Abstract

Amaranthus tricolor L (At), commonly known as Joseph's coat or laal sak, is widely cultivated all over India and is a rich source of red-violet betacyanin. The plant is a common leafy vegetable and the pigment is industrially used as a food additive. The plant has also been reported to have plenty of health care properties, namely antioxidative, anti proliferative, hepatoprotective, anti-microbial, anti-amylase (anti-diabetic) etc. In view of its health care properties and industrial importance as a natural colorant producer this investigation of isolation, purification and characterization of betacyanins and betanidin glycosyltransferase was undertaken.

Betacyanins and other major metabolites were identified from *in vitro* plants using HPTLC, HPLC and LC-MS. Fifteen phenolics were identified by HPLC and another fifteen phenylpropanoids were identified by LC-MS. Total betacyanin content in leaves was found to be ~1.4 mg/g of dry weight. Amaranthin and isoamaranthin were isolated, and further deglycosylated to obtain betanidin and deglycosylation was further confirmed by LC-MS.

Betacyanins, phenylpropanoids and their glycosides identified in At were screened against receptor tyrosine kinases which are potential drug targets in cancer therapy. The investigation predicted that betanin, kaempferol glucoside, quercetin glucoside and rutin were better inhibitors of KIT kinase, Epidermal Growth Factor kinase, Vascular Endothelial Growth Factor r2 Receptor kinase and c Abl kinase than their respective aglycones. *In silico* prediction was also supported by experiments on MDA-MB 231 cell lines which show that rutin has better anti-proliferative properties than its aglycone quercetin.

Betanidin glycosyltransferase (AtUGT), the terminal enzyme for the synthesis of betacyanin was purified and characterized. The enzyme (63.8 kDa) showed optimum activity at pH 8.0 and at 30ºC. AtUGT was found to be active against betanidin, kaempferol, quercetin and caffeic acid among 11 substrates tested and could use UDP-glucose, UDP-galactose, UDP-N-acetylglucosamine and UDP-glucuronic acid as donor substrates. The apparent K_m values for kaempferol and UDP-glucose was found to increase from 344 ± 2.34 to 666.66 ± 3.51 µM and 4.08 ± 0.43 to 5.34 ± 0.23 mM. The apparent rate of reaction for kaempferol and UDP-glucose also increased from 17.24 ± 1.15 to 62.5 ± 1.39 µM/min and 2.51 ± 0.32 to 6.28 ± 0.89 mM/min respectively. Peptide mass fingerprinting analysis showed AtUGT's close resemblance to anthocyanin 3-O-glucosyltransferase from *Zea mays.* AtUGT was also partially sequenced by LC-MS.

Keywords: *Amaranthus tricolor* L; Betacyanin; Phenylpropnanoid; Receptor Tyrosine Kinase; Betanidin Glycosyltransferase.