Biomedical Research 2018; 29 (12): 2459-2464 *ISSN 0970-938X*
www.biomedres.info

Phytochemical analysis and antioxidant activity of *Persia americana* and *Actinidia deliciosa* fruit extracts by DPPH method.

Sadia Rafique*, Naveed Akhtar

Faculty of Pharmacy and Alternative Medicine, the Islamia University of Bahawalpur, Punjab, Pakistan

Abstract

Extracts having antioxidant properties show promising effect on human skin. The main objective of this study was to perform phytochemical analysis to identify active constituents and determine the antioxidant activity through DPPH (2, 2 diphenyl-1-picryl hydrazyl) method in sampled fruit. In this work, extract of *Persia americana* variety 'Hass' and *Actinidia deliciosa* fruit were selected. Various qualitative and quantitative tests were performed for phytochemical analysis. For antioxidant activity, different parts of fruit samples of *Persia americana* were prepared in methanol and in acetone solution while in case of *Actinidia deliciosa* whole fruit was soaked in methanol and in ethanol solution. Phytochemical analysis showed the higher concentration of flavonoids and phenolics contents in both fruit extracts. The analysis also showed the presence of vitamin C, alkaloids, glycosides, amino acids, carbohydrates, proteins, steroids and triterpenoids. The DPPH results using ascorbic acid as standard showed that acetone and methanol extract of *Persia americana* possess higher antioxidant activity (84%) than methanol extract alone (60%) while *Actinidia deliciosa* whole fruit showed better antioxidant activity in ethanol extract (79%) as compared to methanol extract (57%). The extracts possess antioxidant activity can be incorporated in different skin formulations and their *In-vitro* and *In- vivo* studies may be performed for cosmetic market.

Keywords: *Persia americana*, *Actinidia deliciosa*, phytochemical, DPPH, antioxidant, flavonoids and phenolic contents.

Accepted May 08, 2017

Introduction

Persia americana variety 'Hass' belongs to family lauraceae and its fruit is berry shaped [1]. 'Hass' variety changes its colour from green to black upon maturation [2]. It's pulp is rich in unsaturated fatty acids including oleic, linoleic, palmitoleic acid which tend to oxidize during storage [3]. It contain greatest amount of vitamin C, alkaloids, glycosides, flavonoids, carbohydrates, protein, minerals, steroids and triterpenoids [4,5]. It is also the rich source of phytochemical constituents that reduces the risk of cardiovascular disease [6]. These phytochemicals such as flavonoids, carotenoids and antioxidative vitamins found in this fruit reduces the potential risk of cancer disease [7]. *Actinidia deliciosa* belongs to Actinidiaceae family, oval globular shaped fleshy fruit filled with black, small edible seeds [8]. It is a good source of vitamin C, but excellent sources of folate, potassium, vitamin K and vitamin E [9]. The phenolic and flavonoid contents present in highest ratio in fruit juice [10]. *Actinidia deliciosa* fruit is also beneficial in cardiovascular disease [11]. Due to its highest antioxidant activity it may prevent the skin diseases caused by oxidative stress [12].

There are different methods used to test the antioxidant activity. These methods include Total radical-trapping antioxidant parameter assay (TRAP assay), Trolox equivalent antioxidant capacity assay (TEAC I-III assay), N,N-dimethyl-p-phenylendiamine assay (DMPD assay), 2,2 diphenyl-l-picrylhydrazyl assay (DPPH assay), Ferric reducing ability of plasma assay (FRAP assay), β-carotene bleaching test (BCB), Thiobarbituric acid reactive species assay (TBARS assay) and Photochemiluminescence assay (PCL assay) [13,14]. Due to radical scavenging activity, the DPPH is very simple, famous, most sensitive and very convenient method for screening the antioxidant activity [15]. In this method, hydrogen is a donor atom that measures radical scavenger atoms and give antioxidant activity. The changes in colour occur from purple to yellow when antioxidant absorption occurred. The strong absorption seemed at 517 nm. This is stichiometric reaction based upon the absorption of hydrogen atom. When UV absorption falls below 517 nm, the antioxidant effect can be observed [16].

Persia americana antioxidant activity is high and our main study focus was also upon it's antioxidant activity [17]. Every part (peel, pulp and seed) of *Persia americana* has different in antioxidant activity and it changes with the change of solvent [18]. Due to the presence of high contents of oil, it can be used in pharmaceutical and cosmetics industry [19]. *Actinidia deliciosa* also posses higher antioxidant activity because it's

ORAC value is greater when whole fruit was taken for extraction [20]. Our main study objective was to evaluate the total phenolic contents, total flavonoid contents, vitamin C, antioxidant activity and phytochemical analysis of *Persia americana* and *Actinidia deliciosa* fruit extracts.

Materials and Methods

Chemicals and apparatus

Methanol, ethanol (Merck KGaA Darmstadt, Germany), acetone (BDH, England), distilled water (Department of pharmacy, IUB, Pakistan), fruits (Metro, Faisalabad, Pakistan), DPPH (Sigma, USA), rotary evaporator (Eyela, Co. Ltd. Japan), UV-VIS spectrophotometer (U vikon XL, Bio-Tek Instruments, Bad Friedrichshall, Germany), microplate reader (Synergy HT Bio Tek®, USA).

Collection of fruit material

The fruits of *Persia americana* and *Actinidia deliciosa* were collected from local market, Metro, Faisalabad, Pakistan and the specimens were identified from herbarium botany department, University of Agriculture, Faisalabad, Pakistan under voucher no 411-1-16 for *Actinidia deliciosa* and 411-2-16 for *Persia americana*.

Preparation of fruit extract

Different types of fruit extracts were prepared in different solvents. *Persia americana* pulp was grinded into fine particles and then soaked in 70% methanol solution. Similarly, *Persia americana* peel and pulp was also soaked after grinding in 70% methanol solution. *Persia americana* seed alone was grinded and then soaked in 70% methanol solution. *Persia americana* peel and pulp was grinded together and then soaked in 60% acetone and 10% methanol solution. *Persia americana* whole fruit was grinded and soaked in 70% acetone solution.

Similarly, *Actinidia deliciosa* whole fruit was taken and grinded into fine particles by using mixer grinder and then this material was soaked in a solvent mixture containing 70% ethanol solution and another sample was prepared by grinding fruit and then soaking it in 70% methanol solution. Soaking of all samples was done for 48 h then filtered through muslin cloth and finally through whatman No 1 filters paper. The obtained filterate was concentrated in rotary evaporator. The extracts were then kept in refrigerator at 8°C for antioxidant activity and for phytochemical analysis.

DPPH method

The free radical scavenging activity of different extracts of *Persia americana* and *Actinidia deliciosa* was observed. A stable free radical of 2,2-diphenyl-1-picryl-hydrazyl (DPPH) was used with slight modification to determine the antioxidant activity [21]. 100 µM concentration of DPPH in methanol was used. The total volume of assay was 100 µl, in which test solution was 10 µl and DPPH solution was 90 µl in 96 wellplate. Both solutions were mixed in vortex mixer for 30 min and incubated at 37°C. Microplate reader Synergy HT BioTek® USA was used to measure any decrease in absorbance at 517 nm in a UV spectrophotometer (double beam spectrophotometer Uvikon XL, Bio-Tek instruments, Bad friedrichshall, Germany). The reference standard of asorbic acid was used. All results were repeated for three times. The following formula was used to calculate the percentage inhibition.

$$Inhibition\ (\%) = \frac{Abs.\ of\ control\ -\ Abs.\ of\ test\ solution}{Abs.\ of\ control} \times 100$$

Where:

Absorbance of control = Total radical activity without inhibitor.

Absorbance of Test = Activity in the presence of test compound.

Phytochemical analysis

Both fruit extracts were subjected to testing for active principles of vitamin C, alkaloids, glycosides, phenolics, flavonoids, carbohydrates, steroids, triterpenoids, tannins and saponins. Total phenolic contents and total flavonoid contents were find out quantitatively. To find out total phenolic contents, take 50 µl of sample and 50 µl of Folin-Coicalteu reagent. Add it in 750 µl of distilled water and mixture was kept at room temperature for 10 min. Then add 150 µl of 20% Na_2CO_3 in whole mixture. Heat this mixture in water bath at 40ºC for 20 minutes and then cooled in ice bath. Absorbance was measured at 755 nm using spectrophotometer [22]. To test the flavonoid contents, take 0.5 ml of each fruit extract and then it was mixed with 0.5 ml of 2% $AlCl_3$ methanol solution. The mixture was incubated at room temperature for 10 min. Absorbance was measured at 368 nm [23]. Different tests have been performed for qualitative analysis of both fruits. These tests include test for flavonoids, vitamin C, alkaloids, glycosides, amino acids, carbohydrates, proteins, steroids and triterpenoids, tannins and saponins. For qualitative analysis of flavonoids, ferric chloride test has been performed. The appearance of blackish red or green precipitate, upon addition of 2-3 drops of ferric chloride solution in a test sample would indicate the presence of flavonoids [24]. For vitamin C, DNPH test has been performed. Dissolve 2,4 dinitro phenyl hydrazine in concentrated sulfuric acid. Both test solutions were treated separately with this reference mixture and vigorously shake it. The appearance of yellow precipitate would indicate the presence of vitamin C [25].

Hager's test has been performed to find out the presence of alkaloids. In this test, treat both the test solution separately with 3-4 drops of Hager's reagent (Saturated picric acid solution). If yellow precipitate would appear upon shaking then it means alkaloids are present [25,26]. Bromine test has been performed for glycosides. Equal amount of both test solution and bromine water was dissolved in test tubes separately and shake it well. Again the appearance of yellow precipitate

would give the positive response [26]. Another test used for glycosides is keller killiani test. Ferric chloride solution was mixed with 2-3 drops of glacial acetic acid in 2 test tubes and then mix them in a single test tube. When 2-3 drops of concentrated sulfuric acid was added in it, the formation of 2 layers were observed. Reddish brown layer would appear in bottom, while acetic acid layer would appear in top which changes to bluish green indicate the presence of glycosides [27].

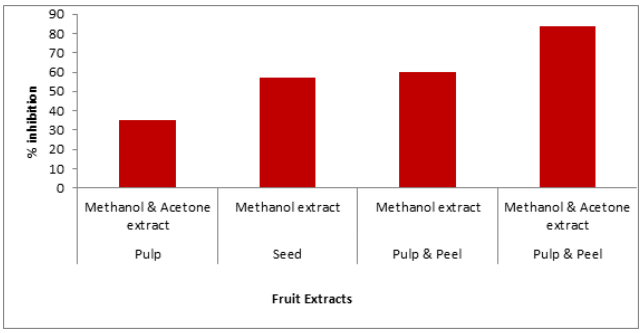

Figure 1. *Antioxidant activity of Persia americana variety 'Hass' by DPPH method.*

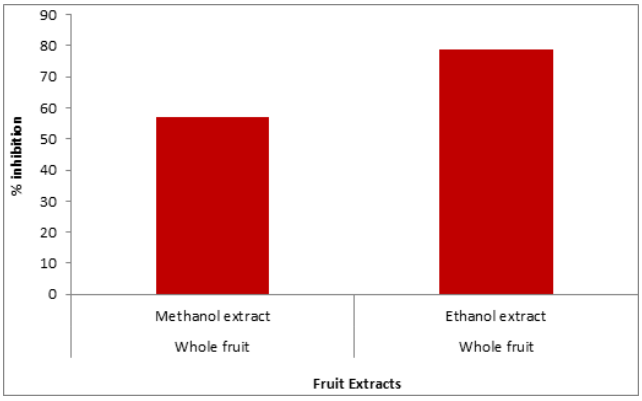

Figure 2. *Antioxidant activity of Actinidia deliciosa by DPPH method.*

Ninhydrin test was performed for amino acids. Boil the test solution with 0.2% Ninhydrin solution. If purple colour appear upon boiling would suggest that free amino acids are present [28]. Benedict's test was performed for carbohydrates. Benedict's solution is the mixture of alkaline solution and complex of cupric citrate. When 2 ml of this reagent is added in a test solution and boil it on water bath for few minutes. If reddish brown precipitate would appear shows the presence of carbohydrates [29]. For proteins detection, biuret test is performed. Treat the test sample with a solution containing two drops of copper sulphate (0.1%) and sodium hydroxide solution (10%). The presence of violet or pink colour would suggest that protein is present [25]. For steroids and triterpenoids, liebermann burchard test was performed. Mix the test sample with 3-4 drops of acetic anhydride and heat the mixture till boiled. Then cool it. Then add concentrated sulphuric acid from the sides of test tube. If brown ring appear at the junction of 2 layers. The upper layer would be of green colour and the lower layer would be of deep red colour would suggest the presence of steroids and triterpenoids [26]. Gelatin test is the conformation of tannins. If small amount of gelatin solution is added on a test sample and white precipitate would appear at the bottom of test tube would give a positive result for tannins [30]. For saponins, foam test is performed. When test sample is mixed with distilled water and shaken contineously for 5 min. If froth appear and remain stable for 15 minutes then it means saponins are present [31].

Results

The antioxidant activity of different parts of *Persia americana* variety 'Hass' is given in Figure 1 and *Actinidia deliciosa* in Figure 2. The quantitative and qualitative analysis of *Persia americana* and *Actinidia deliciosa* is given in Tables 1 and 2.

Table 1. *Quantitative analysis of Persia americana variety 'Hass' and Actinidia deliciosa fruit extracts.*

Chemical tests			*Persia americana* extract	*Actinidia deliciosa* extract
Test for TPC (mg GAE/g extract)	Absorbance		141.14	116.43
	Persia americana	0.994		
	Actinidia deliciosa	0.821		
Test for TFC (mg QE/g)			67.87	61.91

Table 2. *Qualitative anslysis of Persia americana variety 'Hass' and Actinidia deliciosa fruit extracts*

Chemical tests		*Persia americana* extract	*Actinidia deliciosa* extract
Test for Flavonoids	Ferric chloride test	+ve	+ve
Test for Vitamin C	DNPH test	+ve	+ve

Test for Alkaloids	Hager's test	+ve	+ve
Test for Glycosides	Bromine test	-ve	+ve
	Keller Killiani test	+ve	+ve
Test for Amino acids	Ninhydrin test	+ve	+ve
Test for Carbohydrates	Benedict's test	+ve	+ve
Test for Proteins	Biuret test	+ve	+ve
Test for Steroids & Triterpenoids	Liebermann Burchard test	+ve	+ve
Test for Tannins	Gelatin test	-ve	+ve
Test for Saponins	Foam test	-ve	-ve

The results of *Persia americana* variety 'Hass' showed maximum antioxidant activity when peel and pulp of fruit were extracted with different ratio of methanol and acetone solvent. It is reported that 70% of aqueous acetone is more effective for maximum amount of condensed tannins [32]. If 50% of acetone extract is used than it will achieve the greatest level of phenolics as compared to any other solvent [33]. The DPPH results using ascorbic acid as reference standard showed that acetone and methanol extract of *Persia americana* possess 84% antioxidant activity while methanol extract showed 60% antioxidant activity and *Actinidia deliciosa* whole fruit showed 79% of antioxidant activity in ethanol extract as compared to methanol extract which give antioxidant activity of 57%.

The quantitative analysis of both fruits *Persia americana* and *Actinidia deliciosa* showed that both fruits contain excellent total phenolic contents and total flavonoid contents. The TPC (mg GAE/g) of *Persia americana* and *Actinidia deliciosa* are 141.14 and 116.43 while TFC (mg QE/g) of *Persia americana* and *Actinidia deliciosa* are 67.87 and 61.91 respectively. The ferric chloride test confirms the presence of flavonoids in both fruit. Moreover, the presence of vitamin C and phenolic contents also lead to increase the antioxidant activity [6]. The presence of polyphenols and phytochemicals also correlate with antioxidant activity [34].

Discussion

Consumption of such fruits with high antioxidant activity and high value of bioactive compounds give best neutrition results [35].Therefore, most of the scientists recommend to consume such fruits [36]. Two things of Phenolic largely determine the efficiency of extraction process. One is its chemical structure and other is it's polarity and due to this reason, *Persia americana* achieved the highest level of total phenolic contents in acetone solution as compared to methanol solution [37]. While in case of *Actinidia deliciosa*, when whole fruit was extracted in ethanol solution, it showed maximum antioxidant activity than whole fruit is extracted in methanol solution as shown in Figure 2. The hydrophobic property is dominant in ethanol solution because of more number of carbons present in ethanol. The ethanol is also the best extraction solvent for both hydrophilic and hydrophobic extract as compared to methanol solution, so antioxidant activity is higher in it [38].

Naturally, the fruits have higher phenol antioxidant than vegetables and they can reduce the lipoprotein and protect them from oxidantion [39]. The phytochemical contents present in both fruits may be attributable to increase the antioxidant activity of both fruits [40]. The quantitative analysis of both fruits shows that total phenolic contents and total flavonoid contents present in *Persia americana* are greater than *Actinidia deliciosa*, so the antioxidant activity of *Persia americana* is 84% as compared to *Actinidia deliciosa* that have 79% antioxidant activity. But gelatin test shows that tannins are only present in *Actinidia deliciosa*. It was found that total polyphenols and flavonoids contents were significantly higher with the change of extraction solvent. Although the total phenolic contents and total flavonoid contents not only increases the antioxidant activity but there is a linear correlation with phytochemicals such as phenolics, flavonoids and steroids [41]. The fatty acids present in *Persia americana* acts with phytochemicals reduce the risk of cancer disease [19]. These are polyphenols which gives antimicrobial, antioxidant and healing activity to the skin [42]. Several other studies shows that polyphenolic and flavonoid contents may lead to increase antioxidant effect medicinally [43]. *Actinidia deliciosa* healthful atributes related to high contents of ascorbic acid, polyphenols and the presence of flavonoids [44]. The results of *Actinidia deliciosa* showed that ethanol extract have large percent of inhibition in DPPH as compared to other extracts [45]. The antioxidant activity of *Persia americana* and *Actinidia deliciosa* can be incorporated in conventional and sustained release skin formulations and the formulations can be tested in healthy and unhealthy volunteers to explore their effects on different parameters of human skin i.e melanin, erythema, moisture content and transepidermal water loss.

Conclusion

Antioxidant activity of different parts of *Persia americana* variety 'Hass' was taken into consideration by changing the different solvent ratio. The same solvent ratio and method was used for pulp and seed separately and peel plus pulp combined. The pulp showed lowest antioxidant activity then seed but

highest antioxidant activity was observed for peel and pulp when combined together. In case of *Actinidia deliciosa*, the fruit shows highest antioxidant activity of 79% in ethanol solution as compared to methanol solution by DPPH method. Moreover, the skin part of *Actinidia deliciosa* exhibits the higher antioxidant activity as compared to fleshly part, so, the consumption of whole fruit is not only convenient but also beneficial for health promoting effect. This antioxidant activity is very useful and effective which can be used to make various cosmeceuticals formulations that show better anti-acne, skin soothing, anti-aging and skin fairness effects.

It can also be concluded from the present study that total phenolic contents and total flavonoid contents present in *Persia americana* are higher than *Actinidia deliciosa* while vitamin C, alkaloids, glycosides, amino acids, carbohydrates, proteins, steroids and triterpenoids are present in both fruits. The presence of tannins in *Actinidia deliciosa* made it more beneficial for preparation of cosmeceutical preparations.

Acknowledgement

The authors would like to say thank to respected Prof. Dr. Mahmood Ahmad, Dean of Faculty of Pharmacy, IUB, Bahawalpur, Pakistan for providing cosmetic laboratory services.

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***Correspondence to**

Sadia Rafique

Faculty of Pharmacy and Alternative Medicine

The Islamia University of Bahawalpur

Punjab

Pakistan