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EVALUATION OF POLYPHENOL CONTENT IN DIFFERENT PARTS OF *PHYSALIS IXOCARPA*

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Abstract

In the current study extracts of leaf, stem, fruit and calyx with different polarity was investigated for their phenolic content using high performance liquid chromatography and spectrophotometric assay. Among different parts, stem contain high concentration of total polyphenol and gallic acid. The effect of extraction solvent on polyphenol quantification was observed in both assays. Spectrophotometric analysis of the data regarding polyphenol content indicated that among different extracts from the stem, leaf and fruit tissues; ethyl acetate extracted fraction of stem measured maximum polyphenol content of 110.376 mgGAE/g of dry extract. The ethyl acetate extracted sample of leaf showed high polyphenol (Gallic acid) content of 95 mg GAE/g of dry extract using high performance liquid chromatography assay. The amounts of phenolic content (Gallic acid) extracted from the parts of the plant with the different solvent ranged from 0.0354- 95 mg GAE/g of the dry extract using HPLC, however, spectrophotometric assay indicated total polyphenol ranged from 38-110.37 mgGAE g^{-1} of the dry extract. The current study suggested that ethyl acetate is an effective solvent for the extraction of polyphenol in different parts of *P. ixocarapa*.

Key words: Polyphenol; Folin–Ciocalteu reagent; Gallic acid equivalent; Spectrophotometric assay; *P. ixocarapa*

Introduction

A renewed curiosity has occurred to investigate for phytochemicals of native and naturalized plants for pharmaceutical and nutritional purposes (Bilal *et al*., 2016; Chaun *et al*., 2015; Ullah *et al*., 2015; Zakir *et al*., 2015; Bakht *et al*., 2014 a, b, c). Polyphenols are secondary metabolites of plants that provide defense against the ultraviolet radiation and invasion of microbes (Beckman, 2000). They are commonly found in vegetable fruits and seeds. More than 8000 polyphenol have been currently recovered and characterized from different species of plants (Ghosz & Scheepens, 2009). They are produced from phenylalanine and shikimic acid. Structurally polyphenol consist of phenyl rings, connected by basal elements. They are mainly classified into four groups including phenolic acid, stilbenes, lignins and flavonoids based on the number of phenyl rings and structural element (Spencer *et al*., 2008). Phenolic acid is an important group of polyphenol, abundantly found in food. It is divided in to two classes based on their derivatives: derivatives of benzoic acid and derivatives of cinnamic acids. Generally hyrdroxycinnamic acid is abundant in nature as compared to the hydroxybenzoicacid (Pandey & Rizvi, 2009). Recently, due to multiple applications of polyphenol in food and pharmaceutical industry the researchers have become more interested in the study of polyphenol. The health benefit of dietary polyphenol is associated with their potent antioxidant properties and their credible effect in the prevention of various oxidative stresses produced in the body during infection (Manach *et al*., 2004). Modern study reveals that the use of vegetable and fruits in our diet is associated with decrease in threat of stroke and cancer (Beecher, 1999; Kawasaki, *et al*., 2008; Wright *et al*., 2008; Bae *et al*., 2008). Similar observations have been reported about polyphenol rich food and beverages indicating that it protect cell constituents from oxidative stress and, therefore, decrease the threat of various degenerative diseases associated with oxidative stress (Luqman *et al*., 2006; Pandey & Rizvi, 2009). This shielding effect is related to bioactive compounds of plant parts. Different vegetables and fruits showed variation in antioxidant property according to poly polyphenol content, vitamin C, E, carotenoids and flavonoids (Saura-Calixto & Goni, 2006).

The extraction yield and the antioxidant activity of the compound depends on the nature of extracting solvent due to varied chemical characteristics and polarities that may or may not be soluble in a particular solvent. For example, polar solvents are commonly used for the recovery of polyphenol from plant matrix. Selection of the right solvent and extraction procedure greatly affect the quantity and rate of polyphenols extraction (Xu & Chang, 2007). The most favorable solvent for the extraction of antioxidant compound are (hot or cold) water mixtures containing ethanol, methanol, acetone and ethyl acetate (Peschel *et al*., 2006). However, methanol and ethanol have been widely used to extract bioactive compound with antioxidant property from various plants and plant-based foods such as plum, strawberry, pomegranate, wheat grain and bran, mango seed kernel, citrus peel, and many other fruit peels. Recent studies have shown that ethyl acetate is also effective solvent for the extraction of phenolic compounds from onion and citrus peel (Peschel *et al*., 2006; Li *et al*., 2006). Bonoli *et al*. (2004) stated that maximum phenolic compounds were isolated from barley flour with mixtures of ethanol and acetone.

Similarly, water methanol was found to be best solvent for the extraction of phenolic compounds from rice bran (Chatha *et al*., 2006), and *Moringa oleifera* leaves (Siddhuraju & Beaker, 2003). Different methods have been developed to quantify polyphenol content in plant material and correlate their concentration with plant part and extraction solvent (Do *et al*., 2014; Sahreen *et al*., 2010) Most of these methods based on spectrophotometric assay. The color change in these assays is most important indicator for the presence of polyphenol measured spectrophotometrically (Huang *et al*., 2005). The most important spectrophotometric based assay include the Folin-Ciocalteu reagent-based total phenolic assay (FCR) (Shahidi & Naczk, 2004), the orthodihydroxy phenol assay (ODA) (Hendry *et al*., 1994) and the total poly phenol content assay (TPC) (Quinde *et al*., 2004). These methods are relatively simple, inexpensive, and require little specialized equipment or analytical expertise. Now days HPLC and GCMS are used for polyphenol analysis which are more sensitive and accurate methods (Granger *et al*., 2011). *Physalis ixocarpa* belong to genus *physalis* which is the fifth largest genus of family solanacecae (Whitson and Manos, 2005). The most common growing region of genus *Physalis* is the South of America in which Mexico is considered as central of its origin, domestication and diversity (Medina-Medrano *et al*., 2015). Fruits of *Physalis ixocarpa* is consumed fresh or cooked in culinary tradition of people of Mesoamerica. Leaves and calyxes are the key elements of folk medicine (Hernandez & Yanez, 2009). In the light of their medicinal and economic importance, the current study was designed to determine the effect of different solvent on extraction of polyphenol from the different parts (stem, leaves, fruit and calyces) of *P. ixocarpa*, suggest potential solvent for the recovery and isolation of polyphenol.

Materials and Methods

Collection of plant material: Stem, leaves, fruit and calyx were taken from the plant population of *P. ixocarpa* grown in Baylay baba, district Shangla Khyber Pakhtun Khwa Pakistan. Plant parts were dried separately in shady place at room temperature for 10 days. Plant specimens are deposited in the herbarium of Islamia College University Peshawar with voucher No. WD1. The plant material (150 gm) was stirred with 250 ml of ethyl acetate, using hot plate magnetic stirrer for 3 hours and then centrifuged at 10000 rpm for 10 minutes. The supernatant was separated from the residues through filtration using Whatman No. 4 filter paper and again extracted with ethyl acetate. This process was repeated three times. All ethyl acetate extract were then pooled and rotary evaporated to remove the solvents. The same process was carried out for butanol and water extracted samples. The resulting three different extracts, ethyl acetate, butanol and water were then stored at -20□C till analysis (Seeram *et al*., 2001).

Quantitative assay of total polyphenol through spectrophotometer and HPLC: Total soluble polyphenol in extracts were determined according to method of Slinkard & Singleton (1977). The different extracts (0.5ml) with concentrations of 1 mg ml^{-1} were mixed separately with 46 ml of water, followed by the addition of 1 ml of FR reagent (1N) and mixed it thoroughly in volumetric flask. After 3 minutes, 3 ml of sodium carbonate decahydrate (2%) solution was added and the mixture was allowed to stand in shaking incubator for 2 hours. The absorbance was measured at 730 nm. In the same way the absorbance of different concentrations of gallic acid (10, 20, 30, 40, 50, 60, 70, 80 and 90 ppm) were measured and plotted against the concentration for producing the Gallic acid standard equation. The standard curve equation (mentioned below) was used for the estimation of total polyphenol in different samples under study (Fig. 1).

Gallic Acid Standard Equation:

$$y = 0.0008x + 0.0489$$

y ; Absorption and x ; Concentration

HPLC analysis: The phenolic compound (Gallic acid) in different extracts was measured by HPLC, equipped with water diode detector and dualistic pump. The test sample (0.5 mg ml^{-1}) was prepared in acetonitrile and water (1:1), filtered through a 0.45 µm membrane filter (Millipore) and injected directly. Polyphenol standard i.e. Gallic acid of different concentration (10 ppm, 20 ppm, 40 ppm, 80 ppm, 160 ppm, 320 ppm) was prepared in acetonitrile and water (1:1 V/V) run on HPLC using Diamonsil C18 column (4.6 mm, 250 mm, 2.5 µm). The mobile phase consist of Solvent A (Acetonirile) and Solvent B (0.3% Acetic acid) with the Gradient program 0–5 min, 20% B; 5–10 min, 90% B; 10–15 min, 10% B; 15–25 min, 20% B); flow rate 1 ml/min; volume injected 10 µl; temperature 25°C; UV detection wavelength 254 nm (Deng *et al*., 2011). The chromatograms of the gallic acid and water extract of calyx are shown in Figs. 2-4. The gallic acid peak was obtained in at Rt =9.6 ± 0.11 min. Similar peaks were also observed in the different extracts of the subject plant. The peak area of the different concentrations of the standard was noted with respect to time and plotted against the concentration for calculating equation with standard curve. The following equation was used for estimating the gallic acid in the different extracts.

$$y = 46598x + 62967; R^2 = 0.996$$

y ; Area of peaks and x ; Concentration

Statistical analysis: Data are shown as mean values of three replications. MSTATC computer software was used for statistical analysis (Russel & Eisensmith, 1983). Least Significant Difference (LSD) test was applied to separate differences among means (Steel *et al*., 1997).

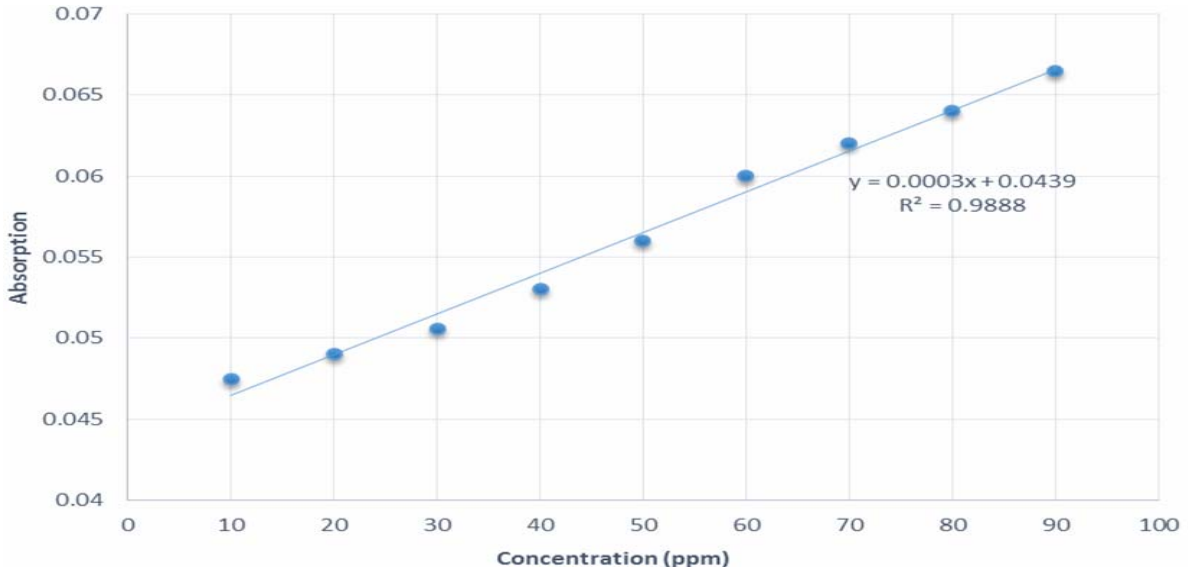

Fig. 1. Standard curve of gallic acid for spectrophotometric analysis of total polyphenol; y= Absorption, x= Concentration of gallic acid, R^2= Corelation coefficient.

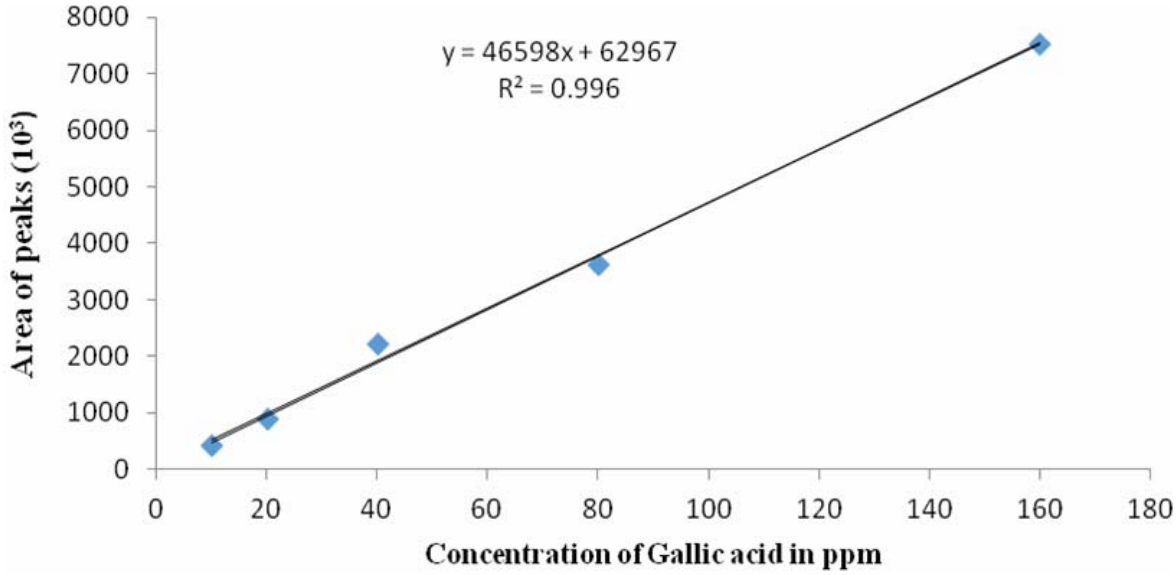

Fig. 2. Standard curve of gallic acid for HPLC analysis; y= Area of peaks, x= concentration of gallic acid, R^2= Correlation coefficient.

Results

Polyphenol quantification and effect of solvent and plant part on the recovery of total polyphenol: Extracts of different polarities from the leaf, stem, fruit and calyx of *P. ixocarpa* were investigated for the quantification of polyphenol. Analysis of the data revealed that different parts of the plant showed varying degree of polyphenol concentrations (Table 1). Among different parts of the plant, extracts from the stem showed highest quantity of total polyphenol content of 328.19 mg g^{-1} followed by extracts from leaf with weight of 245.39 mg g^{-1} of dry weight of the extract. The data showed that the order of total polyphenol content in different parts of the plant were stem > leaf > calyx > fruit. The effect of solvent on the extraction of polyphenol was also observed in the present study (Figs. 4 & 5). Among different extracts from the stem, leaf and fruit tissues, ethyl acetate extracted fraction of stem and leaf measured maximum polyphenol content (i.e. ethyl acetate extracts from leaf contain 110.374 mgGAE g^{-1} and stem produced 75.36 mgGAE $g^{-1}$of dry extract) followed by water extracted sample from the leaf with an average of 73.689mgGAE g^{-1} of dry extract. From these results it can be concluded that ethyl acetate is the most effective solvent for the extraction of polyphenol from different parts of *P. ixocarpa.*

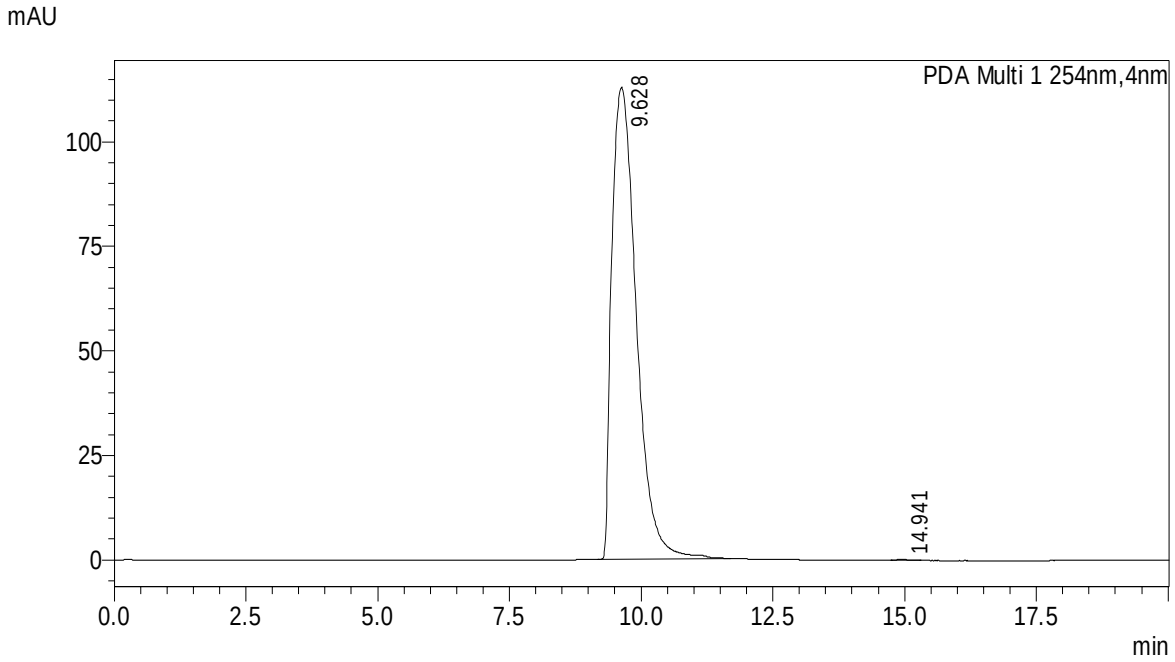

Fig. 3. Chromatogram of gallic acid of 80 ppm concentration.

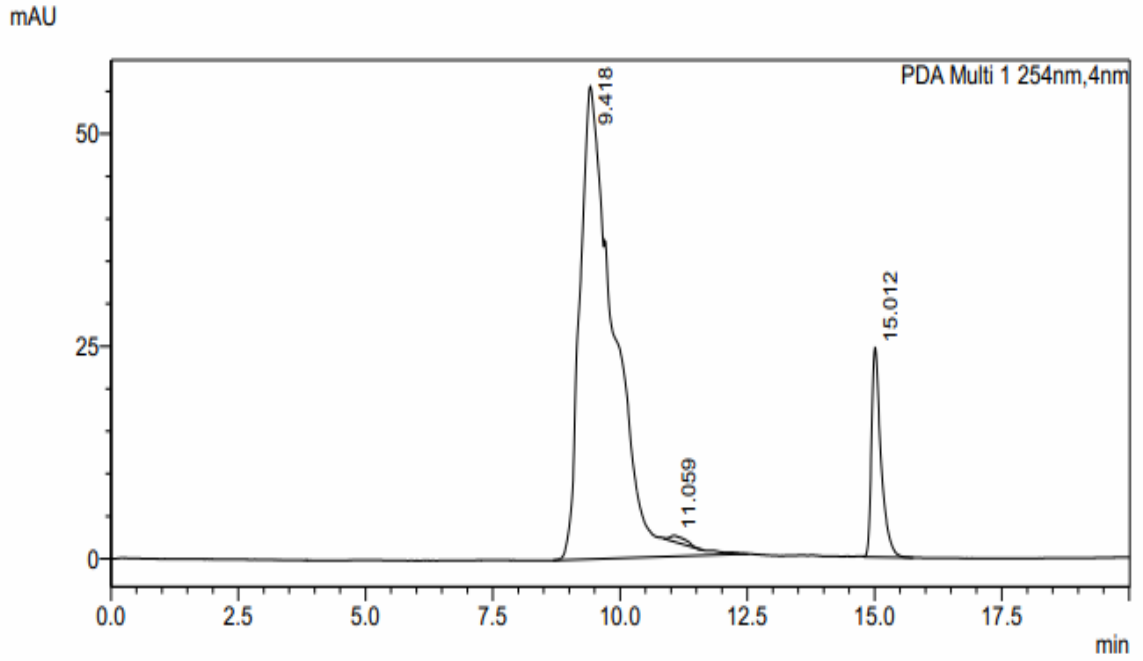

Fig. 4. Chromatogram of water calyx extract showing gallic acid peak.

Quantification of polyphenol through HPLC: Analysis of the data indicated in the Table 1 showed variation in polyphenol content of the different parts of the plant. The order of polyphenol content in different parts of the plant were stem > fruit > leaf > calyx.The solvent effect on the polyphenol quantification through HPLC was observed in this study. The data indicated that the ranking order of solvents for polyphenol recovery were ethyl acetate > water > butanol from the different parts of the subject plant. The amounts of phenolic compound (gallic acid), extracted from the parts of the plant with the different solvent, ranged from 0.0354- 95.0 mg GAE g^{-1} of the dry extract. Among different parts of the plant, the ethyl acetate extracted sample of leaf showed high polyphenol content of 95.0 mg GAE g^{-1} followed by ethyl acetate extract of fruit with polyphenol content of 34.00 mg GAE g^{-1} of the dry extract. The least concentration of polyphenol was found in the butanol extracted sample of calyx (0.0354 mg GAE g^{-1}) (Table 2).

Table 1. Total polyphenol in different parts (Leaf, stem, fruit, calyx) of *Physalis ixocarpa*.

Plant part	Polyphenol (mg GAE/g)
Leaf	245.39 ± 2.7603[c]
Stem	328.19 ± 2.9905[a]
Fruit	165.77 ± 2.0842[d]
Calyx	251.21± 1.9039[b]

Superscriptletters ([a-d]) represent significant difference from one another at p>0.05

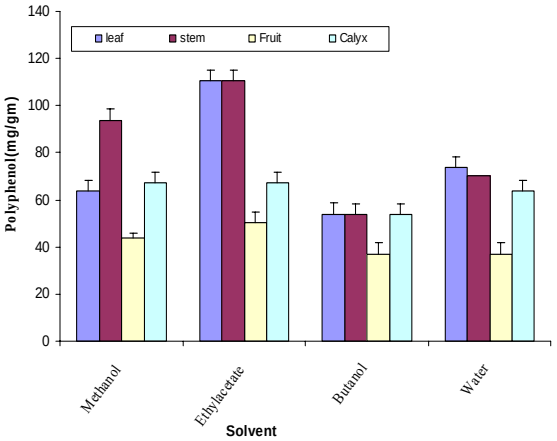

Fig. 5. Total polyphenol contents of different solvent extracted samples from different parts of *P. ixocarpa* (Bar represent ± LSD at p<0.05).

Discussion

Polyphenol is one of the major contributor to the antioxidant and organoleptic characteristics of food, and consider the best nutraceutical in food industry (Tapas *et al*., 2008). Some of phenolic are involved in fruit maturation, food preservation and prevention of enzymatic browning (Robbins, 2003). They are also key element of food tracing and authenticity (Zimmermann & Galensa, 2007). Knowledge regarding abundance, distribution and localization of phenolic in different parts of the plant provide an opportunity to develop different extraction techniques and protocols for their isolation, characterization, and to produce new drugs for improving human health. Different parts of the subject plant were screened for the presence of total polyphenol using spectrophotometric assay. The result of the study showed variation in polyphenol content in different of parts of the plant. Stem contain high concentration of total polyphenol while low concentration was produced in fruits of the subject plant. The differential accumulation of total phenols in studied parts are associated with differential cyto-logical and physiological activities within organs (Itidel *et al*., 2013).The production of these compounds is highly ordered process and regulated by differential expression of genes involved in phenylpropanoid pathway (Mamti *et al*., 2006; Chang *et al*., 2009). The decreasing of phenolic from stem to fruit represents the close association between organs and dissimilar process of biosynthesis /biodegradation and transportation involve in the distribution of these compounds at plant level (Castillo *et al*., 1997). Moreover the solubility and recovery of polyphenol is also governed by the chemical position of the plant sample, as well as the polarity of the solvent used for their extraction (Jin & Russell, 2010). Different solvents such as methanol, ethyl acetate, acetone and n-butanol, alone, and their combination have been used for the recovery of plant phenolic (Xu & Chang, 2007). In the current study ethyl acetate extracted fraction of the different parts of subject plant showed high polyphenol content suggesting that ethyl acetate is an effective solvent for the extraction of polyphenol in different parts of *P. ixocarpa*. These results agrees with recent studies which showed that ethyl acetate is an effective solvent for the extraction of phenolic compounds from onion and citrus peel (Peschel *et al*., 2006; Li *et al*., 2006).

Table 2. Total gallic acid contents of different solvent extracted samples from different parts of *P. ixocarpa*.

Extracts	Concentration (ppm)	Peak area	mg GAE/gm
Gallic acid	10	435103.55	-
Gallic acid	20	907304.208	-
Galic acid	40	2221772.89	-
Gallic acid	80	3643758.65	-
Gallic acid	160	7527088.24	-
Water fruit extract	500	788108.10	31.12 ± 0.80
Water leaf extract	500	247615.34	7.92 ± 0.55
Water calyx extract	500	214087.40	6.48 ± 0.45
Water stem extract	500	240463.60	7.61 ± 0.41
Butanol calyx extract	500	63792.30	0.04 ± 0.01
Butanol fruit extract	500	531563.60	20.11 ± 0.72
Butanol leaf extract	500	124870.40	2.65 ± 0.32
Butanol stem extract	500	136053.30	3.13 ± 0.25
Ethyl acetate fruit extract	500	855212.40	34.00 ± 0.90
Ethyl acetate leaf	500	2290536.16	10.74 ± 0.64
Ethyl acetate calyx	500	2290536.16	7.882 ± 0.32
Ethyl acetate stem	500	313273.80	95.00 ± 1.1

The current trend used for polyphenol analysis is HPLC and GCMS, which are more sensitive and accurate methods (Granger *et al*., 2011). The present study revealed the order of polyphenol (gallic acid) content in different parts of the plant were stem > fruit > leaf > calyx which is not similar to the spectrophotometric quantification of the total polyphenol. This might be due to the differential distribution of individual compound (Gallic acid) in different part of the plant. The solvent effect on the polyphenol quantification through HPLC was observed in this study. The data regarding polyphenol content through HPLC assay ranked the extraction solvent in order of ethyl acetate > water > n-butanol which is similar to the ranking order of spectrophotometric quantification of polyphenol. Among different parts of the plant, the ethyl acetate extracted sample of stem showed high polyphenol content followed by fruit and leaf extracts of the same solvent. The least concentration of polyphenol was found in the n-butanol extracted sample of calyx (0.0354 mg GAE/g). Our finding was similar to the results of (Hu *et al*., 2003) who reported that ethyl acetate is an effective solvent for the extraction of polyphenol in different parts of *P. minima*. Similar results were also reported by Peschel *et al*. (2006) and Li *et al*. (2006). Furthermore, the spectrophotometric analysis of polyphenol content indicated much higher estimates of polyphenol concentration than HPLC analysis. It may be due to sensitivity and identification of individual compound in the test sample. Moreover, the Folin–Ciocalteu reagent is not more specific in nature and may react with many other compounds including those that may interfere with intended reaction, preventing the accurate determination of the concentration of target compounds (Granger *et al*., 2011) Substances like sugar, ascorbic acid and aromatic amines interfere with Folin-Ciocalteu reaction, and sometime the reagent involved in these assays reacts with non-phenolic organic and inorganic substances. The other possible reason for the overestimation of polyphenol quantification may be the reaction of complex phenolic such as flavonoid present in the extracts (Quinde *et al*., 2004; Prior *et al*., 2005).

Conclusion

Phenolic compounds in plant parts have attracted scientific consideration because of their medicinal importance. Current study showed variation in polyphenol concentration depending on plant part and extraction solvent. High concentration of total polyphenol and gallic acid was present in stem of *P. ixocarpa*. The solvent effect on recovery of polyphenol was observed in this study, ethyl acetate is considered as best solvent for the extraction of polyphenol from the different part of the study plant. Regarding the medicinal importance of polyphenol further chemical studies should be conducted for the isolation and characterization of phenolic compound from the subject plant.

References

Bae, J.M., E.J. Lee EJ and G. Guyatt. 2008. Citrus fruit intake and pancreatic cancer risk. A quantitative systematic review. *Pancr.,* 37: 137-146.

Bakht, J., K. Shehla and M. Shaf M. 2014 a. *In vitro* anti-microbial activity of *Allium cepa* (dry bulbs) against Gram positive and Gram-negative bacteria and fungi. *Pak. J. Pharma. Sci.,* 27: 139-145.

Bakht, J., S. Shaheen and M. Shafi. 2014b. Antimicrobial potentials of *Mentha longifolia* by disc diffusion method. *Pak. J. Pharmacet. Sci*., 27: 939-945.

Bakht, J., N. Gohar and M. Shafi 2014c. *In vitro* antibacterial and antifungal activity of different solvent extracted samples of *Alhagi maurorum*. *Pak. J. Pharmacet. Sci*., 27: 1955-1961.

Bilal, M.K. and J. Bakht. 2016. Anti-fungal, anti-yeast, anti-oxidant and HPLC analysis of different solvent extracted samples from *Calmus aromaticus* leaves. *Bangladesh J. Pharmacol*., 11: 91-100.

Beckman, C.H. 2000. Phenolic-storing cells: keys to pro-grammed cell death and periderm formation in wilt disease resistance and in general defense responses in plants? Physiol. *Molec. Plant Pathol.,* 57: 101-10.

Beecher, G.R. 1999. Phytonutrients' role in metabolism: Effects on resistance to degenerative processes. *Nutr. Rev.,* 57: S3-S6.

Bonoli, M., V. Verardo, E. Marconi and M.F. Caboni. 2004. Antioxidant phenols in barley (*Hordeum vulgare* L.) flour: comparative spectrophotometric study among extraction methods of free and bound phenolic acids. *J. Agric. Food Chem.,* 52: 5195-5200.

Castillo, J., O. Benavente-Garcia, F. Sabater, F. Marin, A. Ortuno and J.A. Del Rio. 1997. Flavanone neohesperidosides distribution in *Citrus aurantium* stems. Evidence for a possible translocation process in relation with biosynthesis in leaves and fruits. *Phytochem.,* 1: 77-84.

Chang, J., O.J. Lu and G. He. 2009. Regulation of polyphenols accumulation by combined expression silencing key enzymes of phenylpropanoid pathway. *Acta Biochim. Biophys*., Sin., 41: 123-130.

Chaun, R.Z., K. Wajid, J. Bakht and M.G. Nair. 2015. New inti-inflammatory sucrose esters in the natural sticky coating of tomatillo (*Physalis philadelphica*) an important culinary fruit. *Food Chem*., 196: 726-732.

Chatha, S.A.S., F. Anwar, M. Manzoor and J.R. Bajwa. 2006. Evaluation of the antioxidant activity of rice bran extracts using different antioxidant assays. *Grasas Y Aceites Sevilla.,* 57: 328-335.

Deng, C., C. Lu, X. Wei and Y. Li. 2011. Antioxidant activity, total phenolic, and total flavonoid of extracts from the stems of *Jasmin umnervosum* Lour. *Grasas Y Aceites Sevilla.,* 62: 149-154.

Do, Q.D., A.E. Angkawija, P.L.T. Lien, H. Huynh, F.E. Soetaredjo, S. Ismadji and Y.H. Ju. 2014. Effect of extraction solvent on total phenol content, total flavonoid content and antioxidant activity of *Limnophila aromatic*. *J. Food Drug Anal.,* 22: 296-302.

Ghosz, D. and A. Scheepens. 2009. Vascular action of polyphenol. *Rev. Molec. Nutri. Food Res.,* 53: 322-31.

Granger, K.L., R.S. Gallagher, E.P. Fuerst and J.R. Alldredge. 2011. Comparison of seed phenolic extraction and assay methods. *Methods Ecol. Evol.,* 2: 691-698.

Hernandez, J. and S. Yanez. 2009. Aprovechamiento tradicional de las especies de Physalis en Mexico. *J. Agic. Res*., 43: 81-86.

Hu, Y., J. Xu and Q. Hu. 2003. Evaluation of Antioxidant potential of *Aloe vera* (*Aloe barbadensis* Miller) extracts. *J. Agric. Food Chem.,* 51: 7788-7791.

Huang, D., B. Ou and R.L. Prior. 2005. The chemistry behind antioxidant capacity assays. *J. Agric. Food Chem.,* 53: 1841-1856.

Itidel, C., M. Chokri, B. Mohamed and Z. Yos. 2013. Antioxidant activity, total phenolic and flavonoid content variation among Tunisian natural populations of Rhustripartita (Ucria) Grande and *Rhuspenta phyla* Desf. *Indust. Crops Prod.,* 51: 171-17.

Jin, D. and J.M. Russell. 2010. Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molec.,* 15: 7313-7352.

Kawasaki, B.T., E.M. Hurt, T. Mistree and W.L. Farrar. 2008. Targeting cancer stem cells with phytochemicals. *Molec. Intervent.,* 8; 174-184.

Li, Y., C. Guo, J. Yang, J. Wei, J. Xu and S. Cheng. 2006. Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. *Food Chem.,* 96: 254-260.

Luqman, S. and S.I. Rizvi. 2006. Protection of lipid peroxidation and carbonyl formation in proteins by capsaicin in human erythrocytes subjected to oxidative stress. *Photother. Res.,* 20: 303-6.

Mamti, G.E., Y. Liang and J. Lu. 2006. Expression of basic genes involved in Tea polyphenol synthesis in relation to accumulation of catechins and total tea polyphenols. *J. Sci. Food Agric.,* 86: 459-464.

Manach, C., A. Scalbert, C. Morand, C. Remesy and L. Jimenez. 2004. Polyphenols: food sources and bioavailability. *The Amer. J. Clin. Nutri.,* 79: 727-747.

Medina-Medrano, J.R., N.A. Abarca, M.S. Gonzalez-Elizondo, J.N. Uribe-Soto, L.S. Gonzzlez-Valdez and Y.H. Arrieta. 2015. Phenolic constituents and antioxidant properties of five wild species of Physalis (Solanaceae). *Bot. Study*, 56: 24.

Pandey, K.B. and S.I. Rizvi. 2009. Plant polyphenols as dietary antioxidants in human health and disease. *Oxid. Med. Cellul.Longe.,* 5: 270-278.

Peschel, W., F.S. Rabaneda, W. Dn, A. Plescher, I. Gartzia, D. Jimenez, R.L. Raventos, S. Buxaderas and C. Condina. 2006. An industrial approach in the search of natural antioxidants from vegetable and fruit wastes. *Food Chem.,* 97: 137-150.

Prior, R.L., X. Wu and K. Schaich. 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *J. Agric. Food Chem.*,53: 4290-4302.

Quinde, Z., S.E. Ullrich and B.K. Baik. 2004. Genotypic variation in color and discoloration potential of barley-based food products. *Cereal Chem.,* 81: 752-758.

Robbins, R.J. 2003. Phenolic acids in foods: an overview of analytical methodology. *J. Agric. Food Chem*., 51: 2866-2887.

Russel, D.F. and S.P. Eisensmith. 1983. MSTAT-C. Crop Soil Science Department, Michigan State University USA.

Sahreen, S., M.R. Khan and Khan R.A. 2010. Evaluation of antioxidant activities of various solvent extracts of *Carissa opaca* fruits. *Food Chem.,* 122: 1205-1211.

Saura-Calixto, F. and I. Goñi. 2006. Antioxidant capacity of the Spanish Mediterranean diet. *Food Chem.,* 94: 442-447.

Seeram, N.P., R.A. Momin, M.G. Niar and L.D. Bourquin. 2001. Cyclooxygenase inhibitory and antioxidant cyanidin glycosides in cherries and berries. *Phytomed.,* 8: 362-9.

Shahidi, F and M. Naczk. 2004. Phenolics in food and nutraceuticals. CRC Press, New York.

Siddhuraju, P. and K. Becker. 2003. Antioxidant properties of various extracts of total phenolic constituents from three different agro climatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. *J. Agric. Food Chem.,* 51: 2144-2155.

Slinkard, K. and V.L. Singleton. 1977. Total phenol analyses: automation and comparison with manual methods. *Amer. J. Enol. Viticul.,* 42: 145-152.

Spencer, J.P., M.M. Abd El Mohsen, A.M. Minihane and J.C. Mathers. 2008. Biomarkers of the intake of dietarpolyphenols: strengths, limitations and application nutrition research. *Brit. J. Nutri.,* 99: 12-22.

Tapas, A.R., D.M. Sakakar and R.B. Kakde. 2008. Flavonoids as nutraceuticals: a review. *Trop. J. Pharmaceut. Res*., 7: 1089-1099.

Ullah, R., J. Bakht and M. Shafi. 2015. Antibacterial and anti-oxidant potential of *Periploca hyaspidis*. *Bangladesh J. Pharmacol.,* 10: 645-651.

Whitson, M. and P.S. Manos. 2005. Untangling Physalis (Solanaceae) from the physaloids: A two-gene phylogeny of the Physalinae. *Syst. Bot*., 30: 216-230.

Wright, M.E., Y. Park, A.F. Subar, N.D. Freedaman, D. Albanes, A. Hollenbeck, M.F. Leitzmann and A. Schatzkin. 2008. Intakes of fruit, vegetables, and specific botanical groups in relation to lung cancer risk in the nih-aarp diet and health study. *Amer. J. Epidemiol.,* 1024-1034.

Xu, B.J. and S.K. Chang. 2007. A comparative study on phenolic profiles and antioxidant activities of legumes as affected by extraction solvents. *J. Food Sci.,* 72: S159-166.

Zakir, U.D., A.S. Anwar, J. Bakht, U. Inam and J. Saleem. 2015. *In vitro* anti microbial, antioxidant activity and phytochemical screening of *Apium graveolens*. *Pak. J. Pharmaceut. Sci.,* 28: 1699-1704.

Zimmermann, B.F. and R. Galensa. 2007. One for all-all for one: proof of authenticity and tracing of foods with flavonoids. Analysis of proanthocyanidins in barley and malt. *Eur. Food Res. Technol*., 224: 385-393.

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