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Total phenolic, flavonoid, β- carotene and *in-vitro* anti-oxidant activity of vegetable wastes collected from hotels and food processing centre

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The present study compares Total Phenolic Content, Total Flavonoid Content, β –carotene content and *in-vitro* antioxidant activity of hydro-methanolic (20:80) extract of three commonly available vegetable wastes namely tomato pomace (TP), skin of green pea pod(GP) and beet root peel (BP) from hotels and food processing center nearby Kolkata, India. BP showed significantly (P<0.05) higher level of total phenolic contents followed by GP and TP. On the other hand, GP showed highest (P<0.05) flavonoid content followed by TP and BP. This study revealed that β –carotene content in TP was significantly (P<0.05) more than in GP, although no β–carotene was detected in BP. DPPH assay for *in-vitro* anti-oxidant activity indicated highest antioxidant activity in BP followed by GP and TP. It is concluded that beet root peels might be used for producing functional food/feed additives due to its high anti-oxidant capacity and warrants further study in this regard.

Key Words : Antioxidants, β Carotene, DPPH, Flavonoid, Phenolic, Vegetable wastes

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INTRODUCTION

Recycling of agricultural wastes for producing nutraceuticals has recently attracted the researchers (Sonia *et al*., 2016 and Kumar *et al*., 2017) throughout the world.Plant polyphenols, containing phenolic ring structures with ability to donate H· and β-carotenes with their highly reactive conjugated double bonds,are well known for their scavenging activity on free radicalsthat manifests oxidative stress in living systems.Vegetable wastes have a good treasure of such antioxidant molecules (Peschel *et al*., 2006 and Sonia *et al*., 2016). These antioxidants may be of great use for wellbeing of animal health if judicially incorporated to animal feed as additives (Wadhwa and Bakshi, 2013).In India, huge fruits and vegetables wastesto the tune of 5.6 million tonnes/ annumis dumped regularly (Arvanitoyannis and Varzakas, 2008). Although, some research works (El-Baky and Ahmed, 2010; Babbar *et al*., 2014; Kabir *et al*., 2015) were conducted earlier onantioxidant properties of vegetable wastes, however very little is known, about antioxidant potential of vegetable wastes directly collected from hotels and food processing centers.

In this backdrop, the objective of the present study was to quantify and compare total phenolic content,

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flavonoid content, β-carotene and *in vitro* anti-oxidant activity of three common vegetable wastes, namely- tomato pomace, skin of green pea pods and beet root peels which were collected from hotels and food processing centers.

METHODOLOGY

Sample collection :

Tomato pomace was collected from a food processing center at Narendrapur, Kolkata (22.439^{0}N, 88.396^{0}E). Skin of green pea pod and beet root peel was collected from nearby hotels of Narendrapur. These waste materials (Table A) from vegetables were collected in sterilized plastic bins during the month of February – May'2017.

Sample preparation for extraction :

Following thecollection of samples, tomato pomace and peels were air dried under shade for five days. Peels of green pea and beet root were cut into smaller pieces with a sharp scissor and then made it into coarse powder using anelectrical grinder.

Preparation of liquid extracts for total phenolic content, total flavonoid content and anti-oxidant assay :

One gram of sample powder was extracted with 25ml of 80% (v/v) aqueous methanol (Merck,Germany) in 27°C. The mixtures were kept in anorbital shaker for 4hwith 110 rpm. The extracts were filtered through Whatman No.1 filter paper. The residues were re-extracted again with the same procedure under the same condition and filtered in the same way. The two extract fractions were pooled and final volume was adjusted to 50 ml in a volumetric flask. The extracts of each sample were placed in eppendrof tubes enveloped in aluminium foil in refrigerator (4°C) until further use.

Extraction by Soxhlet apparatus for estimation of -carotene :

Twenty gram of dried sample was extracted with 200 ml of ethanol in Soxhlet apparatus for five cycles. The extract was filtered by Whatman No.1 filter paper and volume was made with ethanol in a 50 ml volumetric flask. Yellow orange colored extract was transferred in dark bottles in refrigerator (4°C) until further use.

Estimation of total phenolic content :

Total Phenolic Contents (TPC) was measured by using Folin-Ciocalteu method (Singleton *et al*., 1999) with slight modifications. Briefly 500 µl of extracts were mixed with 2.5 ml of Folin-Ciocalteu reagent and incubated at 25°C at dark. Then 2.5 ml of 7.5% Na_2CO_3 solution was added and the mixture was incubated again at 25°C for 15 min. The absorbance of the samples were determined at λ_{max} = 765 nm using an automated UV/Visible spectrophotometer (Shimadzu 1800-UV, Japan). Blank was concomitantly prepared, with methanol instead of extract solution. Standard curve (Y=0.00821350X+ 0.0180054; r^2= 0.99850) was constructed by using 10, 20, 30, 40 and 50 mg/ml solutions of gallic acid.The total phenolic content was expressed in terms of gallic acid equivalent (mg of GaE/g of dry weight).

Estimation of total flavonoids content :

Total flavonoids content (TFC) was measured according to Pal *et al*. (2009) and Naskar *et al*. (2011). Briefly, quercetin solution (concentration range 10-60 mg/ml) was used to make a standard curve (equation: Y=0.00433906x; r^2 = 0.99167). The assay was determined using 0.5ml of each extract solution and each dilution of standard quercetin taken separately in test tubes. To each test tube 1.5ml methanol, 0.1ml aluminium chloride solution, 0.1ml potassium acetate solution and 2.8ml distilled water were added and mixed well. The mixture was incubated for 30 min in 27^{0}C and absorbance was measured at λ_{max} =415 nm by UV/Visible spectrophotometer (Shimadzu 1800-UV, Japan). Results were expressed in mg of quercetin equivalent (mg of QE/g of dry weight).

Estimation of carotene using HPTLC assay :

β-carotene of the samples was estimated by the method described by Das *et al*. (2017). High performance thin layer chromatographic (HPTLC) analysis was carried out on a HPTLC plate pre-coated with silica gel. Samples (10µl) and standards (10-25 µl) were applied on plates by Linomat 5 applicator (Camag, Switzerland). The plates were developed to a distance of 90mm in Camag twin-trough chamber withmobile phase of Petroleum ether: Acetone (70:30)in 27°C for 25 min. Afterwards, the plates were scanned for densitometry analysis in CAMAG TLC scanner (Camag, Switzerland) at λ_{max} =450 nm.The chromatograms were finally integrated using Win CATS

4.0 computer programme.

Determination of free radical scavenging activity:

In-vitro antioxidant activity was determined by DPPH assay (Szabo *et al*., 2007). Stock solution (1mg/ml) of extract and standard ascorbic acid (0.5mg/ml) were prepared using methanol. Various concentrations (10-50µg/ml) of the extract and ascorbic acid were taken in test tubes and 1ml of freshly prepared DPPH solution were added, the test tubes were protected from light by covering with aluminium foil. The final volume in each test tube was made to 2ml with methanol and incubated in dark chamber for 30 min in 27°C. After incubation, the absorbance was read at 517nm using a spectrophotometer (Shimadzu 1800-UV, Japan). Control sample was prepared containing the same volume of methanol and DPPH without any extract and reference ascorbic acid. Methanol was served as blank. Radical Scavenging Activity (RSA%) was calculated by using the following equation:

$$\text{Radical scavenging activity \%} = \frac{\text{1 - Absorbacne of sample}}{\text{Absorbance of control}} \text{ x 100}$$

RSA % values were used to calculate Inhibition Concentration at 50% (IC_{50}) values that denote the effective concentration of a sample required to decrease the absorbance at 517 nm by 50%. All measurements were performed in triplicate.

Statistical analysis :

All the measurements were done in triplicates and analysed by one way ANOVA using SPSS software version 16 (SPSS Inc., IBM) The mean values were compared by Duncan Multiple Range Test (Duncan, 1955) at 5% significance level.

OBSERVATIONS AND ASSESSMENT

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Total phenolic content and total flavonoid content :

Polyphenol molecules in plant species are well recognised for their wide and diversified role in maintaining normal plant physiology. In present study (Table 1), beet root peel (BP) showed significantly ($P<0.05$) higher level of total phenolic contents than that of the skins of green pea pod (GP) and tomato pomace (TP) in the order of BP>GP>TP. This result was in contrary to an earlier report (Babbar *et al*., 2014) that demonstrated highest phenolic contents intomato peel (21±0.40 mg GAE/g) followed by pea pod (13.6 ± 0.20 mg GAE/g), cauliflower waste (9.2 ± 0.60 mg GAE/g) and potato peel (5.4 ± 0.60 mg GAE/g). Silva *et al*. (2016) also recorded total phenolic content in tomato pomace in the range of 10.08-22.75 mg GAE/g dry matter. Kujala *et al*. (2001) reported higher total phenolic content (24.1±0.3mg GAE/g) of beet root peel than in the present study. This variation of total phenolic of vegetable waste extracts might be due to varietal difference and prevalent agronomic practices in different regions. Major phenolic compounds present in the beet root is betalains that has significant role in maintaining cellular integrity by free radical scavenging activity in living system (Singh and Singh, 2014). Present study confirmed that beet root peel contains large amount of phenolics.

Flavonoids are most important class of phenolics with proven health benefits. GP showed highest ($P<0.05$) flavonoid content followed byTP and BP in the present study (Table 1). There were dearth of literatures for comparing the result of total flavonoid content of GP with other researchers; however the results of TP were in agreement with Toor and Savage (2005) and Chandra *et al*. (2014). Total flavonoid content of BP was lower compared to an earlier report (EL-Baky and Ahmed, 2010) that stated beet root peel contained 1.17 QE mg/g dry weight.

-carotene content :

β-carotene is the precursor molecule of vitamin A. In poultry diet, dried tomato pomace is frequently used for β–carotene supplementation (King and Zeidler, 2004; Saemi *et al*., 2012). Earlier reports (Baranska *et al*., 2006 and Garande and Patil, 2012) indicated β-carotene content of tomato in the range of 0.23-2.83 mg/100 g fresh weight.

Table 1 : Description of vegetable wastes

Sr. No.	Vegetable wastes	Cultivar	Moisture content (%) after shed drying	Abbreviated As
1.	Tomato pomace	*Pusa Ruby*	7.28	TP
2.	Skin of green pea pod	*Bonneville*	8.46	GP
3.	Peel of beet root	*Local*	14.32	BP

Table 2 : Total phenolic content, total flavonoid content and β-carotene content of three vegetables wastes

	TP	GP	BP
Total Phenolic Content (mg of GaE/g of dry weight)	4.53 [a]±0.031	5.02 [b]±0.309	9.03 [c]±0.223
Total Flavonoid Content (mg of QE/g of dry weight)	0.6 [a]±0.031	1.51 [b]±0.106	0.35 [c]±0.029
β Carotene (mg/100g dry weight)	93.625 [a]±1.103	11.200 [b]±0.679	Not detected

Data (Mean ± SE) in each row with different superscripts differ significantly (P<0.05)

However, research revealed (Assi and King, 2007) as high β-carotene content of tomato pomaceas 4.256mg/ 100g fresh weight.

Densitometry analysis (Fig. 1) and chromatographic study (Fig. 2) in present HPTLC analysis identified β carotene peaks in dried samples and standards. The band for β-carotene in the samples was confirmed by comparing R_f values and spectra of the band with that of the standard (Fig. 3). Quantification of β-carotene concentration was done by five point standard curve (Y= -441.442 + 4.035X; r^2= 0.995).

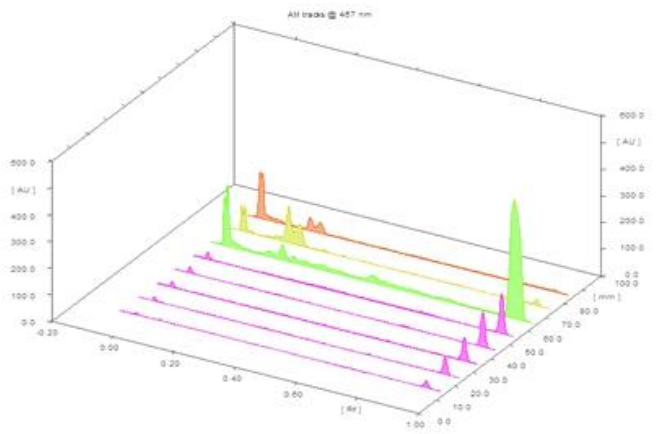

Fig. 1 : HPTLC densitogram at 457 nm in the order of standard -carotene (track 1 to 5), tomato pomace (track 6), skin of green pea pod (track 7) and beet root peel (track 8)

Present study (Table 1) revealed that β-carotene content in TP (93.625mg/100g dry weight)was significantly (P<0.05) higher than in GP (11.2 mg/100 g dry weight). β –carotene was not detected in BP. This result confirms the high β-carotene content of *Pusa Ruby* tomato cultivar. Therefore its pomace may be included as supplement in animal food particularly in the area where hypovitaminosis A is frequently reported.

DPPH assay and IC_{50} value :

DPPH assay was performed to determine antioxidant activity of TP, GP and BP in respect to ascorbic acid. Radical scavenging activity (% inhibition at all concentrations) of all the extracts increased with increasing concentration (Data not shown). IC_{50}values of samples were calculated using radical scavenging activity at various concentrations by linear regression and presented in Fig.4. Lower the IC_{50} value, more is the antioxidant potential of the sample. Among the three vegetable wastes in the present study, BP showed the least IC_{50} value followed by GP and TP. Results of the present study deviated with earlier reports (Babbar *et al*., 2014) that demonstrated highest antioxidant activity of tomato peel followed by pea pod, cauliflower waste and potato peel.

High level of antioxidant activity of BP was earlier

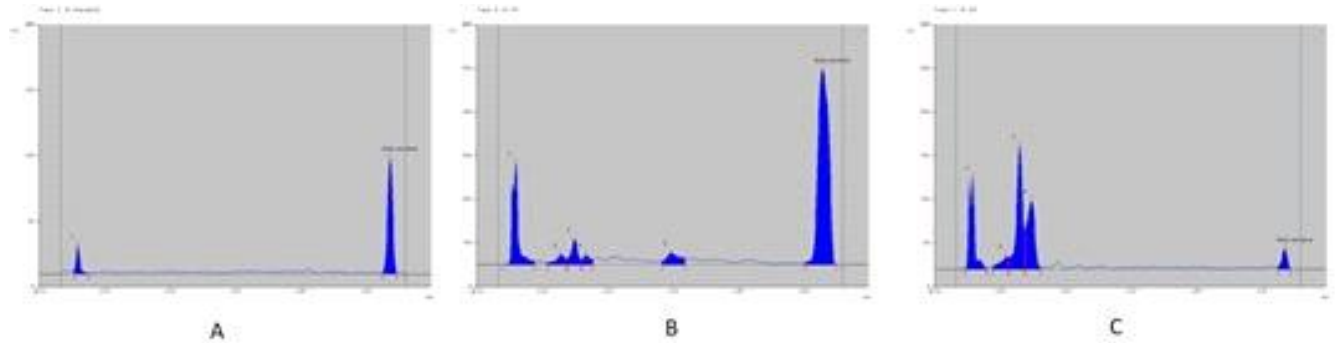

Fig. 2 : HPTLC Chromatograms of A) Standard -carotene (track 3), B) TP (track 6) and C) GP (track 7)

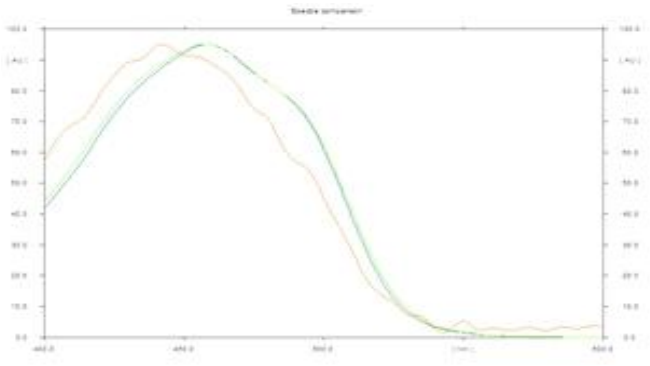

Fig. 3 : Spectra of standard -carotene (Blue), TP (Green) and GP (Red)

reported by Canadanovic-Brunet *et al*. (2011) andthat was in accordance with the present study. High antioxidant activity of the BP may be due to high concentration of total phenolic content (TPC) in it, as high level of correlation exists between antioxidant activity and TPC (Canadanovic-Brunet *et al*., 2011, Hartwig *et al*., 2011 and Zefang *et al*., 2016).

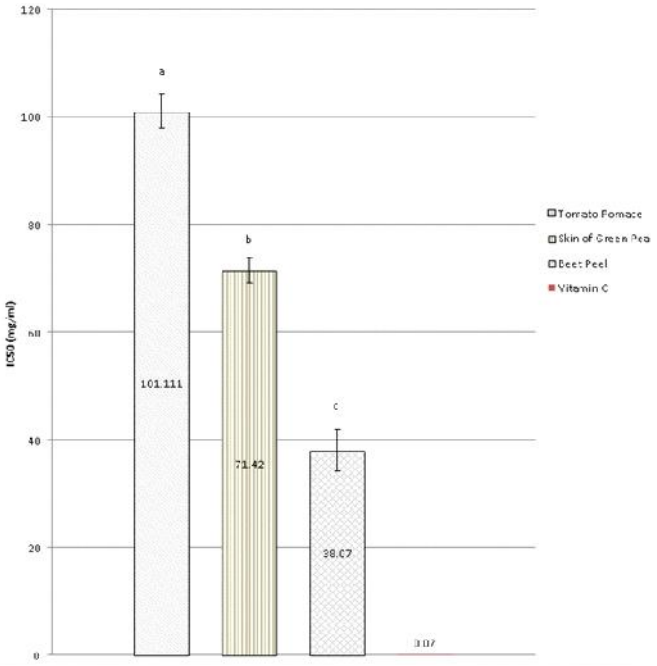

Fig. 4 : IC 50 value of vegetable wastes

Conclusion :

This study clearly revealed that hydro-alcoholic extract of beet root peel contained high level of total phenolic compounds and exhibited high degree of antioxidant activity *in vitro*. Thus beet root peel, which is easily obtained as waste materials from hotels and juice manufacturer, might be used as a feed additive due to its potent antioxidant capacity in human or animal food.

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