The biochemical significances of the proximate, mineral and phytochemical composition of selected vegetables from Pakistan

Asia Atta, Ghalum Mustafa, Munir A Sheikh, Muhammad Shahid, Hang Xiao

1. Introduction

Ethnomedicinal study is considered an important tool for the discovery of plant based medication. Right from the commencement of ethnomedicine, the documentation of folk medicinal acquaintance of plants, has discovered a variety of contemporary medications (Ahmad et al., 2013). Dietary guidelines encouraged the supplementation of plant-based nutraceuticals not only to provide an insight regarding assuaging nature, nutritional worth, sustainability and safety status but also to modulate the onset of chronic ailments (Atta et al., 2016). Epidemiological studies also have correlated the consumption of the phytochemicals-based nutraceuticals particularly those from fruits and vegetables with declining incidences of several physiological threats (Zia-ul-Haq et al., 2014; Zhang and Liu, 2015). These bioactive ingredients like phenolics and carotenoids are diversified in nature which shows considerable antioxidant activity (Shahid, 2009). Understanding the complex role of diet in chronic diseases is challenging since a typical diet provides more than 25,000 bioactive food constituents (Wang et al., 2017). Total energy was calculated according to the following equations:

Energy (kJ) = 17 × (g protein + g carbohydrate) + 37 × (g lipid)

2. Material and Method

2.1. Collection and identification of plant materials

Ipomoea batatas, Trigonella foenum-graecum, Daucus carota, Solanum Melongena and Brassica rapa rapa were purchased from the local market of Faisalabad, Pakistan, washed with distilled water, cut into small pieces and shade dried for several days. All samples were milled to a fine powder, mixed thoroughly and stored at -80 °C. The -20 °C samples were used for the analysis.

2.2. Preparation of crude polyphenol extracts

Total phenolics from the vegetables were extracted by following the method of Noor et al (2014) with slight modifications. Briefly, 25g of plant material was blended in chilled 70% methanol (1:10 w/v) 5min homogenized (5 min) and then filtered under vacuum. The mixture was washed by centrifugation (2500g, 10 min) after adding the 50ul of n-hexane twice. For the biological assay, the crude extracts were evaporated using a rotary evaporator at 45°C to dryness and stored at -40°C until use.

2.3. Proximate analysis of plants materials

The dried plants powder was analyzed for its crude proteins, carbohydrates, crude fats, crude fibers and ash content by the following methods using the standard methods of the AOAC (AOAC, 2003).

2.4. Mineral Analysis

The mineral contents of all the selected plant samples were determined by atomic absorption spectrometry, flame photometry and spectrophotometry according to the methods of AOAC (2010). The selected plants materials were digested in acidic mixture of nitric acid and perchloric acid (1:1), and analyzed on atomic absorption spectrophotometer (Perkin Elmer, A Analyst 300) for Minerals (Iron (Fe), Zinc (Zn), Calcium (Ca), Chromium (Cr), Manganese (Mn) and Magnesium (Mg). Na and K analysis of the sample were done by the method of flame photometry by using the same wet digested food sample solutions. The results are presented in mg/100g of the original sample.

2.5. Phytochemical analysis

Phytochemical analysis for tannins, phenolics, flavonoids, saponins, carotenoids, terpenoids, and alkaloids were carried out according to known guidelines. Tannins and flavonoids were determined according to known methods. Total phenolic contents were determined following the method of Faisalabad, Pakistan, washed with distilled water, cut into small pieces and shade dried for several days. All samples were milled to a fine powder, mixed thoroughly and stored at -80 °C. The -20 °C samples were used for the analysis.

Cite this article as: Asia Atta, Ghalum Mustafa, Munir A Sheikh, Muhammad Shahid, Hang Xiao. The biochemical significances of the proximate, mineral and phytochemical composition of selected vegetables from Pakistan. Mat. Sci. Pharm 1(1) (2017) 06-09
were estimated using the Folin-Denis spectrophotometric method (Shabbir et al., 2013). Saponin content was determined using the method of Khan et al., (2011). Total phenolic contents (TPC) and total flavonoid content (TFC) were determined by following the methods by Ho et al., (2010) and Barros et al., (2011) respectively.

2.6. Antioxidant activity of plant extracts

2.6.1. DPPH radical scavenging assay of selected plant extracts

The antioxidant activity of extracts was assessed by measuring their scavenging abilities to 2, 2-diphenyl-1-picrylhydrazyl stable radical (Ho et al., 2010). Briefly, 50µL aliquot of various concentrations of the samples was added to 5 mL of a 0.004 % methanol solution of DPPH. After 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. The assay was carried out in triplicate.

2.6.2. Determination of reducing power of selected plant extracts

The reducing power of extracts was determined according to the procedure described by Barmos et al., (2011). Briefly, 100 µL of extract was mixed with 0.2 M phosphate buffer (5.0 mL, pH 6.6) and 1% potassium ferricyanide (5.0 mL). After 20 min incubation (50°C) 10 % trichloroacetic acid was added and centrifuged at 12,000 g (10 min, 5°C). The upper layer of the solution (5.0 mL) was diluted with 5.0 mL distilled water and 0.1% ferric chloride (1.0 mL), and read at 700 nm. The experiment was performed thrice.

2.7. Statistical analysis

The mean±SD was calculated for triplicate experiments through Microsoft Excel 7.0. The statistical software Minitab version 13.1 was used for computation and analysis of the antioxidant. Differences were analyzed by considering P<0.05 statistically significant.

3. Results and Discussion

3.1. Proximate analysis of selected vegetables

The proximate analysis of selected vegetables indicate that as usual, there was a variation in crude protein content (Table 1). Fenugreek (28.07±0.3%) showed the maximum protein while turnip and carrot were at lowest position respectively. In sweet potato and eggplant similar percentage of crude protein (12.21±0.11% and 13.85±0.03%) was observed respectively. According to the National Research Council of United States, crude protein less than 20% indicates low protein content of that feed stuff. However, present study was in the harmony with the finding of Sumaya et al., (2012) and Singh et al., (2013). The maximum carbohydrate was observed in carrot whereas it was the minimum in fenugreek. In developing countries 60 to 80% of energy is derived from dietary carbohydrates. The findings are in consonance with Sumaya et al., (2012) and Singh et al., (2013). The crude lipid contents were recorded similar in fenugreek, carrot and eggplant (2-3%), while S. potato and turnip showed slightly less fat contents among the samples. Results of the lipid contents of present investigation were in line with that reported by Dike (2010).

3.2. Mineral analysis of selected vegetables

The mineral analysis of the vegetable species showed significant variability among different macro and microminerals (Table 2). Fenugreek was superior in Cu, Zn, Fe, Ca, P and Mg mineral elements among the edible samples. The trend was seems to be same as reported by Gala and Gujar, (2014). High level of Na and K in carrot and turnip was in accordance to Ekholm et al., (2007). The lowest values of Cu, Zn, Fe, K and Ca were recorded in sweet potato. In turnip P was observed in lowest concentration. According to current data, carrot, eggplant and turnip were also moderately rich source of minerals (Ca, K, Mg, Fe, Cu and Zn) that was in accordance to previous studies (Sharma et al., 2012; Zaccari et al., 2015).

3.3. Phytochemical analysis of selected vegetables

Phytochemicals are compounds, derived from plants hypothesized to confer disease protective characteristic (Berma et al., 2013). They exhibit extensive potential as antioxidants (Khan et al., 2013) antimicrobial agents (Das, 2012), anticancer (Amadi et al., 2013), boosts immune system, decrease platelets aggregation and modulates hormone metabolism. In present study, preliminary screening of phyto-constituents revealed a significant presence of alkaloids, saponins, tannins, flavonoids and β-carotene in all the selected vegetables samples. In quantitative analysis (Table 3) Among the selected vegetables highest amount of alkaloids were obtained from fenugreek (0.07 mg/g) while least were determined for eggplant (0.01 mg/g). In remaining plants the order of crude alkaloids content (mg/g) lie as given carrotturip<s: potato rangeing from 0.04 to 0.02 mg/g.

Recent research indicated that all the tested vegetables were good source of dietary fiber. Certain reports suggested that consumption of high fibrous diet have certain health promoting effects, including improved glucose tolerance in diabetics and the prevention of chronic diseases such as colorectal cancer (EFSA, 2010). Fenugreek and carrot had slightly higher ash content as compare to other samples which showed the similar percentage of ash content. Macronutrients are responsible for energy production in the human organism. In this regard, it should be highlighted that all the edible plants studied are foods with a very moderate energy value (Fig. 1), most
different types of carotenoids in different extents; act as potential antioxidants and a key to preserving health and avoiding different ailments (Krinsky and Johnson, 2005). Dietary β-carotenes have a potential in vitamin synthesis within the human body (Omodamo et al., 2013). The results of present study confirmed that all the selected vegetables are rich source of β-carotenes ranging from 0.01-0.02 (mg/g). In a previous reports, Fesco and Boudion, (2002) suggested that carrots, sweet potatoes and leafy vegetables contain high levels of β-carotene, usually exceeding 8000 IU per 100 g and can therefore cover the recommended daily intakes (5000 to 25000 IU.).

Table 3. Quantitative analysis of phyto-constituents (mg/g) of selected vegetable

| Scientific Name   | Common name | Alkaloids | Tanins | Saponins | β-carotene |
|-------------------|-------------|-----------|--------|----------|------------|
| Brassica rapa     | S. potato   | 0.02±0.00 | 12.5±0.01 | 21.9±0.23 | 0.00±0.25   |
| Foeniculum         | Carrot      | 0.04±0.72 | 7.4±0.23  | 38.3±0.59 | 0.02±0.46   |
| Melongena         | Eggplant    | 0.01±0.55 | 11.6±0.17 | 11.6±0.58 | 0.01±0.57   |
| Daucus carota     | Tomato      | 0.04±0.93 | 16.6±0.25 | 23.8±0.19 | 0.01±0.74   |

Values are mean ±SD. n=5

3.4.3 Reducing power assay of selected vegetables

Among the tested vegetables, the strongest antioxidant property as depicted by the reducing power assay was also observed in T. foenum-graecum (3.44±0.05), which could be due to the presence of total phenolics and flavonoids with respect to other extracts followed by the Solanum Melongena. Daucus carota extract showed moderate while Ipomoea batatas and Brassica rapa rapa gave lowest activities among the samples varied ranging from 1.37±0.13 to 3.44±0.05 in the ascending order (Table 4).

Sahsah et al. (2011) hypothesized that the reducing power of a compound may serve as a significant indicator of its potential antioxidant activity. Previous reports on the relationship between the TPC and antioxidant capacity demonstrated a linear correlation between TPC and the antioxidant capacity (Nisha et al., 2009; Mishra et al., 2010; Atta et al., 2016).

CONCLUSION

Keeping in view the results, the all studied vegetables present good nutritional sources with moderate energy values and rich sources of macronutrients and micronutrients, exhibiting the least toxic risks regarding heavy metals. The phyto-chemical composition revealed the presence of considerable levels of phenolics, flavonoids, alkaloids, and tannins among all the edibles. The biological assay in methanolic extracts of analyzed vegetables also has demonstrated appreciable inhibitory actions against oxidation actions. In this context, nutraceutical worth and exhibiting rich array of biochemicals, nutritional and phytochemicals in of these analyzed edibles especially in T. foenum-graecum, current study also opens new endeavors for the applications of functional/nutraceutical foods and their bioactive moieties as a therapeutic device against maladies and also in the field of nano sciences.

Acknowledgment

The authors are highly thankful to Higher Education Commission (HEC), Government of Pakistan for the financial support under the indigenous PhD program.

References

Atta, A., M. A. Sheikh, M. Shahid and T. Khalid. 2016. Antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants. Turkish Journal of Biology. 30:177–183.

Association of Official Analytical Chemists International. Official method of analysis of AOAC International. 17th ed. Gaithersburg: Association of Analytical Communities; 2003.

Association of Official Analytical Chemists. Official methods of analysis of AOAC International. 18th ed. Washington DC: Association of Official Analytical Chemists; 2010.

Atta, A., M. A. Sheikh, M. Shahid and T. Khalid. 2016. Antioxidant and aglycone potentials of polyphenol extracts of selected vegetables from Pakistan. Oxidation Communications. 39(3-1), 2249–2259.

Barros, L., L. Cabrita, M. V. Boas, A. M. Carvalho and I. C. F. R. Ferreira. 2011. Chemical, biochemical and electrochemical assays to evaluate phytochemicals and antioxidant activity of wild plants. Food Chemistry. 127:1600–1608.

Bermua, S., D. Singh, P. Verma, D. Soni, A. Gupta and R. Nema. 2013. Review Article Boerhavia diffusa: An Important medicinal plant and their phytochemistry. CMBT Journal of Sciences and Technology. 1(1):01-04.

Das, S. 2012. Antimicrobial activity study of ethanolic extract of Boerhavia diffusa: An Important medicinal plant and their phytochemistry. Turkish Journal of Biology. 30:177–183.

Dike, M. C. 2010. Proximate, phytochemical and nutritional compositions of some fruits, seeds and leaves of some plant species at Umudike, Nigeria. ARPN Journal of Agricultural and Biological Science. 5(1):7-16.

EFSA. 2010. Panel on Dietetic Products, Nutrition, and Allergies Scientific opinion on dietary reference values for carbohydrates and dietary fibre. EFSA Journal. 8 (3) 1462.

Ekholm, P., H. Reinivuo, P. Mattila, H. Pahlkka, J. Koponen, A. Happonen, J. Helstro¨m and M. Ovaskainen. 2007. Changes in the mineral and trace element contents of cereals, fruits and vegetables in Finland. Journal of Food Composition and Analysis. 20: 487–495.
Fesco, O. L. and O. W. Bouillon. 2002. Food nutrition security for human security. International Conference on Vegetables, On the occasion of the WFS-51 (World Food Summit - five years later), convened in Rome on 1-13 June 2002.

Gala, B. V. and V. Gujar. 2014. Product development, biochemical, anti-microbial and organoleptic analysis of (Trigonella foenum - graecum) fenugreek seeds and leaves. Plant Sciences. 4 (2): 15-18.

Harborne, J. B. 2008. Phytochemical methods: a guide to modern techniques of plant analysis. 3rd ed. New Delhi: Springer Pvt Ltd.

Ho, S., Y. Tung, K. Cheng and J. Wu. 2010. Screening, determination and quantification of major antioxidants from Balanophora laxiflora flowers. Food Chemistry. 122: 584–588.

Hussain, J., A. L. Khan, N. Rehman, M. Hamayun, T. Shah, M. Nisar, T. Bano, Z. K. Shinwari and I. Lee. 2009. Proximate and nutrient analysis of selected vegetable species: A case study of karak region, pakistan. African Journal of Biotechnology. 8(12):2725-2729.

Khan, A. M., R. A. Qureshi, F. Ullah, S. A. Gilani, A. Nosheen, S. Sahreen, M. K. Laghari, M. Y. Laghari, S. Rehman, I. Hussain and W. Murad. 2011. Phytochemical analysis of selected medicinal plants of Margalla Hills and surroundings. Journal of Medicinal Plants Research. 5 (25): 6017-6023

Khan, M. S., I. A. Ansari, S. Ahmed, F. Akhtar, A. Hashim and A.K. Srivastava. 2013. Chemotherapeutic potential of Bombaxia diffusa Linn: A review. Journal of Applied Pharmaceutical Sciences. 3(01):133-139.

Krisnys, N. I. and E. J. Johnson. 2005. Carotenoid actions and their relation to health and disease. Molecular Aspects of Medicine. 26(6): 459-516.

Mishra, N., A. Dubey, R. Mishra, N. Barik. 2010. Study on antioxidant activity of common dry fruits. Food Chem Toxicol, 48, 3316-3320.

Nisha, P., P. A. Nazar and P. Jayamurthy. 2009. A comparative study on antioxidant activities of different varieties of Solanum melongena. Food and Chemical Toxicology. 47: 2640-2644.

Noor, N., Sarfraz, R. A., Ali, S., Shahid, M. 2014. Antitumour and antioxidant potential of some selected Pakistan honey. Food Chemistry 143, 362-366.

Omodamiro, R. M., S. O. Aifuape, C. J. Njoku, I. I. M. Nwankwo, T. N. C. Echendu and T. C. Edward. 2013. Acceptability and proximate composition of some sweet potato genotypes: Implication of breeding for food security and industrial quality. International Journal of Biotechnology and Food Science. 1(5): 97-101.

Peng, X., Zheng, Z., Cheng, K-W., Shan, F., Ren, G-X., Chen, F., & Wang, M. (2008). Inhibitory effect of mung bean extract and its constituents vitexin and isovitexin on the formation of advanced glycation endproducts. Food Chemistry. 106(2), 475-481.

Raju, J. and C. V. Rao. 2012. Diosgenin, a Steroid Saponin Constituent of Yams and Fenugreek: Emerging Evidence for Applications in Medicine, Bioactive Compounds in Phytotherapy. Prof. Iraj Rasooli (Ed.). ISBN: 978-953-307-805-2, InTech.

Rama, P. J. Sudisha, D. N. Lakshmi and S. M. Aradhyu. 2011. Antibacterial and Anti-oxidant Activities of Fenugreek (Trigonella foenum graecum L) Leaves. Research Journal of Medicinal Plant. 5 (6): 695-705.

Sathisha, A. D., H. B. Lingaraju and K. S. Prasad. 2011. Evaluation of antioxidant activity of medicinal plant extracts produced for commercial purpose. E-J. Chem, 8, 882-886 (2011).

Shabbir M, Khan MR, Saeed N. Assessment of phytochemicals, antioxidant, anti-lipid peroxidation and anti-hemolytic activity of extract and various fractions of Maytenus royleanus leaves. BMC Complement Altern Med 2013; 13:143.

Sharma, K.D., S. Karki, N.S. Thakur and S. Attri. 2012. Chemical composition, functional properties and processing of carrot—a review. Journal of Food Science and Technology. 49: 22-32.

Singh, K. P., B. Nair, P. Chaud and A. K. Naidu. 2013. Contribution of fenugreek (Trigonella foenum graecum L) seeds towards the nutritional characterization. Journal of Medicinal Plant Research. 7(41): 3052-3058.

Singh, P., S. P. Vishwakarma and R. L. Singh. 2014. Antioxidant, oxidative DNA damage protective and antimicrobial activities of the plant Trigonella foenum-graecum. Journal Science Food and Agriculture. 94(12):2497-504.

Sumaya, A. R., S. Swagami and A. Nabeelah. 2012. Screening and Biochemical Quantification of Phytochemicals in Fenugreek (Trigonella foenum-graecum). Research Journal of Pharmaceutical, Biological and Chemical Sciences. 3(1):165.

Wang, Y., S. Huang, S. Shao, L. Qian and P. Xu. 2012. Studies on bioactivities of tea (Camellia sinensis L.) fruit peel extracts: Antioxidant activity and inhibitory potential against α-glucosidase and α-amylase in vitro. Industrial Crops and Products. 37: 520-526.

Zaccari, F., M. C. Cabrera, A. Ramos and A. Saadoun. 2015. In vitro bioaccessibility of β-carotene, Ca, Mg and Zn in landrace carrots(Daucus carota, L.). Food Chemistry. 166: 365–371.

Zhang L., Liu, R. H. (2015). Phenolic and carotenoid profiles and antiproliferative activity of foxtail millet. Food Chemistry 174, 495-501.

Zia-Ul-Haq, M., Riaz, M., De Feo, V., Jaafar, H. Z., Moga, M. 2014. Rubus fruticosus L.: constituents, biological activities and health related uses. Molecules 19(8), 10998-11029.

Shahidi, F. 2009. Nutraceuticals and functional foods: whole versus processed foods. Trends in Food Science and Technology. 20: 376-387.

Ahmad, S., M. Garg, E. T. Tamboli, M. Z. Abdin and S. H. Ansari. 2013. In vitro production of alkaloids: Factors, approaches, challenges and prospects. Pharmacognosy Reviews. 7(13): 27-33.