Research Article

Antibacterial Activity by Chlorogenic Acid Isolated through Resin from Apricot (*Prunus Armeniaca* L.)

Ahmed Mujtaba[1], Tariq Masud[1], Asif Ahmad[1], Waqar Ahmed[3], Saqib Jabbar[3]* and Robert E. Levin[2]

[1]Department of Food Technology, PMAS-Arid Agriculture University Rawalpindi, Pakistan; [2]Department of Food Science, Chenoweth Laboratory, University of Massachusetts, Amherst. USA; [3]Food Science and Product Development Institute, National Agricultural Research Centre, Islamabad, Pakistan.

Abstract | A number of studies concluded the antibacterial activity of apricot (*Prunus armeniaca* L.) and its by-products. The aim of the study were to test the activity of chlorogenic acid isolated from apricot cv. Habi, against different food borne pathogens and quantification of the chlorogenic acid (CA) using High Performance Liquid Chromatography (HPLC). The chlorogenic acid was isolated using resin. The recovered amount of compound was calculated. Aliquots of the extracts at known concentration of chlorogenic acid were then applied against different bacterial strains for antibacterial activity. The minimum inhibitory concentration of isolated compound was also measured. Two antibiotics were used as references compounds for antibacterial activities. The aqueous ethanolic extract having 968.125 µg/ml of chlorogenic acid inhibited significantly the growth of tested bacterial strains. Among the bacterial strain tested *Salmonella entritidis* and *Helicobacter pylori* were even more sensitive to 20.313 (µg/ml) of chlorogenic acid and showed zone of inhibition with the value of 6.0 and 7.0 mm. The HPLC analysis of apricot suggested that 5.0 gm of apricot contain 1.04 µg/ml of chlorogenic acid on dry weight basis. It is concluded that apricot may be an alternative, natural and supplemental treatment for diseases associated with these pathogens.

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***Correspondence** | Saqib Jabbar, Food Science and Product Development Institute, National Agricultural Research Centre, Islamabad, Pakistan; **Email:** saqibjabbar2000@yahoo.com
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Introduction

Apricot (*Prunus armeniaca* L.) is the fruit rosacea tree (Family R*osaceae*) that is domesticated roughly 5000 year ago (Ali et al., 2011; Sochor et al., 2010). The fruits are widely used as a folk medicine especially in Japanese and Chinese prescription (Mitani et al., 2013; Miyazawa et al., 2006). It is often used as anti-asthmatic, anti-anameic anti-cancer, prescribed medicine for disorder of stomach and intestine, quick recovery from fatigue, cough and diarrhea and many other have already been discussed (Garcia Viguera et al., 1997; Yigit et al., 2009). The richness in phytochemical such as polyphenols in addition to other related compounds are responsible for the beneficial effect. Among all phenolic content the chlorogenic acid (CA) is the dominant phenolic compound in apricots (Sochor et al., 2010). The chlorogenic acid possesses the ability of antioxidant, antidiabetic, antivirus, anti-inflammatory and anticancer (Zhang et al., 2013).

The isolation and purification of chlorogenic acid was done through the use of solid liquid extraction, sol-

vent traction, polyamide and gel chromatography (Li et al., 2005). Alternatively, the resins have been proven efficient due to their chemical nature, easy regeneration and porosity and have been used extensively for isolation of active compounds such as flavonoids, saponins and alkaloids (Fu et al., 2005; Zhao et al., 2007). However, no studies have been reported to isolate chlorogenic acid from apricot by resins. Also, in present study, ethanol is used for extraction which could provide a safe and potentially important method for chlorogenic acid production.

Therefore, the present work was designed to investigate the isolation quantity and antibacterial effect of chlorogenic acid from apricot through resin and quantification of chlorogenic acid in apricot.

Material and Methods

Material and reagents

The apricot fruits var. Habi showed the best results in the previous study of Mujtaba et al. (2016). Hence Habi was chosen and procured for isolation of active compound. The fruit was purchased from Zakir Nursary farm, Gilgit-Baltistan, Pakistan during June 2015. The fruit were cleaned, sorted and graded. The fruits were packed in wooden boxes and shifted immediately to Department of Food Science, Chenoweth Lab. University of Massachusetts, Amherst USA. The fruit (3.0 kg) was dried under shade, excluding seed. The dried sample was powdered to Willy Mill at sieve size of 6. The chlorogenic acid standard was purchased from Sigma Aldrich, USA with the purity not less than 98% for High Performance Liquid Chromatography and 95% for total phenolic contents. Other chemical like resin, analytic grade ethanol and High performance liquid chromatography grade methanol and acetic acid were purchased from Fischer Scientific Inc. USA. The samples were passed through 0.45µm membranes (Fischer Scientific Inc. USA) before HPLC and antibacterial testing. The medium for bacterial growth like tryptic soya broth, agar and antibiotics were purchased from Merck and Co. Inc., USA.

Isolation of Chlorogenic acid

Since isolation of chlorogenic acid was done using method of Zhang et al. (2013) with modification. Isolation of chlorogenic acid extract on resin (Rexyn 201, Mesh size 200-400, Fischer scientific Co.) was conducted in 250 mL conical flask with a lid. Flask containing fixed amount of test resin (equivalent to 5.0 g of dry resin) and 20 gm of apricot crude (at the rate of 1: 2 with 0.15 M phosphate buffer at pH 6.5) were sealed tightly and shaken (150 rpm) in a shaker at 20 °C for 1 hr. The residual solution were removed first by washing with deionized water for four times and then desorbed with 20 ml 90% (v/v) aqueous ethanol. The flask was shaken (150 rpm) at 20 °C for 1 hr.

Total phenolics assay (TPC)

The extract and its dilution were analyzed for TPC and recorded in term of µg/ml of chlorogenic acid in term of dry weight basis by Folin-Ciocalteu assay as described by Chandler and Dodds (1993) with some modification. To 400 µl of extract (diluted 1:10), 2 ml of Foiln-Ciocalteu reagent and 1.6 ml of sodium chloride was added and shaked. After mixing it was kept for 30 min at room temperature and absorbance was measured at 750 nm using a spectrophotometer (CE-2021, 2000 series CECIL Instruments Cambridge, England).

Bacterial strains

Antibacterial activity and minimum concentration of chlorogenic acid against four pathogenic bacteria including *Escherichia coli* (O157: H7 ATCC C9490), *Salmonella enteritidis* (ATCC BAA708), *Pseudomonas flourescens* (ATCC 15442) and *Helicobacter pylori* (ATCC 14504) were evaluated.

Antimicrobial test using disc diffusion assay

The antimicrobial test and minimum concentration against chlorogenic acid was performed by the method described by Haghayeghi et al. (2013). Lawns of each bacterial species were prepared as follows: when A_{600} of active cultures reached 0.45 (mid-logarithmic phase of growth), 0.1 ml of each test species with soft agar was spread on plates containing respective media (containing 2.0% agar). Extracts were applied to the discs (6mm in diameter) placed on the inoculated tryptic soy agar. The inoculated plates were incubated at 37°C for *E.coli*, and *S. entritidis* and 20 °C for *P. flourescens* at interval of 24 hr. While the *H. pylori* plates were incubated in Gas-Pak jars (BBL Microbiology Systems, MD, USA) with anaerobic (Gas-Pak) generator envelops at 37 °C for 48 hr. Antimicrobial activity was evaluated by measuring the inhibition zone against test microorganism.

Minimum Inhibitory concentration (MIC)

The minimum inhibitory concentration of the chloro-

genic acid was determined through dilution method. Briefly, serial dilutions, ranging from 20.313 to 968.125 µg/ml of the chlorogenic acid were prepared volume being 30 µl. These dilute on were then applied on disc (6 mm in diameter) placed on tryptic soy agar. All the plates contain lawn of each bacterial species when A_{600} reaches to 0.45 mid of log. Plates were incubated on above mentioned time and temperature. The ethanol used for control. Gentamycin and oxacillin was used as a reference compound for antibacterial activity. Antibiotics were reconstituted according to the manufacturer's direction, filtered through a sterile 0.45 mm pore size polysulfone membrane and used on the same day.

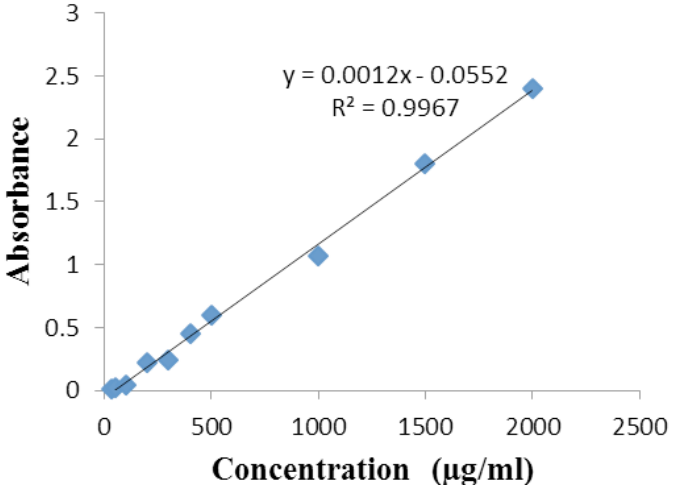

Figure 1: *Calibration curve of total phenolics assay in term of µg/ml of chlorogenic acid.*

High performance liquid analysis of chlorogenic acid (CA)

The chlorogenic acid were then determined in 5.0 gm dry weight of apricot using Agilent 1100 series (Agilent Technologies, Chelmsford, MA, USA) consisted of two solvent delivery pumps (G1310 A), Metachem Polaris C_{18} Aorbax SB C_{18} (150 × 4.6; size of particle 5 µm (Agilent Technologies, Palo Alto, CA, USA), UV detector (G1314 A). Both the detector and the reaction coil/column were thermo stated. The mobile phase was as follows: A: acetic acid (1%) in water and B acetic acid (1%) in methanol at the flow rate of 1.0 ml/min. The detection wavelength was set as 273 nm for acquiring chromatograms for standard curve and quantification of CA (Sochor et al., 2013).

Results and Discussion

Total phenolics content (TPC)

The total phenolics content in term of µg of chlorogenic acid/ml of ethanolic extract in apricot was determined. The calibration curve showed linearity for chlorogenic acid in the range of 35 to 2000 µg/ml, with a correlation coefficient (R^2) 0.9967 (Figure 1). The analysis of extract showed a value of 968.125 µg/ml of CA on dry weight basis (Table 1). This value is much higher than Kan et al. (2014) and Miletic et al. (2014) who reported 5.85 to 152.515 and 0.056 µg/ml of chlorogenic acid respectively in different apricot varieties. These differences may be attributed by cultivar, extraction procedure, climatic condition and stage of ripening (Ali et al., 2011; Kan et al., 2014; Zhang et al., 2013). The higher value suggests the better procedure to extract the chlorogenic acid then others. The extract were diluted and showed a total phenolic contents value of 460.183, 227.313 and 20.313 µg/ml respectively.

Antimicrobial study and MIC of chlorogenic acid

The sensitivity of different pathogens to the phenolic content (chlorogenic acid) was determined (Table 1). All the bacterial strains tested were inhibited by CA extract of apricot. The degree of inhibition of each was similar for all isolates. The three bacterial strains *S. entritidis*, *p. flourescens* and *H. pylori* showed much higher zone of inhibition with the value of 17.0 mm. However *E. coli* is least affected and showed 13.0 mm zone of inhibition. The degree of zone of inhibition varies depending upon concentration of compound. This highlights the importance of extraction procedure, fruit component and tested strain in determining the antimicrobial activity of fruit. It also reflects the distribution and extractability of polyphenolic component involved against microorganism (Hagheghi et al., 2013). The possible mechanism of action of chlorogenic acid describe as the increased outer and plasma membrane permeability, resulting in the loss function, even encouraging slight leakage of nucleotide (Lou et al., 2011).

Table 1: *Antimicrobial activity against E.coli. S. entritidis, P. flourescens and H pylori by chlorogenic acid.*

Chlorogenic acid (µg/mL)	Antibacterial Activity (mm) after 1hr of extraction			
	E. coli	*S. entritidis*	*P. flourescens*	*H. pylori*
968.125	15.0	17.0	17.0	17.0
460.183	11.0	13.0	12.0	13.0
227.313	9.0	10.0	10.0	11.0
20.313	-	6.0	-	7.0

(-) indicates no antimicrobial activity.

The specific sensitivity as the minimum inhibitory

concentration, to chlorogenic acid to three aerobic and one partially aerobic was determined (Table 2). The MIC of CA isolated from Apricot was 227.313 µg/ml. Two bacteria *S. entritidis* and *H. pylori* were even more sensitive with inhibitory concentration of 20.313 µg/ml. At all the extract has lower MIC then gentamycin. While no antibiotic were found to be resistant in case of *P. flourescens*. The MIC value of chlorogenic acid found by Lou et al. (2011) was in the range of 20-80 µg/ml of all tested bacterial pathogens. The MIC values for *E. coli* and *S. entritica* were 5 mm and no (0.0 mm) zone of inhibition with even 500 µg of CA by Puupponen-Pimia et al. (2001). The difference in MIC was due to the cell wall permeability of different organism and source of isolation.

Table 2: *MIC of chlorogenic acid against different bacterial strains*

Test organism MIC(µg/mL)	*E. coli*	*S. entritidis*	*P. flourescens*	*H. pylori*
Ethanol extract	227.313	20.313	227.313	20.313
Gentamycin	460.183	227.313	-	227.313
Oxacilin	-	460.183	-	460.183

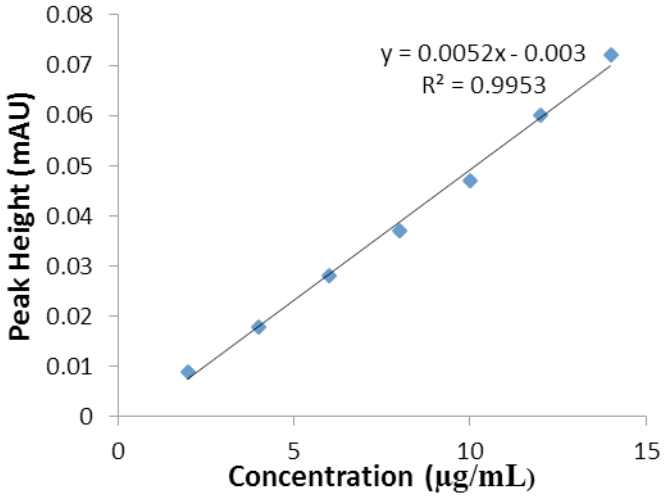

Figure 2: *Calibration curve for chlorogenic acid performed for HPLC*

Chlorogenic acid in apricot (CA)

The ethanolic extract of apricot was quantified for CA. The calibration curve for chlorogenic acid is y = 0.0052x - 0.003 with correlation coefficient (R^2) value of 0.9953 (Figure 2). Chromatogram of apricot showed a value of 1.04 µg/ml for chlorogenic acid which is close to the findings of Sochor et al. (2010) who reported in the range of 1.21 to 9.44 µg/ml of chlorogenic acid in different apricot varieties determined through HPLC. But this value is higher than Donovan et al. (1998) who reported a range from 0.144 to 0.436 µg/g. Many studies prove that chlorogenic acid and its polymer is the dominant ester in apricot (Bors and Michel, 2002; Kan et al., 2014: Fang et al., 2002) and mostly found in flesh of fruit. The reason for the variation in CA content may be due to the variation in extraction procedure, amount of sample, early and late ripening variety and method of determination (Mitani et al., 2013; Zhang et al., 2013; Ruiz et al., 2005).

Conclusion

Human health and nutrition are still interesting and most studied topic around the world. Natural compounds, due to their potential health promoting effect are under detailed investigation. In the present study we aimed to study the antimicrobial effect of chlorogenic acid from apricot and quantification of chlorogenic acid. It can be concluded that CA demonstrated an antimicrobial activity against all tested isolates. Whereas the *S. entritidis* and *H. pylori* are even more sensitive to CA showing zone of inhibition of 6.0 and 7.0 mm to a concentration of 20. 313 µg/ml. On the other hand, a significant quantity of CA is present in apricot fruit.

Authors Contribution

AM and TM conceived the idea of the study. AA provided technical support throughout. AM and REL collected and analyzed data. AM, RM, WA and SJ wrote the manuscript. SJ did overall management of the article.

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