Abstract

*Raphanus sativus*, a common cruciferous vegetable has been attributed to possess a number of pharmacological and therapeutic properties. Water, methanol, acetone, ethyl acetate, chloroform and hexane extracts derived from root, stem and leaves of *R. sativus* were evaluated for the presence of phytochemicals such as isothiocyanates (ITCs) and polyphenolics. These extracts were checked for antioxidant and radical scavenging activity, antimicrobial activity, protective effect against oxidative damage induced by oxidants and anti-proliferative activity towards human cancer cell lines such as HeLa, A549, MCF-7 and PC-3 cells.

Isothiocyanates (ITCs) were detected in considerable amount in root, stem and leaves of *R. sativus*. However, root extracts contained highest levels of ITCs, as compared to leaves and stem extracts. Highest amount of ITCs was extracted from root, when hexane was used as an extraction solvent. However, polar solvents were effective in recovering considerable amount of polyphenolics from *R. sativus*. *R. sativus* had total polyphenolic content, which was comparable to traditional rich source such as green and black tea. HPLC analysis indicated the presence of catechin, protocatechuic acid, syringic acid, vanillic acid, ferulic acid, sinapic acid, *o*-coumaric acid, myricetin and quercetin in *R. sativus*. Among different parts of *R. sativus*, leaves contained highest amount of phenolics and showed strongest antioxidant and radical scavenging activity. Of the different extraction solvents used, methanolic extract showed effective reductive capacity and significantly inhibited linoleic acid peroxidation, displayed metal chelating activity and effectively scavenged free radicals such as DPPH radicals, superoxide radicals, hydrogen peroxide and nitric oxide radicals respectively.

All extracts except water extract of root, stem and leaves had a significant broad spectrum inhibitory activity. Ethyl acetate extract of root had potent antibacterial activity with significant inhibition, comparable to that of standard antibiotics against pathogenic bacteria. This was followed by ethyl acetate extract of leaves and stem. Further, ethyl acetate extract of different parts of *R. sativus* retained their antibacterial activity after heat treatment at 100°C for 30 min and their antibacterial activity was enhanced when pH was maintained in the acidic range, demonstrating their thermal stability and acid tolerance properties. Compositional analysis of ethyl acetate extract of
root by GC-MS revealed the presence of polyunsaturated fatty acids (PUFAs), ITCs such as 4-(methylthio)-3-butenyl isothiocyanate (MTBITC) and 4-(methylthio)-3-butyl isothiocyanate (erucin), alkanes, eugenol and methyl cholesterol. Significant antibacterial activity of ethyl acetate extract of *R. sativus* root could be attributed to complex mixture of phytochemicals present in it.

*R. sativus* extracts per se showed no cytotoxicity and genotoxicity to lymphocytes. Of the different extracts studied, hexane extract of root and methanolic extract of stem and leaves showed significant protective effect against cell death and oxidative DNA damage induced by H₂O₂ in a dose dependent manner. The protective effect afforded by *R. sativus* extracts could be related to presence of isothiocyanates and polyphenolics, as they possess significant capacity to remove reactive species by virtue of their ability to induce antioxidant enzyme system in cells.

Significant growth inhibitory effect towards cancer cells was observed with hexane extract of *R. sativus* root. Analysis of hexane extract by GC-MS revealed the presence of different isothiocyanates (ITCs), with MTBITC and erucin being the predominant ITCs. Hexane extract induced cell death both in p53 proficient and p53 deficient cell lines through induction of apoptotic signaling pathways regardless of p53 status of the cells. The molecular mechanisms underlying *R. sativus*-induced apoptosis may involve interactions among Bcl-2 family genes, as evidenced by up-regulation of pro-apoptotic genes and down-regulation of anti-apoptotic genes along with activation of caspases-3. Our findings present the first evidence that hexane extract of *R. sativus* root exerts potential chemopreventive efficacy through growth inhibition and induction of apoptosis in various human cancer cells.

Our findings suggest the use of *R. sativus* (roots and aerial parts) in functional foods and food supplements designed for prevention of various chronic diseases including cancer. However, further studies are needed to prove that the protective effects observed *in vitro* do indeed translate *in vivo*.