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Short Communication

Anti-Lipoxygenase (LOX) activity of *Dendrobium macrostachyum* Lindl.

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ABSTRACT

The Ethyl acetate (EtOAc), n-Butanol (n-BuOH) and aqueous fractions of ethanolic stem extract of *Dendrobium macrostachyum* Lindl. were screened in vitro for anti lipoxygenase (LOX) property. Anti lipoxygenase activity was carried out as described by Lyckander and Malterud. Although all fractions exhibited anti lipoxygenase activity in comparison with reference standard Non-Steroidal Anti-Inflammatory Drug (NSAID) Indomethcin (IC_{50}=272.73µg/mL), the EtOAc fraction (IC_{50}=232.58µg/mL) showed highest anti lipoxygenase activity than n-BuOH (IC_{50}=629.95µg/mL) and aqueous (IC_{50}=325.87µg/mL) fractions. IC_{50} was calculated from the concentration–effect regression lines. The results obtained in the present study indicates that the anti lipoxygenase property may be one of the mechanisms behind the medicinal claims of the plant being used in the treatment of earache and to relieve pain.

Keywords: *Dendrobium macrostachyum*; Anti lipoxygenase activity; LOX; Orchidaceae; Dendrobium; Radam; Marathilotti

INTRODUCTION

The genus Dendrobium with 1190 species is one of the most important genera in the orchid family Orchidaceae, because of their therapeutic applications (Bulpitt C J *et al*., 2007). *Dendrobium macrostachyum* Lindl (Common name: Radam / Marathilotti) found profusely in plains is one of the widespread Dendrobium species of orchids of South India. It is an epiphytic herb, stem tufted, leaves membranous and deciduous during flowering season with small green flowers and narrow petals (Andre Schuiteman, 2011; Nimisha and Hiranmai Yadav, 2011; Abraham and Vatsala, 1981).

This plant is used as a painkiller by tying plant materials overnight on the parts of body to relieve from pain. The tender shoot tip juice expressed from the plant is warmed and instilled in the ear to mitigate earache (Mohammad Musharof Hossain, 2011; Rama Chandra Prasad P *et al*., 2008). Presence of alkaloids, leaf flavonoids, sterols, glycosides, tannins and phenols were reported on our initial study (Nimisha and Hiranmai Yadav, 2011). Ethanolic stem extract were found to possess good antioxidant activity in all the in-vitro model systems (Nimisha and Hiranmai Yadav, Unpublished data). The possible mechanism and bioactive principles behind the pharmacological potential of this plant is exploring in our laboratory.

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MATERIALS AND METHODS

Plant Material and Extraction

The plant materials collected in June 2011 from Melattur (North Kerala, India), was identified and a voucher specimen (5/23/2011-12/Tech.785) was deposited in the Herbarium, Southern Regional Centre, Botanical Survey of India. Dried ground stem of 50grams were extracted in Soxhlet extractor sequentially in 150ml in varying polarity (Petroleum Ether, Ethanol, Methanol, Water). The successive ethanol extract was selected for further fractionation based on its antioxidant property. The ethanolic residue suspended in water and partitioned with EtOAc and n-BuOH sequentially to give three fractions. All the three fractions were screened for anti lipoxygenase activity.

In vitro Lipoxygenase inhibition assay

Anti-lipoxygenase assay was studied using linoleic acid as substrate and lipoxygenase (Soybean) as enzyme (Shinde U A *et al*., 1999). Samples were dissolved in 2M borate buffer (pH 9.0) and incubated for 5 min at 25°C after adding lipoxygenase enzyme solution. Then substrate linoleic acid solution was added, mixed well and absorbance was measured at 234nm. Indomethcin was used as reference standard. The percent inhibition was calculated from the following equation:

$$\% \text{ Inhibition} = [\{Abs_{Control} - Abs_{Sample}\} / Abs_{Control}] \times 100$$

IC_{50} values, the concentration of sample required for 50% of maximum scavenging capacity was determined

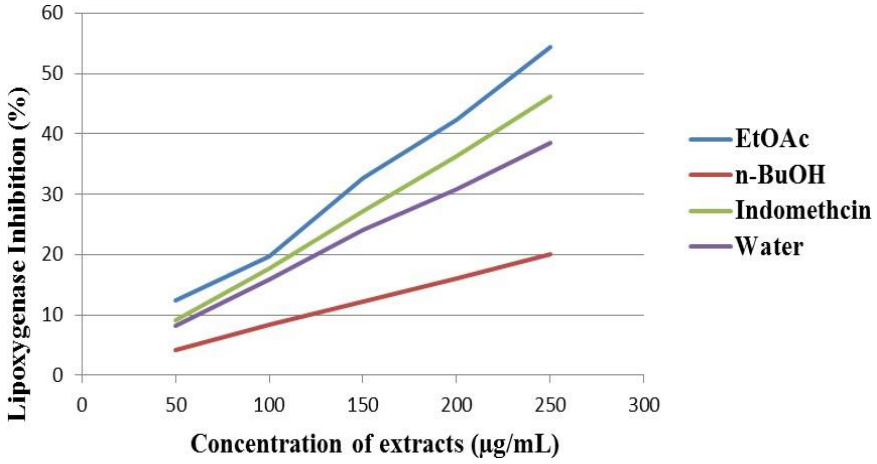

Figure 1: Lipoxygenase Inhibitory Activity of different fractions

Table 1: Anti-lipoxygenase activity of different fractions

Concentration (µg/mL)	EtOAc	n-BuOH	Water	Indomethcin[a]
50	12.4	4.17	8.24	9.16
100	19.72	8.38	15.79	17.65
150	32.64	12.26	24.05	27.09
200	42.31	16.05	30.8	36.22
250	54.39	20.03	38.43	46.17
IC_{50}[b]	232.5846465	629.9459209	325.8696561	272.7286568

[a]Reference Standard; [b]IC_{50} the concentration of extract required for 50% inhibition (µg/mL); Results were expressed as the mean ± S.D. of three replicates.

from the concentration–effect regression Curves. Each sample was done in triplicate and averaged.

RESULTS AND DISCUSSION

Successive Ethyl acetate, n-Butanol and aqueous fractions of ethanolic stem extracts of *Dendrobium macrostachyum* were assayed at 50, 100, 150, 200 and 250 µg/ml. All the fractions significantly inhibited the lipoxygenase activity (Fig 1), but the ethyl acetate fraction exhibited strong lipoxygenase (LOX) inhibitory activity than standard. Successive fractions showed IC_{50} values (Table 1) of 232.58, 629.95, 325.87 and 272.73 µg/ml respectively for EtOAc, n-BuOH, H_2O fractions and Indomethcin (reference standard).

Results of our findings confirmed the presence of active phytochemicals having significant anti-inflammatory properties. These activities may be due to presence of polyphenolic compounds such as alkaloids, tannins and steroids as previously reported (Nimisha and Hiranmai Yadav, 2011). These findings indicate a promising potential for the development of an anti-inflammatory agents from this plant.

CONCLUSION

The present study is hence successful in reporting the lipoxygenase inhibitory mechanism of pharmacologically unscreened plant. Also this study elucidates the possible contribution of *Dendrobium macrostachyum* as an easily accessible source of anti-inflammatory constituents and supports the folkloric usages of this plant. Therefore, further isolation of bioactive elements in the fractions would help to determine the individual potency of the compounds.

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