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Research Article

# Antioxidant activity of ethanolic extract of *Piper betel* leaves

**Ashish Manigauha [1]*, Huma Ali [2] and M. Uma maheshwari [3]**

[*1] NRI Institute of Pharmacy, Sajjan Singh Nagar, Raisen Road, Bhopal – 462021 (India)

[2]Dept. of Research, Jawaharlal Nehru Cancer Hospital & Research Center, Bhopal- 462001

[3]College of Pharmacy, Sri Ramkrishna Institute of Paramedical Sciences, Coimbatore, Tamilnadu. India

*For correspondence:* **Ashish Manigauha ,Department of Pharmacology and Phytochemistry, NRI Institute of Pharmacy, Sajjan Singh Nagar, Raisen Road, Bhopal – 462021 (India)**

*E-mail:* ashish.manigaunha@gmail.com

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## ABSTRACT

The present study was aimed to investigate the antioxidant activity of methanolic extracts of *Piper betel* leaves by different methods like DPPH photometric assay, reductive ability, Deoxyribose degradation assay and Superoxide scavenging activity. It belongs to the family *Piperaceae* used as ingredients in a chew commonly known as pan. Chemically *piper betel* containing chavi betel, chavicol, betel oil, terpene, sesquiterpene. Biologically *Piper betel* have broad spectrum activities like antifungal, antibacterial, antitumour, hypotensive, respiratory depressant, anthelmintic, cardiotonic and antifertility. PBLE showed strong antioxidant activities like reducing power, DPPH radical, superoxide anion scavenging and deoxyribose degradation activities when compared with different standards such as ascorbic acid, DMSO and BHT. Presumably, PBLE functions as an antioxidant to scavenge free radicals and reduces free radical induced cell injury.

**Key words**: *Piper betel* leaves extract, Antioxidant, DPPH, Reducing power, DMSO

## INTRODUCTION

Evidence indicate that harmful free radicals play an important role in most major health problems such as cancer, cardiovascular diseases, rheumatoid arthritis, cataract, alzheimer's disease and other degenerative diseases associated with aging. Antioxidants are beneficial components that neutralize free radicals before they can attack cells and hence prevent damage to cell proteins, lipids and carbohydrates. A wide range of antioxidants both natural and synthetic has been proposed for use in treatment of human diseases. Interest in the role of antioxidants in human health has prompted research in the fields of food science and medicinal herbs to assess the role of herbs as antioxidants. Antioxidant action includes free radical scavenging capacity, inhibition of lipid peroxidation, metal ion chelating ability and reducing capacity [1].

The significant contribution made by herbal medicines to human health has lead to increased popular, official and commercial interest. *Piper betel* plant is one among the herbals used in folk medicines all over the World. It belongs to the family *Piperaceae*, used as ingredients in a chew commonly known as pan [2]. Chemically *piper betel* has light yellow aromatic essential oil with sharp burning taste. Aromatic odor containing chavi betel, chavicol, eugenol, cadinene, alkaloids, sugar, tannin, diastase, betel oil, terpene, sesquiterpene. Biologically *Piper betel* is aromatic, stimulant, carminative, astringent and antiseptic [3, 4]. Leaf possesses activities like antidiabetic [5], antiplatelet [6], antiulcer [7], antifertility [8, 9], cardiotonic [10], antitumour [11, 12], antimutagenic [13], hypotensive, respiratory depressant and anthelmintic [3, 4].

## MATERIALS AND METHODS

### Plant material

*Piper betel* leaves (Local name - Karpoora vettillai) was purchased in the local market, Coimbatore. The herbarium of this plant was identified and authenticated (Herbarium No. BSI / SC/5/21/04-05/Tech -1374) by the taxonomist, Botanical Survey of India, Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamilnadu, India.

### Preparation of plant material

Fresh leaves were collected and air dried in shade at room temperature. Dried leaves were powdered mechanically through mesh sieve. 100 g of freshly powdered leaves were evenly packed in soxhlet apparatus and the extraction was done with 70% alcohol. Then solvent was evaporated at low temperature under reduced pressure.

### Drugs and chemicals

2,2diphenyl-1picrylhydrazylhydrate (DPPH) and nitroblue tetrazolium (NBT) were obtained from Himedia, Mumbai. 2-Deoxy 2-ribose, xanthine oxidase and hypoxanthine oxidase were obtained from Sisco research laboratory Mumbai. Ascorbic acid and potassium ferricynide were obtained from SD Fine Ltd, Baisar. All other chemicals used were obtained commercially and were of analytical grade.

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## *IN VITRO* ANTIOXIDANT ACTIVITY [14]

### i. DPPH photometric assay [15]

The hydrogen donating ability of *Piper betel* extract was examined in the presence of 2, 2-diphenyl-1-picrylhydrazylhydrate (DPPH) radical. It is produces a violet solution in ethanol.

Stock solution of the extract (1.0mg/ml) diluted to final concentration of 5, 10, 15, 20 and 25 µg/ml in ethanol. One ml of 0.3 mM DPPH ethanol solution was added to 2.5 ml of sample solution of different concentration and allowed to react at room temperature. After 30 minutes the absorbance values were measured at 518 nm. Ethanol (1.0 ml) plus plant extract solution (2.5 ml) was used as blank. DPPH solution (1.0ml: 0.3mM) plus ethanol 2.5 ml was used as a negative control. The positive control was those using the standard solutions (Ascorbic acid).

The percentage antioxidant activity (%AA) was calculated using the following formula:

$$AA\% = 100 - \left\{ \frac{\text{Abs Sample - Abs blank}}{\text{Abs control}} X100 \right\}$$

The concentration required to inhibit or change the absorbance at 50%. ($IC_{50}$) was also calculated.

### ii. Measurement of the reductive ability [16]

For the measurement of the reductive ability, the $Fe^{3+}$ - $Fe^{2+}$ transformation in the presence of the extract was investigated. 1 ml of plant extract (1 mg/ml), 2.5 of 0.2M phosphate buffer (pH 6.6), 2.5 ml of 1% potassium fericynide [$K_3Fe(CN)_6$] were incubated at 50°C for 20 minutes. 2.5 ml of 10% Trichlo acetic acid (TCA) were added to the mixture and centrifuged for 10 minutes at 3000 rpm. 2.5 ml of the supernatant were diluted with 2.5 ml water and is shaken with 0.5 ml of freshly prepared 0.1 % Ferric chloride. The absorbance was measured at 700 nm. The reference solution was prepared as above, but contained water instead of extract.

Increased absorbance of reaction mixture indicates increased reducing power.

### iii. Scavenging of hydroxyl radical [17]

Hydroxyl radicals (OH) were generated by the reaction of ferric EDTA together with $H_2O_2$ and ascorbic acid to attack the substrate deoxyribose. The resulting products of the radical form pink chromogen when heated with TBA in acid solution.

The reaction mixture contained in a final volume of 1:0 ml, 100 µl of 2-deoxy-ribose (28mM in $KH_2PO_4$, $K_2HPO_4$ buffer, pH 7.4), 500 µl solution of various concentrations of the extract in buffer, 200 µl of 1.04 mM EDTA and 200 µM $FeCl_3$ (1:1v/v), 100 µl of 1.0 mM $H_2O_2$ and 100 µl of 1.0 mM ascorbic acid. PBLE were kept at 37°C for 1 hour. One ml of 2.8 %TBA was added to the test tubes and was incubated at 100°C for 20 minutes. After cooling, absorbance was measured at 532 nm against a blank containing deoxyribose and buffer. DMSO is used as standard.

Inhibition of deoxyribose degradation was calculated in percentage (I %) using the following formula,

$$I\% = 100 X \left( \frac{Ao - At}{Ao} \right)$$

Where $A_o$ is the absorbance of the control and $A_t$ is the absorbance of the test compound.

### iv. Scavenging of Superoxide anion radical [18, 19]

The superoxide anion radical was generated *in vitro* with hypoxanthine and xanthine oxidase. A reaction mixture with a final volume of 1 ml per tube was prepared with 50 mM $KH_2PO_4$ – KOH pH 7.4 containing 1 mM EDTA. 100 mM hypoxanthine, 100 mM NBT. 0.066 U per tube of xanthine oxidase diluted in 100 ml of phosphate buffer and the PBLE in 10 ml of saline. The xanthine oxidase added last. The reaction mixture incubated at 25°C for 5 minutes and absorbance was measured at 560 nm.

Decrease in the absorbance of reaction mixture indicates an increase in superoxide anion scavenging activity. The results are expressed as the percentage inhibition of NBT reduction rate with respect to the reaction mixture without PBLE (saline only). Inhibition of reaction mixture was calculated in percentage (I %) using the above formula.

## RESULTS:

### i. DPPH photometric assay

DPPH was reduced in the addition of the extract in a concentration dependent manner. When compared by $IC_{50}$, the free radical scavenging activity of the extract was 12.0 mg/ml, which was greater than to that of the ascorbic acid 5.35 mg/ml, the standard drug, indicating potential antioxidant activity of the extract. (table.1)

**Table. 1. Measurement of DPPH photometric assay**

| Treatment | Concentration (µg/ml) | %AA | $IC_{50}$ |
|---|---|---|---|
| PBLE | 05 | 44.07 ± 1.44 | 12.00µg/ml |
| | 10 | 47.05 ± 3.59 | |
| | 15 | 60.34 ± 5.20 | |
| | 20 | 66.50 ± 2.90 | |
| | 25 | 72.02 ± 3.86 | |
| Ascorbic acid | 05 | 47.60 ± 9.84 | 5.35µg/ml |
| | 10 | 55.39 ± 14.4 | |
| | 15 | 67.42 ± 7.07 | |
| | 20 | 72.81 ± 5.33 | |
| | 25 | 76.20 ± 6.50 | |

Results are mean ± SD of the three parallel measurements. %AA – Percentage of antioxidant activity. $IC_{50}$ – 50% inhibitory concentration.

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### ii. Measurement of reductive ability

The extract could reduce the most $Fe^{3+}$ ions and possess reductive ability when compared to the standard (Table.2). Increased absorbance of reaction mixture indicates increased reducing power.

**Table. 2. Measurement of Reductive ability of PBLE**

| Treatment | Concentration (µg/ml) | Absorbance |
|---|---|---|
| PBLE | 10 | 0.4403 ± 0.23 |
| | 20 | 0.4831 ± 0.42 |
| | 30 | 0.5570 ± 0.12 |
| | 40 | 0.8087 ± 0.78 |
| | 50 | 0.8092 ± 0.65 |
| BHT | 10 | 0.5071 ± 0.24 |
| | 20 | 0.5823 ± 0.31 |
| | 30 | 0.6068 ± 0.28 |
| | 40 | 0.7535 ± 0.35 |
| | 50 | 0.8562 ± 0.29 |
| Blank | | 0.4279 ± 0.18 |

Results are mean ± SD of the three parallel measurements

### iii. Deoxyribose degradation assay

The concentrations for 50% inhibition were 100 µg/ml for *Piper betel* leaf extract and 90 µg/ml for Dimethyl sulphoxide (DMSO) and the results are shown. The percentage inhibition of deoxyribose degradation (I %) and concentrations for 50% inhibition of the extract and standard is listed in Table 3.

**Table. 3. Measurement of Deoxyribose degradation assay**

| Treatment | Conc. (µg/ml) | %AA | $IC_{50}$ |
|---|---|---|---|
| PBLE | 050 | 39.71 ± 0.82 | 100mg/ml |
| | 100 | 49.75 ± 0.31 | |
| | 150 | 57.92 ± 3.76 | |
| | 200 | 67.09 ± 1.37 | |
| | 250 | 69.22 ± 1.15 | |
| DMSO | 050 | 42.75 ± 1.08 | 90mg/ml |
| | 100 | 51.06 ± 0.64 | |
| | 150 | 55.19 ± 0.67 | |
| | 200 | 67.54 ± 0.80 | |
| | 250 | 75.12 ± 1.08 | |

Results are mean ± SD of the three parallel measurements

### iv. Superoxide scavenging activity

The alcoholic extract of *Piper betel* leaves and ascorbic acid at 50 µg/ml, inhibited NBT reduction by 66% and 77% respectively. $IC_{50}$ values were 20 µg/ml for the extract and 22 µg/ml for the standard as shown in Table 4. This shows that the extract inhibited xanthine oxidase activity.

**Table. 4. Scavenging of Superoxide anion radical (NBT)**

| Treatment | Conc.(µg/ml) | %AA | $IC_{50}$ |
|---|---|---|---|
| PBLE | 10 | 28.01 ± 3.16 | 20mg/ml |
| | 20 | 50.77 ± 7.70 | |
| | 30 | 57.01 ± 5.50 | |
| | 40 | 60.50 ± 4.72 | |
| | 50 | 66.24 ± 3.57 | |
| Ascorbic Acid | 10 | 34.72 ± 7.43 | 22mg/ml |
| | 20 | 48.44 ± 4.05 | |
| | 30 | 56.12 ± 12.5 | |
| | 40 | 68.28 ± 4.96 | |
| | 50 | 77.82 ± 9.89 | |

Results are mean ± SD of the three parallel measurements

## DISCUSSION

The *in vitro* antioxidant activity of PBLE shows that it has potential free radical scavenging effect on DPPH. The extracts reduced most of the Fe3+ ions and possess strong reductive ability. The extract also showed strong hydroxy radical and superoxide anion radical scavenging property when compared with different standards such as ascorbic acid and BHT. Presumably, PBLE functions as an antioxidant to scavenge free radicals and reduces free radical induced cell injury.

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