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Antioxidant potential of some agri-horticultural wastes

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Abstract

Free radicals are known inducers of cellular and tissue pathogenesis leading to several human diseases such as cancer, inflammatory disorders, as well as in aging processes. Antioxidants provide protection to living organisms from damage caused by uncontrolled production of reactive oxygen species and the concomitant lipid peroxidation, protein damage and DNA strand breakage. There has been an increased concern over management of agri-horticultural waste to develop useful product and to prevent environment pollution. Some selected agri-horticultural wastes like left over residuals from *Trigonella foenum-graecum* (Fenugreek), *Brassica rapa* (Turnip), and *Brassica oleracea* (Cabbage) were studied for their antioxidant activity alone and in combination to find synergistic effect if any and to determine their nutraceutical potential. The antioxidant activity of residuals of fenugreek, turnip, and cabbage was 816, 864 and 545.6 µmTE/g whereas the antioxidant activity of their synergistic combinations of (fenugreek plus turnip), (fenugreek plus cabbage), (turnip plus cabbage) and all the three (fenugreek plus turnip plus cabbage) was found to be 808, 1140, 2456 and 1048 µmTE/g respectively. The synergy fold was found to be maximum in combination of turnip and cabbage with 3.4 fold. The present studies showed that agri-horticultural wastes have tremendous potential to be used as nutraceutical in the prevention and management of free radicals, oxidative stress and associated disorders.

Introduction

Oxidation is essential to many living organisms for the production of energy to fuel biological processes. Free radicals are produced in normal and/or pathological cell metabolism as a result of cellular oxidation and are known inducers of cellular and tissue pathogenesis leading to several human diseases such as cancer, inflammatory disorders, rheumatoid arthritis, cirrhosis, arteriosclerosis as well as in degenerative processes associated with aging processes. Exogenous chemical and endogenous metabolic processes in the human body or in the digestive system might produce highly reactive free radicals, especially oxygen derived radicals, which are capable of oxidizing biomolecules, resulting in cell death and tissue damage (Mau *et al*., 2001).

Antioxidants provide protection to living organisms from damage caused by uncontrolled production of reactive oxygen species and the concomitant lipid peroxidation, protein damage and DNA strand breakage. Several epidemiological and in vitro studies have supported the idea that plant constituents with antioxidant activity are capable of exerting protective effects against oxidative stress in biological systems which is among the major causative factors in induction of many chronic and degenerative diseases (Prakash *et al*., 2012; Sharma *et al*., 2014).

Food industry produces large volumes of wastes, both solids and liquid, resulting from the production, preparation and consumption of food. These wastes pose increasing disposal and severe pollution problems and represent a loss of valuable biomass and nutrients. Although beside their pollution and hazard aspects, in many cases, food processing wastes might have a potential for conversion into useful products of higher value as by-product, or even as raw material for other industries, or for use as food or feed after biological treatment (Schieber *et al*., 2001). This depends on the composition of wastes emerging from food processing factories and on the nature of the product and the production technique employed. Large amounts of fruit and vegetable processing wastes are produced from packing plants, canneries, etc., which may be disposed in several ways including immediate use for landfill or drying to a stable condition (10% moisture) in order to use an animal feed during out of season, or which, alternatively, may be processed biotechnologically in

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order to produce single cell protein. First choice is not economical, and the second one is expensive due to drying cost. Industry continues to make progress in solving waste problem through recovery of by products and waste materials such as peel, pulp, or molasses by the employment of fermentation process (Schieber *et al*., 2001).

From the above it is pertinent that there has been an increased concern over management of agri-horticultural waste to develop useful product and to prevent environment pollution. Thus present studies three agri-horticultural wastes like left over residuals from *Trigonella foenum-graecum* (Fenugreek), *Brassica rapa* (Turnip), and *Brassica oleracea* (Cabbage) were studied for their antioxidant activity alone and in combination to find the synergistic effect if any and to determine their nutraceutical potential.

Materials and Methods

Chemicals and materials

All the chemicals used in the assay are of analytical grade and were purchased from Sigma–Aldrich and E. Merck, India. The left over residuals of vegetables like *Trigonella foenum-graecum* (Fenugreek), *Brassica rapa* (Turnip), and *Brassica oleracea* (Cabbage) were collected from the local market of Delhi, NCR region (India) in the month of October- November 2014. The left-over residuals/ discards of these vegetables were cleaned, washed with sterile distilled water, chopped, dried, powdered (40-mesh) and stored in polythene bags at 4°C until further use.

Extraction of polyphenols from plant residuals

For the extraction of polyphenols from the agri-horticultural wastes of selected vegetables, 0.5g of each dried & grounded vegetable residuals (Fenugreek, Turnip and Cabbage) were soaked overnight in 10mL of 50% aqueous methanol at room temperature (cold maceration). The residues were filtered and the filtrate was used for the antioxidant assay. Before the assay, 1:50 dilutions of all the sample extracts were prepared.

Estimation of antioxidant activity

The antioxidant activity of selected plant residuals was determined by CUPRAC assay (Apak *et al*., 2004) along with some modifications. This method measures the copper (II) or cupric ion reducing ability of polyphenols, vitamin C and vitamin E. This is a simple and widely applicable antioxidant capacity index for dietary polyphenol, vitamin C and vitamin E. It makes use of the copper (II)-neocuproine [Cu(II)-NC] reagent as the chromogenic oxidizing agent.

Preparation of CUPRAC reagents

The CUPRAC reagents were prepared by method of Apak *et al*. (2004) along with some modifications. Cupric chloride (copper chloride) 10-2M was prepared by dissolving 0.1705 g of copper chloride in 100mL distilled water. This solution should always be freshly prepared before use. Neocuproine (Cu^{+2}) (7.5×10^{-3}M) was prepared by dissolving 0.1562g of neocuproine in 100mL ethanol. Ammonium acetate buffer (pH=7) was prepared by dissolving 7.7 g of ammonium acetate in 100mL distilled water.

Method of estimation

In a test tube, 1mL copper chloride solution, 1mL Neocuproine, 1mL ammonium acetate buffer and 1 mL of diluted (1:50) sample solution (total volume 4 mL) was added in the same sequence. The test tubes were kept at room temperature for 20 minutes and the absorbance was measured at 450 nm in a UV-Vis spectrophotometer against 50% aqueous methanol as a blank. Butylated Hydroxy Anisole (BHA), a synthetic antioxidant was used as a reference standard.

The antioxidant activity of residuals of fenugreek, turnip, and cabbage were first determined individually and then their combinations like fenugreek and turnip; fenugreek and cabbage; turnip and cabbage; and finally the combination of all three viz. fenugreek, turnip, and cabbage. This was done to check the synergistic effect in their antioxidant activity.

Calculation of antioxidant activity

The antioxidant activity was calculated in terms of Trolox equivalent (TE) through CUPRAC assay by using the following formula.

Capacity (in mmol TE/g) = $(A/\varepsilon\ TR)\ (V_f/V_s)\ r\ (V_t/m)$

Where A- absorbance

ε TR – Molar Absorptivity of Trolox = 1.67×10^4

V_f - Final volume made = 4 mL

V_s - Sample volume taken from diluted extract (mL)

Vt - Total volume (mL)

r - Dilution factor (if made)

m - Weight of the sample taken (g)

Table 1. Antioxidant activity of plant residuals of Fenugreek, Turnip and Cabbage

S. No.	Plant	Antioxidant activity $AOA_{(cal.)}$ (µmTE/g)	Synergistic AOA $_{(obser.)}$ (µmTE/g)	Synergistic fold (AOA_{obs}/ $AOA_{cal.}$)
1.	*Trigonella foenum-graecum* (Fenugreek residuals)	816	-	-
2.	*Brassica rapa* (Turnip)	864	-	-
3.	*Brassica oleracea* (Cabbage)	545.6	-	-
4.	*Trigonella foenum-graecum + Brassica rapa*	840	808	0.96
5.	*Trigonella foenum-graecum + Brassica oleracea*	680.8	1140	1.67
6.	*Brassica rapa + Brassica oleracea*	704.8	2456	3.4
7.	*Trigonella foenum-graecum + Brassica rapa + Brassica oleracea*	741.8	1048	1.4
8.	Butylated Hydroxy Anisole (Standard)	5028	-	-

Where TE- Trolox equivalent; AOA- Antioxidant activity

Results and Discussion

The antioxidant activity of the residuals of fenugreek, turnip, and cabbage calculated individually and synergistically is presented in Table 1. The antioxidant activity (AOAcal.) of residuals of *Trigonella foenum-graecum, Brassica rapa* and *Brassica oleracea* as calculated by CUPRAC assay was 816, 864 and 545.6µmTE/g respectively; whereas the observed antioxidant activity (AOAobs.) of their synergistic combinations of (*Trigonella foenum-graecum* + *Brassica rapa*); (*Trigonella foenum-graecum* + *Brassica oleracea*); (*Brassica rapa* + *Brassica oleracea*) and all three (*Trigonella foenum-graecum* + *Brassica rapa* + *Brassica oleracea*) was found to be 808, 1140, 2456 and 1048 µmTE/g respectively. The synergy fold was found to be maximum in combination of (*Brassica rapa* + *Brassica oleracea*) with 3.4 fold.

Although numerous studies have reported the antioxidant activity of the commonly used south Asian herbs but to the author's knowledge, this is the first study to report the antioxidant activity of plant residuals of commonly used vegetables fenugreek *(Trigonella foenum-graecum)*, turnip *(Brassica rapa)* and cabbage *(Brassica oleracea)*. In a study, the antioxidant activities of fenugreek seeds were reported. Their effects on reactive oxygen species (ROS) and superoxide dismutase (SOD) activity were also investigated. It was found that fenugreek extract had not increased the SOD activity of the HUVECs (human umbilical vein endothelial cells) (Waisundara and Hoon, 2013).

In another study on fenugreek seeds, three types of solvent extracts of fenugreek seeds were used to examine the effects of extraction solvent on total phenolics content (TPC), 1,1-diphenyl-2-picryl hydrazyl radical scavenging (DPPH) and ferric reducing antioxidant power (FRAP). The TPC for fenugreek seeds were found from 25.90 to 15.45mg GAE/100g DW and antioxidant activity FRAP ranged from 47.49 to 31.85mg TE/100 g DW, DPPH were from 67.30% to 43.61% (Mashkor, 2014).

Similarly, there are various studies that has proved that high intake of Brassica vegetables reduces the risk of age-related chronic illness such as cardiovascular health and other degenerative diseases including cancer. This is because of the antioxidant properties of different compounds present in this group. Brassica vegetables contain bioactive substances with a potential for reducing the physiological as well as oxidative stress-induced DNA damage. Brassica vegetables commonly include crops such as cabbage, broccoli, cauliflower, turnip, Brussel sprouts that are consumed all over the world (Soengas *et al*., 2011).

Keck and Finley (2006) showed that broccoli extracts reduced oxidative stress and inhibited DNA single-strand breaks in cultured cells (Hepa 1c1c7). The effect of broccoli extracts on the prevention of oxidation in a cellular system was also investigated by Roy *et al.* 2009. These authors demonstrated that broccoli extract gives significant cytoprotection in PC-12 cell line (neuroblastoma) and therefore it showed neuro-protective effect.

In yet another study, antioxidant potential of ethanolic root extracts of *Brassica rapa* were studied.

The ethanol extract possessed antioxidant potentials such as free radical scavenging, nitrite scavenging, and lipid peroxidation inhibitory activities as well as reducing power. The antioxidant potential showed a positive correlation with total phenolic content (Ryu *et al*., 2012).

They contain a variety of organic compounds with biological activity such as glucosinolates, phenylpropanoids, flavonoids, phenolics and organic acids (Fernandes *et al*., 2007). Turnip extract has been reported to possess hepatoprotective, nephron-protective, and anticancer effects (Rafatullah *et al*. 2006; Kim *et al.* 2006; Hong and Kim, 2008). The phenolic compounds such as kaempferol 3-O-soporoside-7-O-glucoside and isorhamnetin 3, 7-O-diglucoside were identified from leaves, stems and flower buds of turnip. In addition, 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity was screened in edible parts of a turnip (Fernandes *et al*., 2007).

Conclusions

Thus from the foregoing discussion, it may be safely concluded that the agri-horticultural waste residues of *Trigonella foenum-graecum, Brassica rapa* and *Brassica oleracea* possess antioxidant activity because of the presence of several bioactive phenolic constituents. Therefore, their left-over residuals of these vegetables can also be used as a potential source of nutraceuticals and for designing the various functional foods for regulating oxidative stress and other degenerative diseases. This would also help in the management of agri-horticultural wastes to combat pollution problems and waste disposal. However, more detailed studies are further required to identify the specific bioactive constituent(s) in these residuals.

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