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## EVALUATION OF ANTIOXIDANT POTENTIAL OF BETEL (*PIPER BETEL*) LEAVES EXTRACT

ASHISH SAINI\*, ANURAG PANDEY, SANJITA SHARMA,  
UMESH S. SURADKAR, YELLAMELLI R. AMBEDKAR AND  
PRIYANKA MEENA

DEPARTMENT OF LIVESTOCK PRODUCTS TECHNOLOGY, POST  
GRADUATE INSTITUTE OF VETERINARY EDUCATION AND  
RESEARCH, JAIPUR, RAJASTHAN, INDIA 302031.

Corresponding author email: [ashish29vet@gmail.com](mailto:ashish29vet@gmail.com)

### ABSTRACT:

Different type of biochemical test (Antioxidant ability assays, Hydrogen peroxide scavenging assay, Nitric oxide radical scavenging assay and 2, 2'-azino-bis (3 ethylbenzothiazoline-6-sulfonic acid (ABTS) were exercised to evaluate antioxidant activity of betel leaves extract (BE). The result showed that BE exhibit high Antioxidant ability assays (303.55±5.18 µg ascorbic acid) and IC<sub>50</sub> value of BE for Hydrogen peroxide scavenging assay, Nitric oxide radical scavenging assay and ABTS were found 37.48±2.85, 53.24±2.39 and 57.74±2.05 respectively. It can be concluded that betel leaves can be use as antioxidants in food industry to prevent the dilemma of lipid oxidation and rancidity.

**KEYWORDS:** *Antioxidant properties, Antioxidant ability assays, Betel leave, Nitric oxide radical scavenging assay.*

### INTRODUCTION:

The most common form of deterioration in any food products is oxidative rancidity; which leads to extensive flavor changes, structural damage to proteins leading to loss of freshness that discourages repeat purchases by consumers (Saini *et al.*, 2019). The most effective approach to avoid oxidative deterioration in food products is to integrate antioxidants into formulations. Antioxidants either synthetic or natural have become an indispensable group of food additives mainly because of

their unique properties of enhancing the shelf life of food products without any damage to sensory or nutritional qualities (Nanditha and Prabhasankar, 2008). In industrial processing, mainly synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) are used in food industry. However, increasing concerns over the safety of synthetic food additives has resulted in a trend towards “natural products”. Plants are persistently the liberal source to furnish man with valuable bioactive substances (Tayel and El-Tras, 2012) and thus different plant products are being evaluated as natural antioxidants to preserve and improve the food quality. Natural antioxidants extracted from herbs and spices exhibit various degrees of efficacy when used in different food applications (Bowser *et al.*, 2014).

Among natural antioxidant sources, betel is more potent source of natural antioxidants. Betel (*Piper betel*) belongs to the genus *Piper* of the family *Piperaceae*. It is distributed throughout east Africa and the tropical regions of Asia. The antioxidant activity of betel is due high phenolic content such as catechol, hydroxy-chavicol, chavibetol, allylpyrocatechol, chavibetol acetate, and allylpyrocatechol diacetate (Dasgupta *et al.* 2014) responsible for the antioxidant activity. Betel is an eminent beneficial medicinal herb that is commonly used in pharmaceutical products and traditional medicine as a digestive, tonic, astringent, diuretic, diaphoretic and useful for urinary ailments (Mahomoud *et al.*, 2005). Therefore, the present study has been undertaken to explore antioxidant activity of betel leaves *in-vitro*.

## ***MATERIAL AND METHODS:***

### **Extract preparation:**

The betel leaves were oven dried at 50°C for 12 hrs followed by grinding and sieving. Pre-weighed powdered leaves were extracted with 70% ethanol for 24 hrs at 40°C. The extract was collected and concentrated under reduced pressure in a rotary vacuum evaporator (Labconco Corporation, USA) until semi solid consistency. The semisolid mass was oven dried at 50°C overnight to obtain dried extract. The extract were reconstituted with the same solvent as used for extraction to obtain 5% solutions and stored at 4°C.

### ***In-vitro* antioxidant assays analysis of betel leaves extract:**

#### **Antioxidant ability assays of betel leaves extract**

The antioxidant activity of the betel leaves extracts (BE) was evaluated by the phosphomolybdenum method according to the procedure of Prieto *et al.* (1999). Briefly, 0.3 ml of BE was combined with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate

and 4 mM ammonium molybdate). The tubes containing reaction solution were incubated at 95°C for 90 min and cooled to room temperature. The absorbance of the solution was measured at 695 nm using spectrophotometer against the blank. Ascorbic acid was used as reference standard. The antioxidant activity is expressed as the number of equivalents of ascorbic acid (AscAE).

#### **Hydrogen peroxide scavenging activity of betel leaves extract:**

Hydrogen peroxide scavenging potential of the BE was determined using the method described by Jayaprakasha *et al.* (2004). A solution of hydrogen peroxide (20 mM) was prepared in phosphate buffer saline (PBS, pH 7.4). Different concentrations of the BE (10-50 µg/ml) were added in ethanol (1 ml) along with 2 ml of hydrogen peroxide solution in PBS. After 10 min, the absorbance was measured at 230 nm against the blank solution containing hydrogen peroxide solution without the extract. The percentage of Hydrogen peroxide scavenging of the plant extract was calculated as follows:

$$\% \text{ scavenged} = [(\text{Abs control} - \text{Abs sample}) / \text{Abs control}] \times 100$$

#### **Nitric oxide radical scavenging assay betel of leaves extract:**

The method of Garrat (1964) with slight modification was used to determine the nitric oxide radical scavenging activity of the extract. A volume of 2 ml of 10 mM sodium nitroprusside prepared in phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of BE and ascorbic acid at various concentrations ranging from 20-100 µg/ml. The mixture was incubated at 25°C for 150 min, then after 0.5 ml of the incubation solution was withdrawn and mixed with 0.5 ml of Griess reagent [1.0 ml sulfanilic acid reagent (0.33% prepared in 20% glacial acetic acid at room temperature for 5 min with 1 ml of naphthylethylene diamine dihydrochloride (0.1% w/v)]. The mixture was incubated at room temperature for 30 min, followed by the measurement of absorbance at 540 nm using a spectrophotometer (spectramax plus).

$$\% \text{ scavenged} = [(\text{Abs control} - \text{Abs sample}) / \text{Abs control}] \times 100$$

#### **ABTS<sup>•+</sup> discoloration assay of betel leaves extract:**

Method of Dahiya and Puniya (2015) was followed to analyze 2, 2'-azino-bis (3 ethylbenzothiazoline-6-sulfonic acid (ABTS) free radical scavenging activity. ABTS<sup>•+</sup> radicals were generated by admixing 5 ml (7 mM) of the ABTS<sup>•+</sup> solution to 88 µL (140 mM) of potassium persulfate solution. The mixture was placed in the dark at room temperature for 16-18 hrs to accomplish the reaction. Before use, the OD of the solution was adjusted to 0.7±0.2 at 750 nm with PBS (pH 7.2). A 10 µL of aliquot extracts and standard of various concentrations was

separately added to 200  $\mu$ L of ABTS $\cdot^+$  solution and absorbance was taken at 750 nm after 10 min. Trolox (Sigma) was used as a standard to analyze the antioxidative capacity. For per cent ABTS $\cdot^+$  radicals scavenged, the values were subtracted from control ABTS $\cdot^+$  and further used to calculate trolox equivalent antioxidative capacity (TEAC) from trolox (5 mM) standard curve.

$$\% \text{ scavenged} = [(\text{Abs control} - \text{Abs sample}) / \text{Abs control}] \times 100$$

#### **Statistical analysis:**

All experiments were conducted in triplicate and data expressed as mean  $\pm$  SD.

### ***RESULT AND DISCUSSION:***

#### **Antioxidant ability assays of betel leaves extract**

Total antioxidant capacity of BE was found to be  $303.55 \pm 5.18$   $\mu$ g ascorbic acid equivalents at 100  $\mu$ g/ml (Fig.1). The phosphomolybdenum method is an important antioxidant assay based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of green phosphate/Mo (V) complex with a maximal absorption at 695 nm (Deepa *et al.*, 2013). Many studies have reported a positive correlation between phenolic compounds in plants and their antioxidant activities showing the importance of phenolic compounds as antioxidants. The antioxidant capacity of betel is might be due to its phenolic compound. Alam *et al.* (2012), Putri and Farida, (2013) and Prayitno *et al.* (2016) reported total antioxidant capacity of betel extract was 81.72, 10.74 and 127.35  $\mu$ g ascorbic acid equivalents, depending on the concentrations and solvent medium.

#### **Hydrogen peroxide scavenging activity of betel leaves extract:**

Increasing the concentration of the extract significantly increase the radical-scavenging activity. The alcoholic BE and Ascorbic acid at the concentration of 20, 40, 60, 80 and 100  $\mu$ g/ml, inhibited H<sub>2</sub>O<sub>2</sub> reduction by 42.25, 53.47, 61.89, 66.89, 72.58% and 49.78, 59.45, 69.12, 72.59, 78.10 respectively (Fig. 2). The IC<sub>50</sub> values of BE and ascorbic acid were found to be  $37.48 \pm 2.85$  and  $19.45 \pm 3.66$   $\mu$ g/ml (Table 1). Abraham *et al.* (2012) and Chakraborty *et al.* (2013) they were observed the IC<sub>50</sub> values of H<sub>2</sub>O<sub>2</sub> scavenging activity of betel extract was 313.3 and 72  $\mu$ g/ml respectively.

#### **Nitric oxide radical scavenging assay betel of leaves extract:**

Alcoholic BE and ascorbic acid at a concentration of 20, 40, 60, 80 and 100  $\mu$ g/ml inhibited nitric oxide radical by 30.99, 43.23, 51.87, 60.45, 74.89% and 40.23, 53.25, 64.89, 78.45, 89.45%

respectively (Fig. 3). The IC<sub>50</sub> values of BE and ascorbic acid were found 53.24±2.39 and 32.93±2.42 µg/ml respectively (Table 1). Abraham *et al.* (2012) and Alam *et al.* (2012) reported IC<sub>50</sub> values of nitric oxide radical scavenging assay of betel extract was 143.3 and 25 µg/ml respectively.

#### **ABTS·<sup>+</sup> discoloration assay of betel leaves extract:**

The alcoholic BE and ascorbic acid at a concentration of 20, 40, 60, 80 and 100 µg/ml, inhibited ABTS radical by 12.23, 27.69, 47.78, 57.56, 80.91% and 19.12, 40.25, 58.78, 78.89, 94.20 % (Fig. 4) where the IC<sub>50</sub> value of BE and trolox were found 57.74±2.05 and 49.51±2.12 µg/ml (Table 1). Teruel *et al.* (2015) found IC<sub>50</sub> value of ABTS·<sup>+</sup> radical scavenging activity betel extract was 811.60 µg/ml.

#### **CONCLUSION:**

After the results interpretation of the current study, it can be concluded that plant based natural antioxidants like Betel (*Piper betel*) leaves could be use as alternative to synthetic antioxidants in food industry to overcome the problem of lipid oxidation and rancidity.

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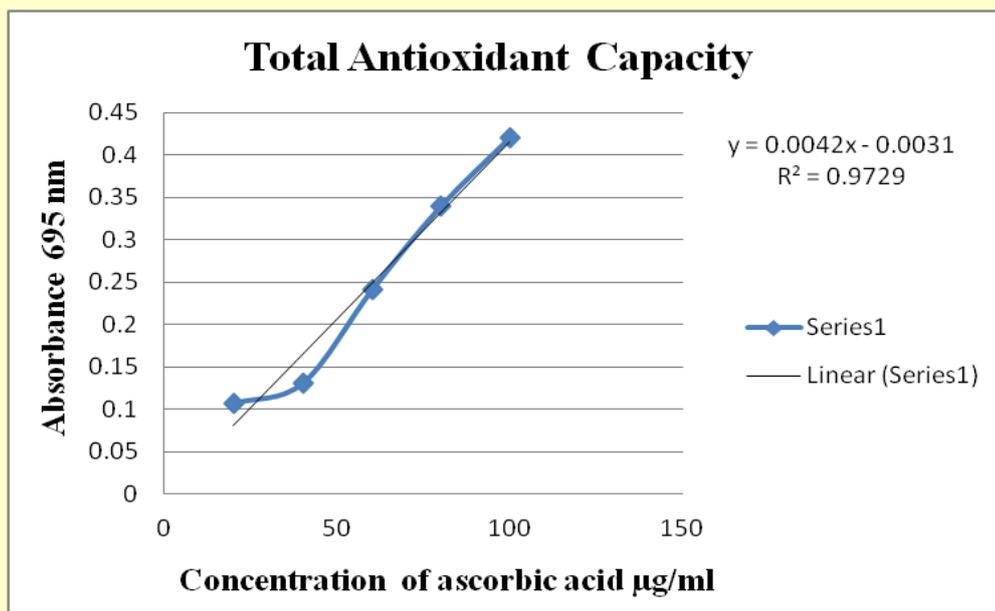
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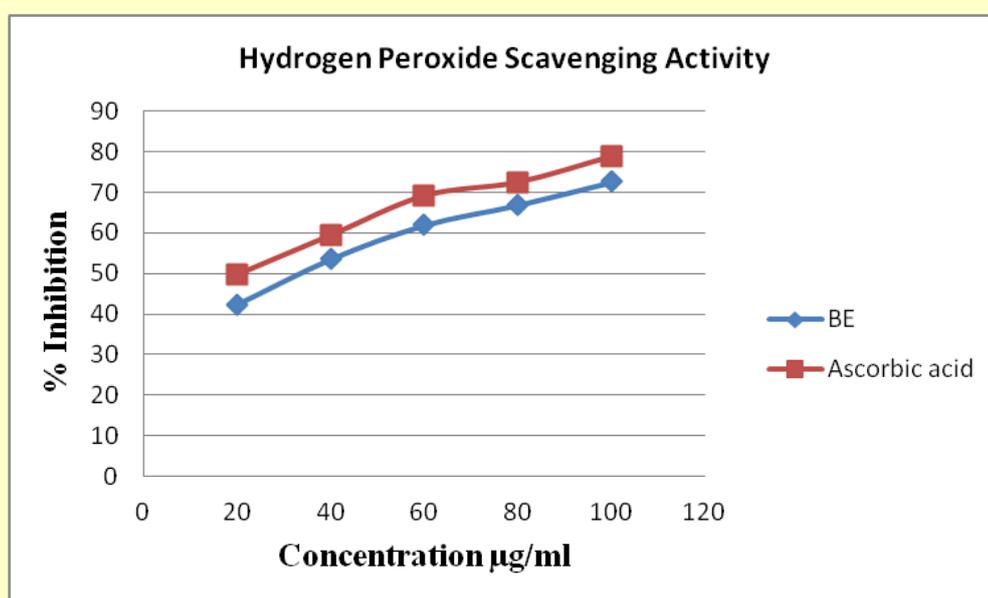
**Table 1: IC<sub>50</sub> value of *in-vitro* antioxidant activities betel leaves (BE) extract**

S.No.	Sample	H <sub>2</sub> O <sub>2</sub> (µg/ml)	Nitric oxide (µg/ml)	ABTS (µg/ml)
1	BE	37.48±2.85	53.24±2.39	57.74±2.05
2	Ascorbic acid	19.45±3.66	32.93±2.42	-
3	Trolox	-	-	49.51±2.12

Mean ± SD, (n=3)



**Fig. 1: Antioxidant ability assays of betel leaves extract**



**Fig. 2: Hydrogen peroxide scavenging assay betel of leaves extract**

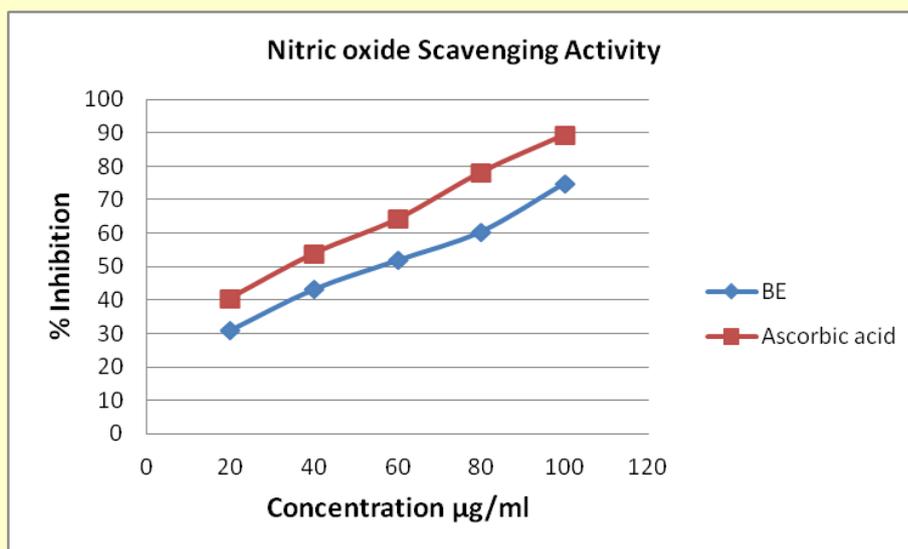


Fig. 3: Nitric oxide radical scavenging assay betel of leaves extract

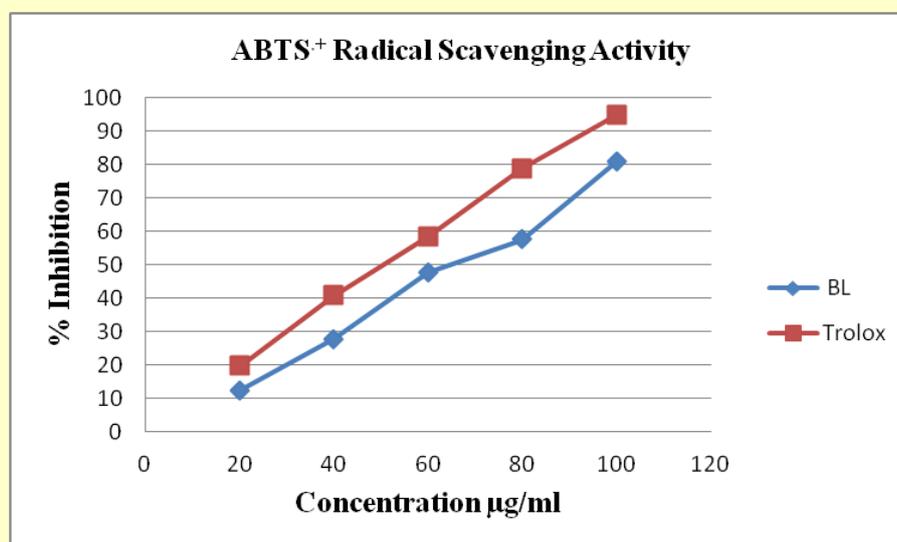


Fig. 4: ABTS. + discoloration assay of betel leaves extract