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Characterization of phenolics by HPTLC and evaluation of antioxidant activity in millet grains

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Several scientific evidences have proved that millets as commonly consumed in India reduce the risk for chronic diseases including cancer and heart disease. Millets provide a wide range of nutrients and phytochemicals that may work synergistically to optimize human health. It is believed that high content of antioxidant compounds is the key to such protection. There is a need to optimize selective and sensitive methodologies to accurately quantify the levels of these phytochemicals such as phenolic acids and flavonoids in millets. The present study was undertaken to evaluate millet grains viz. foxtail, little, finger & barnyard millet, these grains were analyzed for the Total Phenolic Content (TPC), Total Flavonoid Content (TFC) and Antioxidant Activity (AOA) by spectroscopic method. The antioxidant activity of the samples were analyzed by radical scavenging activity by using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay, expressed in terms of IC50 value. The identification and characterization of phenolic acids was determined by chromatographic analysis with the aid of analytical equipment High Performance Thin Layer Chromatography (HPTLC). TPC was analyzed by Folin-Ciocalteu reagent assay method and expressed in terms of gallic acid equivalent (GAE µg/g) and TFC was analyzed by spectroscopic method and expressed in terms of rutin equivalent (RE µg/g) using ethyl alcohol (70% v/v) as extracting solvent. High amount of TPC (410 µg/g) and TFC (358 µg/g) was observed in finger millet, whereas low amount of TPC (275 µg/g) and TFC (199.1 µg/g) was observed in barnyard millet. However, the sequence of radical scavenging activity (IC50 value) observed in the samples was in the sequence finger millet>foxtail millet>barnyard millet>little millet. HPTLC was performed on silica gel 60 F254 (0.25 mm) aluminum backed TLC plates as stationary phase with automatic sample applicator using the 10 µl micro syringes. The chromatographs were developed in the mobile phase comprising of ethyl acetate, toluene, formic acid, methanol (6:6:1.6:0.4 v/v), chromatographs obtained were evaluated using TLC scanner with scanning at λ=254 nm. Post-chromatographic derivatization was done with natural product reagent (spraying) and the Rf values obtained for reference standards (ferulic acid, caffeic acid and syringic acid) were compared with the Rf values of the bands for the sample extracts. The peak height and area were utilized for evaluation and quantification of phenolic acids. An attempt has been made here to develop a simple, precise and accurate HPTLC chromatographic method to evaluate the phenolics in millet grains, which can be easy and fast for the quantification of phenolic compounds.

Biography

Giridhar Goudar has obtained his MSc (Biochemistry) degree from Karnataka University Dharwad. He is working as a Research Associate in ICAR-NAIP project on Enrichment and Popularization of Potential Food Grains for Nutraceutical Benefits at University of Agricultural Sciences, Dharwad.

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