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Isolation and Chemical Characterization of Individual Bioactive Compounds in Selected Malaysian Plants

Muhammad Khan, Azhari Hamid Nour, Farhan Haidar Bangash
Faculty of Industrial Sciences and Technology
University Malaysia Pahang Gambang, 26300, Pahang, Malaysia
muhammadkhan1985@gmail.com; azharyhamid@yahoo.com

Ahmad Ziad Sulaiman
Faculty of Chemical and Natural Resources Engineering,
University Malaysia Pahang Gambang, 26300, Pahang, Malaysia
ziad@ump.edu.my

Faridah Kormin
Faculty of Science, Technology and Human Development,
University Tun Husseion Onn Malaysia, 86400 Parit Raja,
Batu Pahat, Johor, Malaysia
faridah_k@hotmail.com

Muhammad Mumtaz Khan
Faculty of Health Sciences, University of Haripur
Hattar Road Haripur, 22620, Pakistan
mumtaz.muhammadee@gmail.com

Extended Abstract

This study is concerned with the assessment of antioxidant activity and chemical composition of extracts made from nine little used legume seeds. The legume seeds include: *Lablab purpueus, Phaseolus vulgaris, Vigna radiata, Cicer arietinum, Vigna radiate, Vigna unguiculata*, *Cicer arietinum, Trigonella foenumgraecum,* and *Phaseolus vulgaris.* Two methods were used to evaluate antioxidant activity of ethanol extracts of these selected species: β-carotene bleaching assay and 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) method. Beta carotene bleaching assay was performed according to the method of Charoensiri et al. (2009). B-carotene solution was prepared by dissolving 0.2 mg of beta carotene in 1 mL chloroform, in the round bottom flask containing 0.02 mL linoleic acid and 0.2 mL 100% Tween 20. The mixture was evaporated at 40 ºC for 10 min using a rotary evaporator to remove chloroform. Then 100 mL of distilled water was added with vigorous shaking to form an emulsion. Then 5 mL of the emulsion was taken out and transferred into test tubes containing 0.2 mL of sample. All test tubes were placed in a water bath at 45 ºC for 2 h. The radical scavenging activity of the extracts from nine legume seeds under investigation was estimated according to the procedure modified by Braca et al. (2001). A 4 mL of 0.004% DPPH (dissolved in methanol) was mixed with 1 mL of plant extract of different concentrations (100, 200, 400, 600, 800 and 1000 µg/L). Butylated hdroxyanisole (BHA, 10-100 µg/mL) was used as reference standard. The mixture of 1 mL methanol and 1 mL DPPH solution was used as a control. The mixture was vigorously shaken and kept at room temperature for 30 min in a dark place. The experiment was carried out in triplicates and absorbance was recorded at 517 nm by using UV-visible spectrophotometer. DPPH was also used as a TLC spray to detect separated antioxidant compounds. The method was based on Gu, et al. (2009) experiment. The extracts were subjected to TLC on silica gel using the developing solvent system, n-hexane-toluene-ethyl acetate-formic acid (2:5:2.5:0.5). The

developed plates were allowed to stand in a fume hood until the solvents evaporated off. The plates were then sprayed with 2.0 mM DPPH solutions in methanol, when bands or spots with radical scavenging (antioxidant) activity immediately gave white and sometimes yellow colors on a purple background. Ethanol extracts of the five legume seeds showed antioxidant activity with chick pea (*Cicer arietinum*) exhibiting the highest activity. Germination did not increased antioxidant activity as measured by the β-carotene method, in five legumes. However, TLC bioautography clearly indicated that at least four compounds with radical scavenging activity have been biosynthesized during the germination of chick pea seeds (Fernandez-Orozco et al, 2009). The biosynthesized compounds were more polar compared to those present in ungerminated seeds. This broadens the solubility spectrum of chickpea antioxidants, an advantage for the food industry. The potential of commercially using legume flours in processed baked and meat products are indicated, adding antioxidant activity to other functionalities. Synergistic studies using the legumes of this study showed that the antioxidant activity of chick pea could be synergistically increased by addition of the extract of roots of G. gynandra.

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