

ABSTRACT

The Indian Sarsaparilla, *Hemidesmus indicus* (Asclepiadaceae) locally known as “Anantamul” is an endemic medicinal plant. The roots of *H. indicus* are widely used in traditional Indian systems of medicine as blood purifier, diuretic, antirheumatic and antidiarrheal. Rootstock is woody and fragrant. An unusual phenolic compound, 2-hydroxy-4-methoxybenzaldehyde (MBALD) was found to be the principal compound responsible for the root fragrance. Structurally MBALD is analogous to another fragrance compound vanillin, which is a 4-hydroxy-3-methoxybenzaldehyde. No information is available on the biosynthesis of MBALD in *H. indicus* roots. Even, the biosynthesis of the well known vanillin is incomplete at the enzymatic level. Many bioactive properties of overall root extract of *H. indicus* were explored, however, limited reports are available on MBALD as a potent bioactive compound. Therefore, the aim of this thesis is to elucidate the enzymatic route to MBALD biosynthesis in *H. indicus* root along with evaluation of bioactive capacity of this phenolic aldehyde, MBALD. Profiling of methanolic extract of this root showed high accumulation of MBALD. Shikimate pathway inhibition with glyphosate showed lower accumulation of MBALD, thus indicating MBALD biosynthesis was modulated by shikimate pathway. Further studies using *in vitro* assays with desalted protein extract showed involvement of shikimate dehydrogenase (SKDH), phenylalanine ammonia-lyase (PAL), a C₂-chain cleaving hydroxybenzaldehyde synthase (HBS), hydroxybenzaldehyde-2-hydroxylase (HB2H) and 4-*O*-methyltransferase (4-OMT) enzymes in MBALD biosynthesis. Reduced accumulation of MBALD upon inhibition of PAL with aminooxyacetic acid (AOAA) and inhibition of HB2H with 1-aminobenzotriazole (1-ABT) confirmed the involvement of these two enzymes in MBALD formation. Profiling of major cell wall-bound phenolics of *H. indicus* roots were also carried out and their accumulation in the *H. indicus* root was correlated with the MBALD biosynthetic pathway. In this context an unusual coniferaldehyde dehydrogenase activity was found in cell free enzyme extract of the root. Bioactivity studies showed that both the crude alcoholic root extract and MBALD have potential tyrosinase and acetylcholinesterase inhibitory properties. Exact inhibition type for tyrosinase inhibition could not be explored but kinetic study showed acetylcholinesterase inhibition with MBALD was mixed type of inhibition.

Key words: Elicitation, *Hemidesmus indicus*, 2-hydroxy-4-methoxybenzaldehyde, anti-tyrosinase, anti-acetylcholinesterase