

**ACETYLCHOLINESTERASE INHIBITORY PROPERTY OF *PIPER BETLE* L. LEAVES**

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35 Ballygunge Circular Road, Kolkata 700019\*Author for correspondence: [bratatide@hotmail.com](mailto:bratatide@hotmail.com)**Summary**

*Piper betle* L. (Piperaceae) leaves are widely used as masticatory in Asia. The leaves have many medicinal properties. Acetylcholinesterase inhibitory properties of three local varieties of *P. betle* leaves, Kaliganga, Meetha and Haldi are reported. Aqueous extracts of both fresh and dry leaves of all the varieties of *P. betle* leaf studied inhibited acetylcholinesterase activity in a dose dependent manner. The activities in different species were significantly different. It was observed that the local variety kaliganga had the highest activity. Lowest activity was observed in Haldi variety.

**Key words:** Acetylcholinesterase inhibition, *Piper betle*, leaf

**Introduction**

*Piper betle* L. (Piperaceae) leaves are widely used as masticatory in Asia. Medicinally the leaves are useful in catarrhal and pulmonary affections (1). The leaves showed activity against obligate oral anaerobes responsible for halitosis (2) and antifungal activity (3). The leaf extract has significant stimulatory influence on pancreatic lipase activity in experimental rats (4). The leaf extract inhibited the radiation induced lipid peroxidation process. The extract also increased the activity of superoxide dismutase activity in a dose dependant manner indicating elevation of antioxidant status in Swiss albino mice (5). *P. betle* leaves also afforded a significant hepatoprotective effect and improved the tissue antioxidant status by increasing the levels of nonenzymatic antioxidants (reduced glutathione, vitamin C and vitamin E) and the activities of free radical detoxifying enzymes in liver and kidney of ethanol treated rats (6). *P. betle* leaf extract inhibited platelet aggregation via both antioxidative effects and effects on thromboxane B2 (TXB2) and prostaglandin-D2 (PGD2) production (7). Piperbetol, methylpiperbetol, piperol A and piperol B, isolated from *P. betle*, are effective platelet activating factor (PAF) receptor antagonists *in vitro* (8). The free radical scavenging effect and prevention of lipid peroxidation by three varieties of *P. betle* leaf have been reported (9). The leaf extract also demonstrated significant schizonticidal activity in all three antimalarial evaluation models (10), antileishmanial activity (11), antifilarial activity and immunomodulatory efficacy (12), anti-giardial activity (13), anti-amoebic activity (14). The leaves are reported to possess anti-inflammatory (15),

antidiabetic activity (16), have chemopreventive potential against liver fibrosis (17). In this paper we report acetylcholinesterase inhibitory property of *P. betle* leaf.

### Materials and methods

#### Plant material

The three local varieties of *P. betle* leaves, Kaliganga, Meetha and Haldi, were collected from Kolkata and Midnapore (Fig. 1).



Fig. 1 Three local varieties of *P. betle* leaf

#### Preparation of plant extract

The infusion, prepared from the fresh and air dried leaves by boiling in distilled water for 5 minutes, centrifuged and the supernatant was used for analyzing acetylcholinesterase inhibitory activity *in vitro*. Each experiment was repeated three to five times.

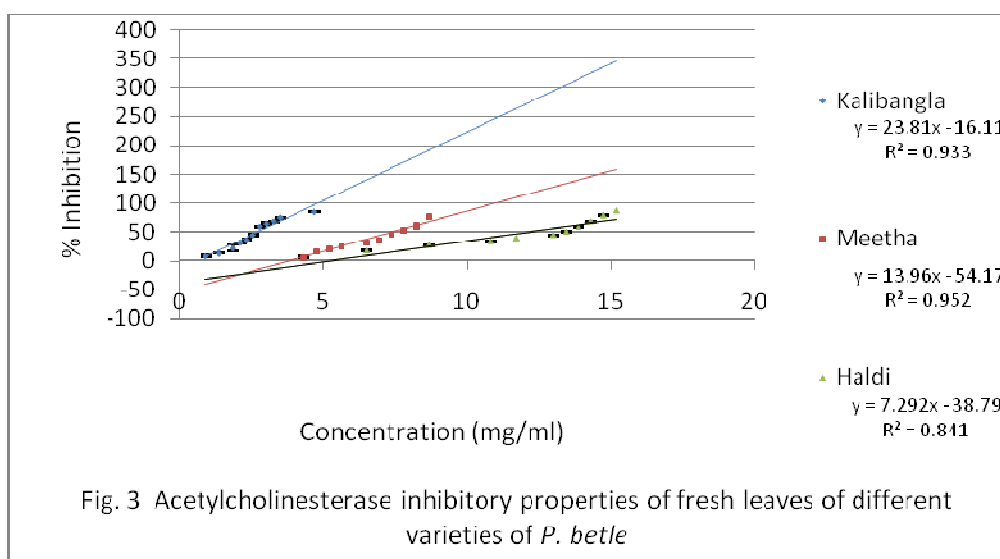
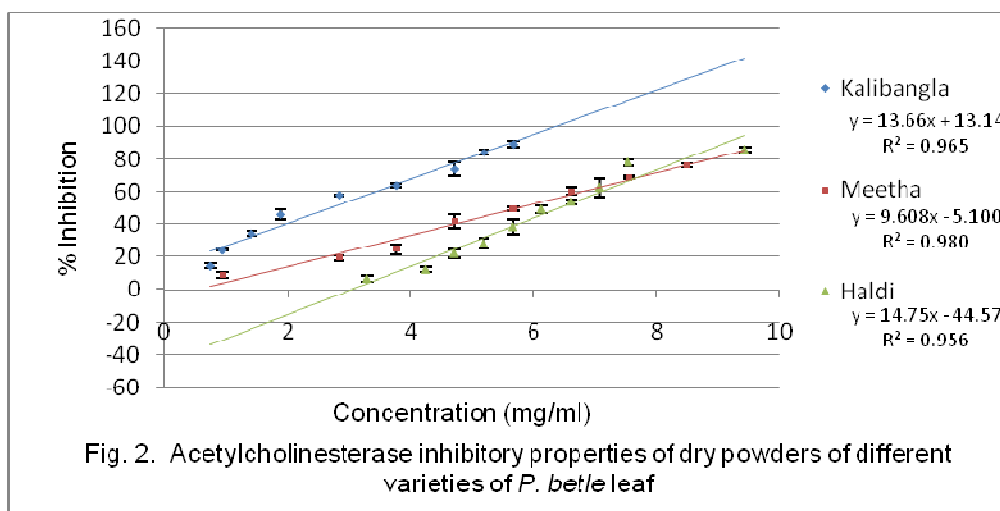
#### Acetylcholinesterase inhibitory activity

Acetylcholinesterase inhibitory property was measured modifying the method of Ellman et al.(18) following Oh et al. (19) and Siqueira et al. (20). AChE from electric eel was used for assay. Different concentrations of aqueous extracts of plant extract (0.01ml) were added to 0.02 ml AChE (19.93 unit/ml buffer, pH 8) and 1ml of buffer. The reaction was started by adding 0.01 ml 0.5 mM 5,5' dithiobis (2 nitrobenzoic acid) (DTNB) and 0.02 ml. 0.6mM acetylthiocholine iodide solution. The reaction mixture was incubated at 37<sup>0</sup>C for 20 min. The optical density was measured at 412 nm immediately. The percentage inhibition of AChE activity by plant extract was calculated.

### Results and discussion

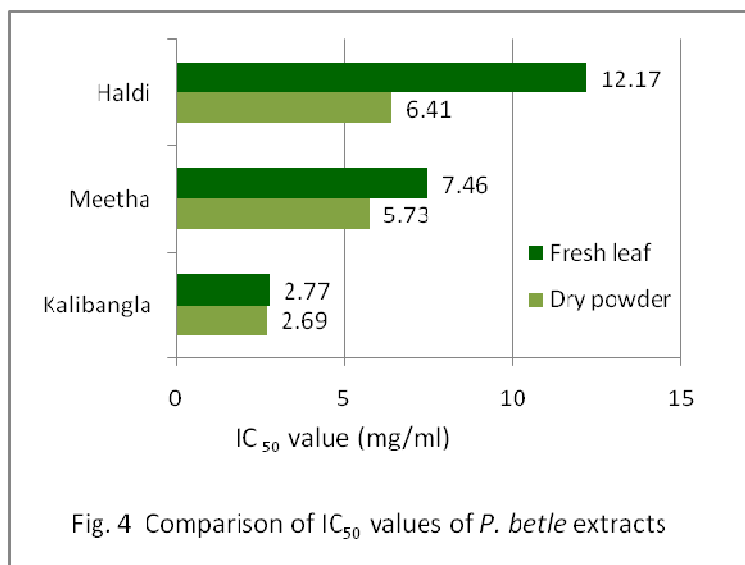
Regression equations were prepared from the concentrations of the extracts and percentage inhibition of AChE activity. IC<sub>50</sub> values (concentration of sample required for 50% inhibition of enzyme activity) were calculated from these regression equations. Aqueous extracts both fresh

and dry leaves of all the varieties of *P. betle* leaf studied inhibited AChE activity in a dose dependent manner (Fig. 2, Fig. 3) (squared correlation being > 0.9). One-way ANOVA and Dunnett's Multiple Comparison Test reveal that the activities in different species were significantly different. IC<sub>50</sub> values of the three varieties of *P. betle* leaves were compared (Fig. 4). IC<sub>50</sub> value is inversely related to the activity. It was observed that the local variety kalibangla had the highest activity. Lowest activity was observed in Haldi variety.



The neuropathological occurrence associated with memory loss is a cholinergic deficit which has been correlated with the severity of Alzheimer's disease (AD) (21-23). Approaches to enhance cholinergic function in AD have included simulation of cholinergic receptors or prolonging the availability of acetylcholine (ACh) released into the neuronal synaptic cleft by inhibiting ACh hydrolysis by acetylcholinesterase (AChE); the latter may be achieved through the use of AChE

inhibitors (24). The AChE inhibitory property of *P. betle* may have beneficial effect on memory function. The activity should be further studied in vivo.



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