ABSTRACT

Hoppea fastigiata, an annual medicinal herb belonging to Gentianaceae is mostly found in South-Asian countries. The genus possesses unique class of compounds called xanthones, which are known for their potential against Alzheimer's and Parkinson's diseases. Limited availability and potential pharmacological significance of plants leads to the establishment of *in vitro* cultures of *H. fastigiata*. Three major xanthones were isolated and structurally confirmed as 1,5,7-trihydroxy-3-methoxyxanthone (1), 1,5-dihydroxy-3,7-dimethoxyxanthone (2) and 1,3,5-trihydroxy-8-methoxyxanthone (3) from shoot cultures which showed potential inhibitions against AChE, MAO A and MAO B enzymes. Upon treatment with yeast extract (YE), a 20-fold increase of 1,3,5-trihydroxy-8-methoxy xanthone was observed. We demonstrated that the YE treatment was followed by a rapid burst of reactive oxygen species (ROS, O_2^- and H_2O_2) and the subsequent increase in xanthone contents. The antioxidant enzymes (NADPH oxidase, superoxide dismutase, peroxidase and catalase) followed a similar kinetics as that of ROS, depending on their role in production or degradation. Upon YE treatment, shikimate dehydrogenase (SKDH) and shikimate kinase (SK) activities were enhanced after 8 h, benzophenone synthase (BPS) activity continued to rise after elicitation and peaked at 18 h. Phenylanine ammonia-lyase (PAL) activity suppressed and 4-coumarate: CoA ligase (4CL) activity remained unaffected after elicitation. This suggested phenylalanine-independent biosynthesis of xanthones. Subsequent treatment of shoots cultures with a NADPH-oxidase inhibitor diphenylene iodide (DPI) and a ROS-scavenger dihydrolipoic acid (DHLA) showed inhibition in the accumulation of O_2^- and H_2O_2 and decrease the activities of SKDH and SK enzymes, leading to a lesser amount of xanthones formation. Although O_2^- showed continuous increase upon treatment with a SOD inhibitor diethyldithiocarbamic acid (DIECA), the contents of H_2O_2 and xanthones were found to be decreased which correlates well with the reduced activities of SKDH and SK enzymes. Treatment with calcium antagonists, such as, lanthanum chloride (La) and ethylene glycol-bis-(β-aminoethyl) ether-N, N, N', N'-tetracetic acid (EGTA) were also shown to block the activities of SKDH, SK, NADPH-oxidase and SOD, leading to suppressed accumulation of O_2^- and H_2O_2 and xanthones. Thus, calcium mediated generation of H_2O_2 followed by the activation of shikimate pathway enzymes are the key early steps of xanthone biosynthesis in *H. fastigiata*.

Keywords: *Hoppea fastigiata*, Shoot cultures, Xanthones, Acetylcholinesterase inhibitory activity, Monoamine oxidase inhibitory activity, Reactive oxygen species