### 2.2.1 Total phenolic contents

Total polyphenols were estimated by Folin-Ciocalteau method. [8] Different aliquots of standard tannic acid were taken and volume made up to 1ml distilled water. Samples were taken accordingly and 1ml of FC regent (1:2 v/v), 10% $Na_2CO_3$ (2ml) were added incubated at room temperature for 1hour. Absorbance was assured against blank at 765 nm and concentration of phenolic content was calculated using standard graph.

#### 2.2.2 DPPH scavenging activity

Extracts of *persea macrantha* were tested for antioxidant activity. [9] Different aliquots of standard BHA and samples were taken in test tubes and the total volume was made upto 250µL with water/methanol respectively. To this 1mL of DPPH was added and the tubes were incubated in dark at room temperature, for 20minutes. Absorbance was assured against the blank at 517nm. Percentage of free radical

scavenging activity was calculated based on the degree of reduction in the colour.

### 2.2.3 Reducing power assay

Different aliquots of standard tannic acid and samples were taken in test tubes and the volume was made upto 0.5ml with buffer (pH 6.6). To this 0.5ml potassium ferricyanide was added and incubated for 20minutes at $50^0$C in water bath. 0.5ml of 10% TCA was added to all the tubes, which was then centrifuged at 2000g for 10minutes. About 1.5ml of supernatant was obtained.and 1.5ml of distilled water, 0.3ml of 0.1% ferric chloride was added. The colour obtained was assesed spectrophotometrically at 700nm. [10]

### 2.2.4 DNA protection studies using gel electrophoresis [11]

The reaction mixture (20µl) contained calf thymus DNA, 20mM PBS (pH 7.4). Different amounts of extracts were added prior to $FeSO_4$ and ascorbic acid. The samples were incubated for 2 hours at $37^0$C and the reaction mixture was added with loading buffer (xylene cyanol 0.25%), bromophenol blue dye (bromophenol blue 0.25% and glycerol 30%). Electrophoresis was carried out by using 40mM TAE (Tris-Acetate-EDTA buffer) (pH 8) as running buffer at 50V for 2 hours. Comparitive difference in the migration between the native and oxidized DNA was ensured on 1% Agarose gel after staining with Ethidium bromide. Gels were documented and the intensity of the bands was identified. DNA protection was estimated on the basis of the DNA band comparable to that of native in the presence and absence of extracts.

### 2.2.5 Cytoprotective studies using RBCs [12]

The blood was obtained from healthy donors. Heparinized blood was centrifuged at 3000g for 10minutes. After removal of plasma and buffy coat, the RBCs were washed thrice with PBS (pH 7.4) at room temperature and resuspended in PBS four times its volume for subsequent analysis. 100µl of RBC were incubated with 50µl of phenolic fractions in presence of oxidants and the total volume was made up to 400µl with PBS. This was incubated at $37^0$C for 20minutes and centrifuged. Haemolysis was spectrophotometrically evaluated at 410nm as haemoglobin released from cells in the supernatant. The comparative effect of selected sources in protecting RBC against oxidative damage was calculated.

### 2.2.6 Lipid peroxidation inhibition [13]

*In vitro* lipid peroxidation levels in rat liver homogenate were measured as thiobarbituric acid reactive substances (TBARS). 10% fresh liver homogenate was prepared in 20mM phosphate buffer saline (PBS- pH 7.4) (Ohkawa *et al*, 1979). 0.5ml of liver homogenate was incubated with different amounts of extracts and total volume was made upto 1ml with PBS. After 5 minutes of pretreatment, 50µl of ferric chloride and 50µl of ascorbic acid were added and incubated at $37^0$C for 1hour. The reaction was ceased by the addition of 2ml of TBA reagent (15% TCA, 0.375% TBA in 0.25N HCl), and the tubes were boiled for 15minutes at $95^0$C, cooled and centrifuged; their absorbance was then evaluated at 532nm. TBARS were measured by using a standard 1, 1, 3, 3- tetramethoxypropane (TMP).

## 3. Results

Aqueous and methanolic extracts were employed to extract polyphenols efficiently. In this case methanolic extracts possessed more phenolic content than that of aqueous extract (Table 1). Aqueous/Methanolic extracts of *persea macrantha* contained 8.80 and 5.57 mg TAE (Tannic Acid Equivalents)/g respectively.

**Table 1:** Total Phenolic contents of *persea macrantha* extract

| *Extract* | *Total phenols present (mg/g of leaves)* |
|---|---|
| Aqueous extract | 5.57 |
| Methanolic extract | 8.80 |

Free radical scavenging potential of the extracts was tested against a methanolic solution of α, α, diphenyl- β picryl hydrazyl (DPPH**).** Antioxidants react with DPPH and convert α, α, diphenyl- β picryl hydrazine. The degree of decolouration indicated the scavenging potential of the antioxidants in the extract. Data indicated in **Figure-1** indicated concentration dependence, suggesting that the activity is due to the phenolic constituents present in the extract. In this case PMME exhibited 72% radical scavenging activity when compared to PMAE which has ~60%.

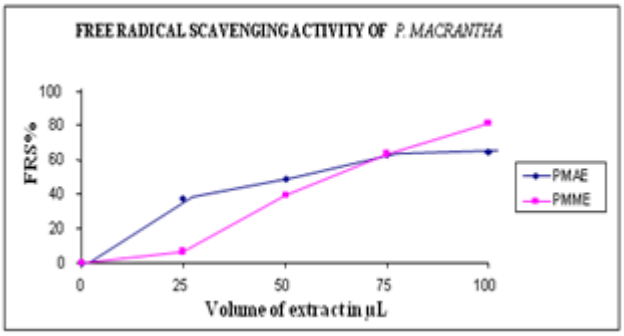


**Figure 1**: Free radical scavenging activity of *persea macrantha* extracts.

Aqueous and methanolic extracts were examined for free radical scavenging activity. The $IC_{50}$ values are shown in **table-2**

| *Source* | *Extracts* | *IC50 in µg of phenols/ml* |
|---|---|---|
| *P.macrantha* | PMAE | 8.7 |
| | PMME | 8.2 |

The absorbance of the assay mixture are recorded spectrophotometrically and represented graphically in figure-2. The activity of the extracts was calculated as total absorbing units/g of sample.

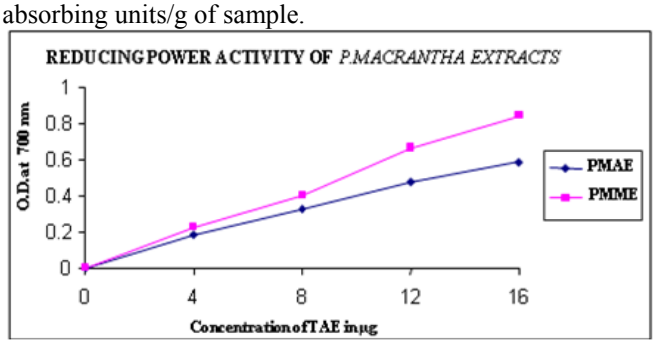


**Figure 2:** Reducing power activity of *persea macrantha* extracts.

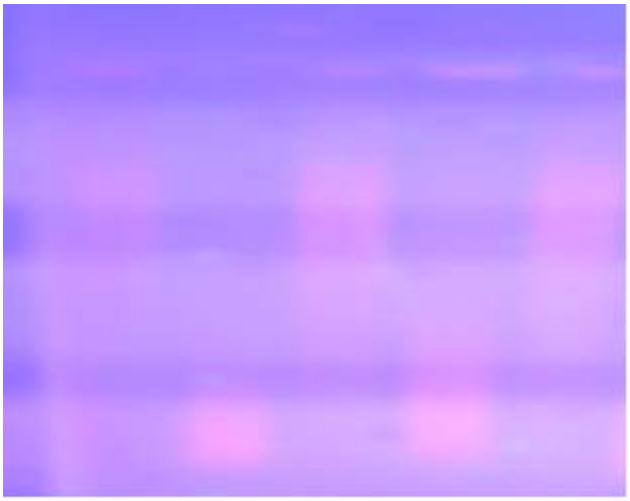


**Figure 3:** Showing electrophoretic mobility of DNA with different extracts.

Total reducing power units of extracts of *persea macrantha* is shown in **table-3**.

| *Source* | *Extracts* | *Total reducing power units activity/g of dry weight* |
|---|---|---|
| *P.macrantha* | PMAE | 615 |
| | PMME | 610 |

Native DNA (lane 1) was subjected to oxidation (lane 2) and studied its protection by Tannic acid, PMAE, PMME, (lane 3 to lane 5) was evaluated by agarose gel electrophoresis, bands were viewed by Ethidium bromide.

The DNA samples, preincubated with the phenolic fractions and then treated with oxidants (lane 3- lane 5) were run on an electrophoresis gel shown in figure-3. The relative fast mobility of the DNA indicates DNA damage. The DNA incubated with sample extracts regressed the migration indicating protection to DNA against damage by selected extracts.

Determination of comparative RBC protection potential of PMAE and PMME is shown in **table-4.**

| Source | Extracts | IC50 in mg of phenols/ml |
|---|---|---|
| *P.macrantha* | PMAE | 1.57 |
| | PMME | 1.05 |

The protection of RBCs by the Phenolic fractions of the selected sources was observed and $IC_{50}$ of the extracts indicated is the concentration required to inhibit 50% of RBC damage.

Comparitive lipid peroxidation inhibition by PMAE and PMME extracts shown in **table-5**.

| Source | Extracts | IC50 in µg of phenols/ml |
|---|---|---|
| *P.macrantha* | PMAE | 17.89 |
| | PMME | 8.88 |

The TBARS method was carried out to examine the lipid peroxidation. The spectrophotometric values of the fluorescent pink obtained were recorded and the inhibition constant is expressed as $IC_{50}$ which is concentration equivalent of phenolics required to inhibit 50% of lipid peroxidation.

## 4. Discussion

Plants are extensively used to treat and combat various types of diseases. Over the past decade, the demand for herbal remedies has dramatically increased, both from medicinal as well as economic point of view.

Oxygen is essential for the production of energy, oxidation of endogenous compounds and detoxification of xenobiotics. On the other hand, its incomplete reduction leads to the formation of a series of reactive chemical intermediates including ROS. These radicals are highly capable of widespread, indiscriminate oxidation and peroxidation of proteins, lipids and nucleic acids which can lead to significant cellular injury and even organ failure.

Therefore substantial interest continues to revolve around the aetiological significance of ROS. The regulation of the oxidative environment of cells is a highly complex process in which a fine balance exists between free radical generation via metabolism and the activity of a multilevel antioxidant system. Now it is widely accepted that free radicals are solely responsible for the chronic diseases. Although list of synthetic drugs are available in the market, there are limitations and side effects associated with it. Hence there is an urge for side effect free natural antioxidants. During the current investigation, *persea macrantha* a rich source of phytochemicals was selected. The leaves and bark of *persea macrantha* are extensively used in traditional medicines. They exhibit various properties such as anti-rheumatic, anti-asthmatic, anti-arthritic, anti-inflammatory, anti-tuberculosis, wound healing and microbicidal. [14] In our study we examined the radical scavenging, cytoprotective, inhibition of lipid peroxidation and DNA protective properties associated with aqueous and methanolic leaf extracts.

In order to know their bio potency *in vivo*, where they have to work both in the polar such as cytosolic compartment as well as non polar area such as at the cellular membrane level; both aqueous and methanol extracts were prepared. Our studies revealed that methanolic extract of *persea macrantha* contains more phenolics when compared to aqueous extract. Methanolic extract possess 8.8mg of phenolics per gram of leaves and aqueous possess 5.57 mg phenolics per gram of leaves.

In case of radical scavenging activity, methanolic extract possess 8.2 µg of phenolics/ml of the extract which is sufficient to inhibit 50% of radical scavenging activity. But aqueous extract exhibited at 8.7 µg/ml, suggesting that phenolics of methanolic extract were more efficient than aqueous.

In case of reducing power activity methanolic extracts exhibited more reducing power when compared to aqueous extract. This suggests that PMME are more potent. From table-3 it is clear that 610 reducing power units activity of

PMME per gram of leaf is more than enough to reduce ferricyanide. In order to verify the antioxidant property of the extracts, calf thymus DNA used as a standard i.e., without any reagent. In the next lane it is subjected to oxidation with $FeSO_4$ and ascorbic acid (minimum concentration) which acts as a fenton's reagent [15]. Next lanes are followed by Tannic acid (standard antioxidant), PMAE (*persea macrantha* aqueous extract) and PMME (*persea macrantha* methanolic extract).

More the oxidation more will be the relative fast movement (lane-2) of the DNA. This indicates DNA damage. Results of the gel electrophoresis is tabulated in the below table.

**Table 6:** Electrophoresis results demonstrating Antioxidant properties

| Lane no. on gel strip | Antioxidant source | Oxidation reagent | DNA protection |
|---|---|---|---|
| 1(native DNA) Standard | Absent | Absent | Positive |
| 2 DNA | Absent | $FeSO_4$+Ascorbic acid= fenton's reagent | Negative |
| 3 DNA | Tannic acid | $FeSO_4$+Ascorbic acid= fenton's reagent. | Positive |
| 4 DNA | PMAE | $FeSO_4$+Ascorbic acid= fenton's reagent | Positive |
| 5 DNA | PMME | $FeSO_4$+Ascorbic acid= fenton's reagent | Positive* |

* indicates more potentcy.

From the figure-3 and table-6 we can conclude that methanolic extracts of *persea macrantha* more potent source of antioxidants than aqueous extract. The protection of RBCs by the phenolic fractions suggests that methanolic extracts of persea macrantha is more potent when compared to aqueous extract. (Refer table-4) Since methanolic extract contains 1.05 mg of phenolics /ml. This was sufficient to prevent 50% of RBC damage. When the bio potency evaluated for lipid peroxidation inhibition, methanolic extracts exhibited more potency. (Refer table-5). This is because only 8.88 µg of phenolics/ ml of extract inhibited 50% of lipid peroxidation. This was very less when compared to $IC_{50}$ values of aqueous extract.

## 5. Conclusions

*Persea macrantha* exhibited more phenolics content in both the extract. They also exhibited various antioxidant properties such as radical scavenging activity, ferricyanide reduction, cyto protection, DNA protection and lipid peroxidation inhibition.
In comparison methanolic extracts of *persea macrantha* exhibited more biopotency when compared to aqueous extract. The cytoprotective and DNA protection studies may have promising role as an anti-ulcer and anti-cancerous agent.

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