ISOLATION OF NADP+ GERANIOL DEHYDROGENASE FROM *Polygonum minus*.

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INTRODUCTION

Polygonum minus, locally named ‘kesum’ in Malay, is a plant having a kind of sweet and nice aroma. It comes from the family of Polygonaceae (Burkill 1966). From metabolite profiling results, long chain aldehydes and terpenoids, including geraniol and geranial were found in the essential oils of kesum. These secondary metabolites have many useful applications in the manufacture of food, pharmaceuticals, natural pesticides, and medicines. Therefore, the isolation and characterisation of geraniol dehydrogenase is important in order to enhance the economical values of these monoterpenes especially in nutraceutical and biopesticide industries. Geraniol and geranial are usually used as flavour and fragrance compounds in food, beverages, perfumes, cosmetics and in a broad range of cleaning products and also exert antimicrobial, antioxidant, immunostimulant and anticarcinogenic effects [4]. The conversion of geraniol into geranial is catalysed by $NADP^+$-geraniol dehydrogenase (EC 1.1.1.183). In plants, geraniol dehydrogenase activities were found only in *Citrus sp., Cymbopogon flexuosus, Ocimum basilicum, Vitis vinifera* and *Zingiber officinale* [2]. The objectives of this research are to identify and optimise the extraction method of geraniol dehydrogenase from *P. minus* leaves.

METHODS

Enzyme extraction was performed from leaves using modification of Sangwan et. al. (1993). *P. minus* geraniol dehydrogenase was extracted with 50 mM, 100 mM and 200 mM tricine NaOH buffer (pH 7.5) containing antioxidants (mercaptoethanol), osmotic reagent (glycerol), protease inhibitor (PMSF), chelating agent (thiourea) and a mixture of additives for adsorption of phenolic compounds, half of the tissue weight of PVPP or PVP-10 and quarter of the tissue weight, half of the tissue weight and without amberlite XAD-4. The standard enzyme reaction mixture (1.5 ml) contained 100 mM glycine-NaOH (pH 9.5), 0.2 mM $NADP^+$ and 2 mM geraniol and an appropriate amount enzyme. One unit activity is defined as the amount of enzyme that catalyses the formation of 1 µmol of NADPH per min under assay conditions. The activity will be calculated using extinction coefficient of 6,200 $M^{-1} \cdot cm^{-1}$ for NADPH at 340 nm. To chemically identify the products formed in the enzyme-catalysed reactions, the same enzyme assay described above was performed. After 30 min, 3 h, and overnight reactions at 30 °C, hexane was added to the tube, vortexed briefly, and centrifuged at 1500 x *g* for 30 min to separate the phases. The hexane layer was directly placed into a gas-chromatography-mass spectrometry (GC-MS) analysis. Compounds separated on the column were identified by comparing their retention time and mass fragmentation patterns with those of with those of authentic standards and library matches.

RESULTS AND DISCUSSION

P. minus geraniol dehydrogenase was best extracted with a 50 mM tricine NaOH buffer (pH 7.5) containing antioxidants (mercaptoethanol), osmotic reagent (glycerol), protease inhibitor (PMSF), chelating agent (thiourea) and a mixture of additives for adsorption of phenolic compounds, half of the tissue weight of PVPP and amberlite XAD-4. The optimum pH for geraniol dehydrogenase is 7.5 using 50 mM tricine-NaOH buffer (Fig. 1.a) while the optimum pH for enzyme assay is pH 9.5 using 100 mM Glycine-NaOH buffer (Fig. 1.b). This enzyme was highly thermostable, particularly at temperature above 30ºC (Fig. 2). The presence of geraniol was considerable and protects the enzyme from thermal inactivation [3].

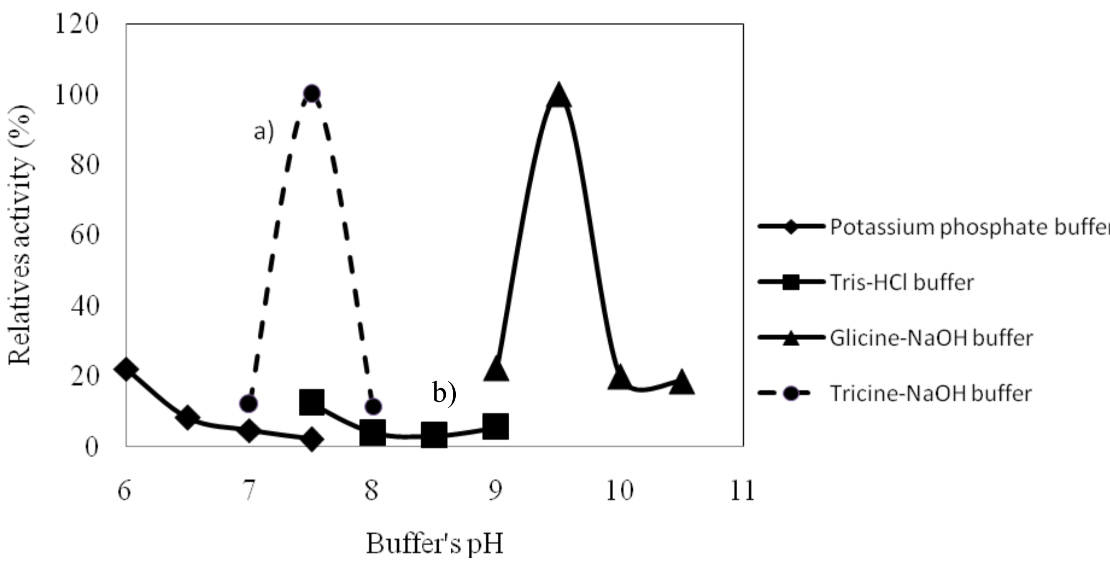

Figure 1. Effects of pH on the enzyme activity. Enzyme activity was assayed under the standard assay condition except that:

a) pH stability: Geraniol dehydrogenase was extracted by using 2.5 mM mercaptoethanol, 15% glycerol, 1 mM PMSF, thiourea and half of the tissue weight of PVPP and amberlite XAD-4 by using 3 different tricine-NaOH buffer pHs, pH 7, 7.5 and 8 (●).
b) pH optimum: Geraniol dehydrogenase was extracted by using 50 mM tricine-NaOH buffer, pH 7.5 containing 2.5 mM mercaptoethanol, 15% glycerol, 1 mM PMSF, thiourea and half of the tissue weight of PVPP and amberlite XAD-4. Enzyme activity was assayed under the standard assay condition except that the following buffers were used at a final concentration of 100 mM in the incubation mixture: potassium phosphate buffer (♦), tris-HCl (■) and glycine-NaOH (▲).

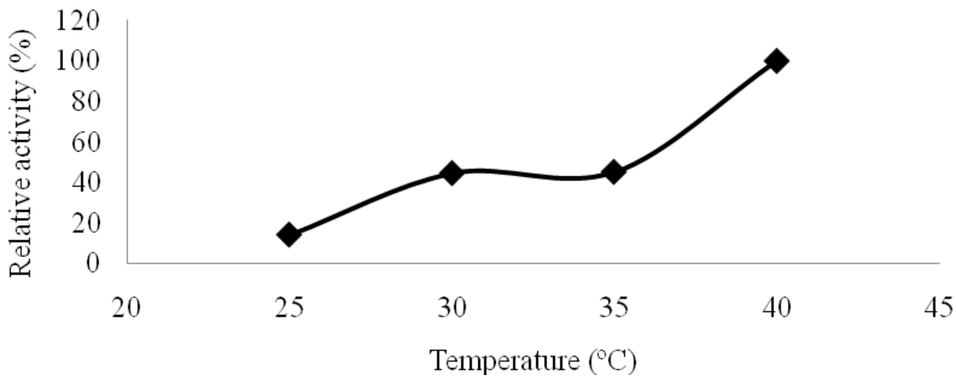

Figure 2. Effect of temperature on geraniol dehydrogenase activity. The enzyme activity was assayed under the standard assay condition except that the reaction temperature was varied.

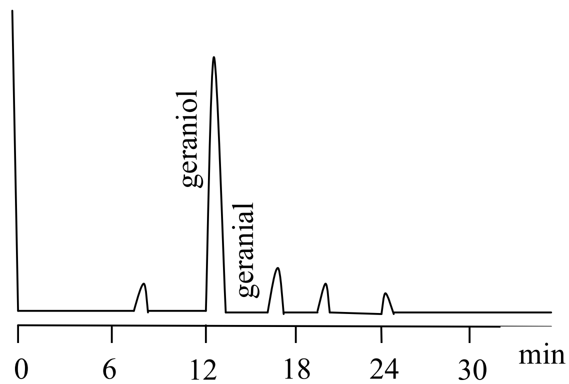

Figure 3. GC separation products generated by crude extract of geraniol dehydrogense from *P. minus* leaves.

The products from the oxidation of geraniol by crude enzyme extract of *P. minus* leaves with $NADP^+$ cofactor were analysed by gas chromatography mass spectrophotometer (GC-MS). The product in the reaction, geranial, confirmed that geraniol dehydrogenase was successfully extracted (Fig. 3).

CONCLUSION

Geraniol dehydrogenase from *P. minus* leaves were successfully extracted and optimised. The enzyme reaction product was identified by direct comparison with authentic geranial and library matches by GC-MS.

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