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Journal of Biochemistry and Analytical studies
ISSN 2576-5833 | Open Access

RESEARCH ARTICLE

Volume 4 - Issue 1

Phytochemical Studies and *In Vitro* Evaluation of Antioxidant & Antihyperglycemic Activities of *Actinidia deliciosa* (A. Chev.) C.F. Liang & A.R. Ferguson (Kiwifruit)

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Received: 09 Jan, 2020 | **Accepted:** 25 Mar, 2020 | **Published:** 31 Mar, 2020

Citation: Dhiman VK, Chauhan V, Kanwar SS (2020) Phytochemical Studies and *In Vitro* Evaluation of Antioxidant & Antihyperglycemic Activities of *Actinidia deliciosa* (A. Chev.) C.F. Liang & A.R. Ferguson (Kiwifruit). J Biochem Analyt Stud 4(1): dx.doi.org/10.16966/2576-5833.119

Abstract

The fruit of *Actinidia deliciosa* (A. Chev.) C.F. Liang & A.R. Ferguson, kiwifruit also known as 'China's miracle fruit' and 'Horticulture wonder of New Zealand' is a very well-known fruit with excellent bioactive properties associated with good health attributes such as healthful skin, better sleep contributed by antioxidants and serotonin content of kiwifruit. It also maintains cardiovascular health and blood pressure as kiwifruit is a good source of potassium, fibre and antioxidants. Antioxidant properties help in anti aging and blood cleansing activities while antihyperglycemic compounds may lead to antidiabetic effects. The purpose of the present study was to investigate the biochemical constituents of the kiwifruit and to establish its antioxidant and antihyperglycemic effects. The chemical profiling of kiwi fruit extracts in two solvents (Ethanol and Methanol) indicated the presence of steroids, cardiac glycosides, terpenoids, flavonoids and carbohydrates. Antioxidant capacity was performed using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical inhibition whereas, α-amylase inhibitory assay was performed using α-amylase from *Aspergillus oryzae*. Methanolic extracts of kiwifruit (*Actinidia deliciosa*) possessed highest inhibitory activities out of the two extracts (compared to ethanolic extracts) against DPPH free radical and α-amylase. IC_{50} (concentration of extract which results in 50% inhibition) values were calculated for both Methanol (MKE) and Ethanol Extract (EKE) of kiwifruit. IC_{50} values for DPPH free radical scavenging assay was 4.2 ± 0.073 mg/mL for MKE and for 3.14 ± 0.153 mg/mL fir EKE. On other hand, the alpha-amylase assay IC_{50} value was found to be 1.53 ± 0.158 mg/mL for MKE and 2.05 ± 0.639 mg/mL for EKE. Kinetic analysis revealed that the EKE and MKE displayed competitive mode of inhibition toward α-amylase. In the study, kiwifruit proved to have health beneficial effects as both antioxidant and antihyperglycemic properties were found in its extract.

Keywords: *Actinidia deliciosa* Chev.cv. Abbot; DPPH; α-amylase; IC_{50} value; Phyto-chemical screening; Antioxidant capacity; Antihyperglycemic

Introduction

Kiwifruit is a worldwide known fruit among the genus Actinidia (*Actinidiaceae*). It is a dioecious plant, widely distributed in Asia. It is native to China but most of the species are cultivated in the southwest part of China. Today kiwifruit is cultivated in many countries, notably Italy, New Zealand, China, Chile, France, Greece, Japan and United States. Kiwifruit consists of various phyto-constituents belonging to category of steroid, triterpenoids, flavonoids, quinones, polysaccharides and phenylpropanoids such as β-sitosterol, stigmastane sterols, asiatic acid, arjunolic acid, 9'-cis-violaxanthin, pheophytin b, pheophytin q, lutein epoxide, pyrrolo quinoline quinone, rhamnose, arabinose, xylose, glucose, mannose and phytoalexins [1-3]. The fruits, seeds, stems and roots naturally possess diuretic, natural blood thinner, febrifuge and sedative properties [4]. It has been reported to be beneficial in the treatment of diseases like rheumatoid arthritis, hepatitis, edema, gingivitis, pyorrhea, etc. Studies have reported kiwifruit to possess immunomodulatory and anticancerous activities [5,6]. A study performed in Norway revealed that consuming 2-3 kiwifruit daily for 28 days can replace aspirin therapy and can significantly thin the blood. Thus, it can help in reducing the risk of clots and in lowering the fats in the blood which are prominent causes of blockages [7].

According to many studies, the intake of kiwifruit plant products caused reduced risk of chronic diseases that have been associated with the compounds possessing antioxidant activities [8]. Carotenoids and Vitamin E contributes to the first defence system against oxidative stress by quenching singlet oxygen [9]. These antioxidants act together and reduce free radicals or reactive oxygen species which are harmful to the living system [10]. Furthermore, the methanolic extracts of kiwifruit showed regulation of adipogenesis in adipocyte differentiation and function which is important in prevention of diabetes [11]. Keeping in view an array of medicinal benefits of kiwifruit, the present study was performed to check the phyto-constituents present in the fruit. However, some of phyto-constituents such as cardiac glycosides have not been quantified in previous

studies and the antidiabetic potency of *Actinidia deliciosa* Chev. and its mechanism of inhibition have not been extensively studied till now. Therefore, the antioxidant and alpha amylase-inhibitory activities of the *Actinidia deliciosa* Chev. Crude fruit extracts were determined to establish its health promoting nature.

Materials and Methods

Plant material

The fruits of *Actinidia deliciosa* Chev. cv. Abbot were obtained from Kiwifruit Orchard (30°.51.44N; 77°.09.32E; 1290 amsl), Department of Fruit Science, Dr. Y. S. Parmar University of Horticulture & Forestry, Nauni-Solan (India) in the month of October 2019. All the samples were washed properly and stored under chilling conditions (0°C temperature).

Chemicals and reagents

DPPH and alpha-amylase from *Aspergillus oryzae* were procured from Sigma-Aldrich Co., St. Louis, USA, while starch soluble (analytical grade) was obtained from HiMedia Laboratories Pvt. Ltd., Mumbai, India. Other chemicals and reagents were of analytical grade and water used was glass distilled.

Preparation of kiwifruit extract

Actinidia deliciosa Chev. Whole kiwifruits were washed with water to remove all the contaminants followed by surface cleansing with 70% ethanol. The fruits were weighed and grinded into fine particles by using blender. Then this slurry (14 g each) was soaked in 350 ml of selected solvent (70% ethanol and 70% methanol) separately in 1:25 (w/v) ratio. Soaking of all samples was done for 48 h under dark condition. Both slurries (Ethanolic kiwifruit extract: EKE; Methanolic kiwifruit extract: MKE) were kept under constant shaking (200 rpm) for 48 hours at 30°C. Then, the contents of the mixtures were filtered through a muslin cloth and finally through Whatman No.1 filter paper. The extraction solvents were evaporated at 30°C under reduced pressure in a rotatory vacuum evaporator until sample was solvent free. The dried residues were weighed and their stock solution were made in distilled water. The yield (percentage) was calculated using the following equation:

Yield (%)=(initial weight of extract in g/final weight of concentrated extract in g) × 100.

The alcoholic extracts were stored in a refrigerator for assay of antioxidant activity and for phytochemical analysis.

Preliminary phytochemical screening of kiwifruit extracts (EKE and MKE)

Phytochemical composition of the *A. deliciosa* Chev. Ancoholic fruit extracts were determined using different methods. Extracts (EKE and MKE) were treated with few drops of 2% (w/v) ferric chloride solution. Formation of blue-black colouration indicated the presence of phenols [12]. A few drops of Molisch's reagent was added into fruit extracts followed by concentrated sulphuric acid slowly along the walls of the test tube. A reddish violet ring showed the presence of carbohydrate in the test sample [13]. Extracts were treated with1.0 ml of chloroform followed by a few drops of concentrated sulphuric acid along the sides of test tube. A reddish-brown colouration at the interphase indicated the presence of terpenoids [14]. Extracts were treated with a few drops of acetic anhydride, shaken and added with 1.0 ml of concentrated sulphuric acid, a colour change from violet to blue green indicated the presence of steroids [15,16]. Extracts were treated with 1.0 ml of glacial acetic acid, mixed well, added a few drops of 5 percent ethanolic ferric chloride solution, shaken, slowly and added 1.0 ml of concentrated sulphuric acid. An appearance of a brownish ring between the two layers with lower acidic layer turning blue-green upon standing indicated the presence of cardiac glycosides [17,18]. Extracts were treated with 1.0 ml of Wagner's reagent, mixed well, added 1% (v/v) hydrochloric acid. The reaction mixtures were incubated in hot water bath for 5 minutes. Appearance of precipitate in the reaction mixture indicated the presence of alkaloids [19]. Extracts were also treated with 1.0 ml of 10% (w/v) sodium hydroxide solution. The yellow colouration which appeared later turned colorless upon addition of dilute acid indicated the presence of flavonoids [20].

Quantitative determination of phytochemical constituents of kiwifruit extracts (EKE and MKE)

Determination of total flavonoid content: The total flavonoid contents of *A. Deliciosa* Chev. Crude fruit extracts were measured using a colorimetric assay developed by Stankovic MS, et al. [21]. A known volume (1.0 ml) of extract (EKE and MKE) was added to a 10 ml volumetric flask followed by addition of 0.15 ml of 5% (w/v) sodium nitrite$_2$. After 5 min, 2% (w/v) of 0.15 ml aluminium chloride was added. Further, after 6 min, the reaction mixture was neutralized with 1.0 ml of 1 M NaOH solution and total volume was made up to 10 ml with distilled water, mixed well and monitored spectrophotometrically at 510 nm. The flavonoid content was expressed as micrograms of quercetin equivalents per milligram of fresh material.

Quantitative estimation of steroids: The amounts of total steroids in *A. Deliciosa* Chev. Crude fruit extracts were determined according to the procedure reported by Patra A, et al. [22]. Each of kiwi extracts (1.0 ml, EKE and MKE) was transferred into 10 ml volumetric flask followed by addition of 2.0 ml of 4N sulphuric acid and 2.0 ml, 0.5% (w/v) iron (III) chloride. Added 0.5 mL of 0.5% (w/v) potassium hexacyanoferrate (III) solution into the reaction mixture. Heated the reaction mixture in a water-bath set at 70 ± 2°C for 30 minutes with constant shaking and the content was diluted with deionised water. Absorption at 780 nm was measured against a reagent blank. The steroid content was determined in kiwifruit extract using cycloartenol as a standard. Total steroid content was expressed as micrograms of cycloartenol equivalents per milligram of fresh material.

Quantitative test for cardiac glycosides: The total cardiac glycoside contents of *A. Deliciosa* Chev. Crude fruit extracts were measured using a colorimetric assay developed by Muhammad SA, et al. [23]. Eight millilitres of kiwifruit extract (EKE and MKE) was transferred to a 100 ml volumetric flask followed by addition of 60 ml of deionised water and 18 ml of 12.5% (w/v) lead acetate, the content was mixed well and filtered. Then, 50 ml of filtrate were transferred into another 100 ml flask and 8 ml of 47% (w/v) disodium monophosphate was added to precipitate excess Pb^{2+} ion. The contents were mixed well and completed the final volume with deionised water. The mixture was filtered twice through same filter paper to remove excess lead phosphate. The 10 ml of filtrate transferred into a clean Erylen-Meyer flask was treated with 10 ml of Baljet reagent. A blank titration was carried out using 10 ml of distilled water and 10 ml of Baljet reagent. This mixture was allowed to stand for one hour for complete colour development. The colour intensity was measured colorimetrically at 495 nm using the following equation:

$$TotalGly\,\ddot{u}\quad ide = \frac{A_{\ddot{u}}\ \times \ddot{u}\quad g}{77}$$

Quantitative estimation of carbohydrates by Phenol-sulphuric acid method: The total carbohydrate contents of alcoholic *A. Deliciosa*

Chev. Crude fruit extracts were determine using a modified method of Nielsen [24]. Aliquot of kiwifruit extracts (2.0 ml, EKE and MKE) were added into two sets of test tubes containing 5% (w/v) phenol (2.0 ml), the contents were mixed well, to which slowly added 5 ml of concentrated sulphuric acid and vortexed the contents. The reaction mixture was allowed to stand for 10 minutes followed by incubation of 10 min at 25°C. Vortexed the mixture again and measured the absorbance at 490 nm. The total carbohydrate content determined in extract using glucose as a reference compound was expressed as micrograms of glucose equivalents per milligram of fresh material.

Antioxidant assay

Free radical-scavenging activity: The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging capacity of *A. Deliciosa* Chev. crude fruit extracts were measured according to the method of Rajurkar NS, et al. [25].The stock solution (100 mM) of DPPH (4 mg/10 ml) was diluted to the ratio of 1:10. Aliquots (0.5 ml) of DPPH stock were added into different test tubes followed by increasing concentrations (10-100 mg/ml) of kiwifruit extract. Deionised water was added to make the final volumeto1.0 ml. incubated the content for 30 min. The absorbance was measured at 517 nm (A_{517}) against a reagent blank on a UV-Visible spectrophotometer. The radical scavenging activity was measured as a decrease in the absorbance of DPPH in the presence of test sample/compound. The DPPH radical scavenging capacity was estimated from the difference in absorbance for the sample and blank, expressed in terms of percentage of DPPH scavenging. The extent of discoloration of the violet colour of DPPH radical, as it gets reduced, indicated the radical scavenging potential of the antioxidant [26]. The reaction mixture was vortexed vigorously followed by incubation period of 1 h in a hot-water bath at 37°C, after which the A_{517} of the reaction mixture was recorded. The absorbance decreased due to colour change from purple to yellow as a result of radical scavenged by the antioxidant through the donation of hydrogen to form the stable DPPH molecules. Quercetin was used as a reference molecule for calibration of a reference profile.

Scavenging (%) of kiwifruit extract=$(C_{Abs} - S_{Abs})/C_{Abs} \times 100$

Where C=A_{517} of Control and S=A_{517} of test sample.

The IC_{50} (concentration of extract resulting in 50 % reduction of the DPPH radical) values were calculated using a linear regression analysis which indicated antioxidant capacity of kiwifruit extract(s).

Alpha-amylase inhibitory assay: *In vitro* alpha-amylase inhibition assay was performed according to the method of McCue PP, et al. [27]. A total of 200 µl of *A. Deliciosa* Chev. Crude fruit extracts (20-100 mg/ml) was taken in a test-tube followed by addition of 200 µl of alpha-amylase solution (0.5 mg/ml) prepared in 20 mM sodium phosphate buffer (pH 6.9). The contents were pre incubated at 25°C for 15 min, after which 200 µl of 1% (w/v) starch solution prepared in 2 mM sodium phosphate buffer (pH 6.9) was added. The mixture was further incubated at 25°C for 10 min. To the reaction mixture 500 µl of dinitro salicylic acid (DNS) reagent was added to stop the reaction and then incubated the test-tube in hot boiling water for 5 min followed by cooling at room temperature. The control was prepared in distilled water instead of fruit extract using the above procedure. The reaction mixtures were finally diluted using 5 ml of distilled water and A_{540} values were recorded. The alpha-amylase inhibitory activity was calculated as:

$$PercentageInhibition = \left(\frac{ControlA_{540} - ExtractA_{540}}{ControlA_{540}} \right) \times 100$$

The IC_{50} (concentration of extracts resulting in 50 % inhibition of enzyme activity) values were calculated using a linear regression analysis that indicated the inhibitory potential of kiwifruit extracts.

Calculation of IC_{50}: The IC_{50} for each sample were calculated according to the following procedure:

The inhibition ratio (y) and sample concentration (x) were plotted against each other. Respective regression line was drawn to obtain a regression equation (*y*=mx+c). The sample concentration (x) was calculated at y=50 in the regression equation. This calculated value was set as the IC_{50} (mg/ml) value of each analytical sample.

Mode of α-amylase inhibition: *A. Deliciosa* Chev. Crude fruit extracts (EKE and MKE) were used for kinetic study of α-amylase inhibition according to the modified method by Ali H, et al. [28]. A total of 250 µl of kiwifruit extract was pre-incubated with 250 µl of alpha-amylase solution (0.5 mg/ml) in 20 mM phosphate buffer (pH 6.9) for 10 min at 25°C in a set of tubes. In another set of tubes 250 µl α-amylase was pre-incubated. To the reaction mixtures 250 µl of starch solution was added to both sets of reaction mixtures at increasing concentrations (0.30, 0.90, 1.50, 2.50 and 5.0 mg/ml) to start the reaction. The reaction mixtures incubated for 10 min at 25°C were boiled for 5 min after the addition of 500 µl of DNS. The amount of reducing sugars released in each case was determined spectrophotometrically at A_{540} using a maltose standard curve and converted to reaction velocities as follows:

Activity (U=mmoles/min/mL)=S_{Abs}- B_{Abs}/Std_{Abs}-B_{Abs} × $Conc._{std}$/ Incubation time X $n/MW_{maltose}$;

where S=Sample, B=Buffer, Std=Standard, Conc.=Concentration, n=Dilution factor and MW=Molecular weight.

A double reciprocal plot ($1/V°$ *versus* $1/[S]$) where *V* is reaction velocity and [*S*] is substrate concentration was plotted. The type or mode of inhibition and kinetic parameters of the *A. Deliciosa* Chev. Fruit extracts on α-amylase activity was determined by analysis of the double reciprocal (Lineweaver-Burk) plot using Michaelis-Menten kinetics. Kinetic parameters were calculated by adjusting curves to the Lineweaver-Burk kinetic equation:

$1/V=(K_m/V_{max})\ (1/S) + 1/V_{max}$

Starch-agar gel diffusion test: Starch-agar plates (containing 0.5% of soluble starch with 2.5% of agar) were prepared [29]. Five holes were punched in the agar medium of the plate followed by addition of 30 µl of each of the reaction mixtures containing increasing concentrations of EKE and MKE (60, 80 and 100 mg/ml) treated α-amylase; a positive control containing alpha α-amylase in sodium phosphate buffer (0.5 mg/ml) and a negative control containing only buffer. Plates were incubated overnight for diffusion to happen at 20°C, followed by detection and measurement of starch hydrolysed zones using iodine solution.

Statistical analysis

Statistical analysis was performed using Microsoft excel. All the results were expressed as Mean ± SE for triplicate determinations. Values of $p \leq 0.05$ which were considered as significant.

Results and Discussion

Fruit material preparation and yield of the extract

To investigate the efficiency of solvents in extraction of specific components, the yields of each of the specified biochemical constituents of *A. Deliciosa* Chev. Fruit extracts was determined. The

A. Deliciosa Chev. Fruit extracts gave yield in EKE (3.47 g) and MKE (1.66 g) extract of 24.83 and 11.8%, respectively. Yang H, et al. [30] reported 7.9% and 8.6% yield (w/w) of ethanol extracts whereas 4.1 and 13.0 yield (w/w) of solvent fractions (Ethyl acetate and *n*-Butanol) from *Actinidia deliciosa* and *Actinidiachinensis* fruit peel, respectively. Prior investigations assessed the impact of different types of solvents on extraction of bioactive compounds from various plant parts, such as fruits, leaves and seeds [31]. Interestingly, the extraction procedure attempted in the present study appeared to be quite efficient than the earlier investigations. The variation in extracted residue(s) amount and yield was mostly dependent on use of good solvent with lesser toxicity, quick mass transfer, lower boiling point and preservative action. Furthermore, other essential factors which determined the efficiency of extraction procedure were the type of extract, temperature and the extraction time involved [32].

Qualitative analysis

Primary phytochemical investigation: In a preliminary approach, *Actinidia deliciosa* Chev. Crude fruit extracts were employed for preliminary phytochemical screening of various secondary metabolites by reported chemical assays (Table 1). The phytochemical analysis of both alcoholic extracts revealed excellent presence of steroids, cardiac glycosides, terpenoids, flavonoids and carbohydrates. Several studies have been conducted on kiwifruit, in order to determine their bioactive compounds [33-38]. EKE and MKE extracts gave positive results for the presence of most of the phytoconstituents.

Quantitative analysis

Total flavonoid content: Flavonoids are most important among plant secondary metabolites with miscellaneous bioactive, antioxidant effects and are considered indispensable in its pharmaceutical, medicinal, nutraceutical and cosmetics applications [39]. The amount of flavonoids in the *A. Deliciosa* Chev. Fruit extracts were measured by the development of flavonoid-aluminium-complex during the reaction between sodium nitrite and aluminium chloride. Total Flavonoid Contents (TFC) in EKE and MKE were calculated using quercetin analytical curve (10-100 µg/ml) based on the UV-Vis signal (Y= 0.007x+0.159; R^2= 0.982).The determination of the TFC of EKE and MKE extracts (100 mg/ml), expressed as quercetin equivalents (µg/mg), established flavonoid abundance in methanolic extract (0.37) than that of ethanolic extract (0.28). Fiorentino A, et al. [38] quantified TFC in *A. Deliciosa* cv. Hayward crude extracts. Their analysis reported massive amounts of flavonoids in polar extracts (catechin equivalent; 131.7 to 108.9 mg/100 g of fruit weight) whereas non-polar extract showed limited or no results. TFC (catechin equivalent; mg/100 g of fruit weight) of *A. kolomikta* (69.05), *A. arguta* (188.43) and *A. chinensis* (67.63) were established using an aluminium colorimetric assay which showed three *Actinidia* extracts in decreasing order *A. arguta*>*A. kolomikta*>*A. chinensis* [40]. Flavonoids identified during mass spectrometric studies of *A. Deliciosa* fruit extracts characterized 11 flavonoid molecules as naringenin, quercetin, tricin,3-O-α-L-rhamopyranosyl derivatives of flavones kaempferol and quercetin, quercetin-3-O-β-D-glucopyranoside, kaempferol-3-O-βrutinoside,rutin, epicatechin, catechin and gallocatechin [38].

Table 1: Qualitative phytochemical analysis of *Actinidia deliciosa*.

Phytochemicals	Chemical test	Methanolic extract	Ethanolic extract
Flavonoids	Sodium hydroxide test	+	+
Terpenoids	Salkowaski test	++	+
Steroids	Lieberman Burchard's test	++	++
Carbohydrates	Molisch's test	++	++
Cardiac glycosides	Keller-Killani test	+	+
Tannins	Ferric Chloride test	-	-
Alkaloids	Wagner's test	-	-

+ Indicated presence of phytochemicals
++ Indicated excellent presence of phytochemicals
- Indicated absence of phytochemicals

Total steroid content: Steroids are cholesterol derived, lipophilic, low-molecular weight compounds which are used in many food additives, cosmetics and as ingredient in formulation of various medicines or drugs [41]. The phytosterols in the *A. Deliciosa* Chev. Fruit extract reacts with the ferric chloride and potassium hexacyanoferrate in the presence of concentrated sulphuric acid to give a pink-red coloured complex whose intensity is directly proportional to the amount of steroids present. Total Steroid Content (TSC) in EKE and MKE (100 mg/ml) were calculated using cycloartenol analytical curve (4-28 µg/ml) based on the UV-Vis signal (Y=0.0029x+0.107; R^2=0.9989). The determination of the TSC of EKE and MKE extracts (100 mg/ml), expressed as cycloartenol equivalents (µg/mg), established higher steroid content (13.50) in ethanol extract compared to methanolic extract (12.58). However, limited studies have been done to estimate total steroid content of Actinidia fruits. Fiorentino A, et al. [38] characterized 7 types of phytosterols in *A. deliciosa* 'Hayward' organic fruit extracts identified as stigmasterol, β-sitosterol, campesterol, stigmast-7-en-3β-ol, ergosterol, its peroxide derivative and 5,7,14,22-ergostatetraen-3β-ol *via* GC-MS analysis. These phytosterols contributes in reduction of the hematic cholesterol level and as a chemo preventive agent to inhibit carcinogenesis processes.

Total cardiac glycoside content: Cardiac glycosides are one of several classes of drugs or medicines used in treating myocardial infarction, certain irregular heartbeats and various heart related conditions [42]. Kiwifruit is enriched with glycosides and its total cardiac glycoside content was estimated in EKE and MKE (100 mg/ml) [23]. The methanolic extract showed higher amount of cardiac glycoside content (4.7 µg/mg) as compared to ethanolic extract (3.7 µg/mg). However, prior investigations only featured preliminary detection of cardiac glycosides but not its quantity in Actinidia fruits.

Total carbohydrate content: Carbohydrates contribute in sweetness, appearance and texture of most of foods. Apart from this each food type has a carbohydrate 'fingerprint', nutrient content, identity and various other factors. Therefore, it is essential to determine carbohydrate content. Total carbohydrate contents (TCC) in EKE and MKE (100 mg/ml) were calculated using a glucose analytical curve (10-90 µg/ml) based on the UV-Vis signal (Y=0.0047x + 0.438; R^2=0.9638) and phenol-sulphuric acid colorimetric assay. The concentrated sulfuric acid breaks down the polysaccharides, disaccharides and oligosaccharides into monosaccharides. Pentoses are then dehydrated into furfural and hexoses into hydroxymethyl furfural. These compounds imparted yellow-gold colour when they react with phenol. The determination of the TCC of EKE and MKE extracts (100 mg/ml), expressed as glucose equivalents (µg/mg), established higher steroid content (0.24) in methanol extract compared to ethanolic extract (0.22). Deters AM, et al. [43] studied the molar composition of kiwifruit polysaccharide *via* GC/MS-analysis which identified which identified 9 different types of carbohydrates in *A. chinensis*: rhamnose, fructose, ribose, arabinose, xylose, mannose, galactose, glucose and uronic acid.

Antioxidant (DPPH free radical scavenging activity) assay

DPPH free radical scavenging activity is a sensitive method to analyse the antioxidant activity of a specific compound or plant extract [44]. Determination of *A. deliciosa* Chev. Fruit antioxidant property was carried out by measuring its ability to scavenge the DPPH radical. DPPH is a free radical that readily undergoes scavenging when in the vicinity of an antioxidant molecule which shows maximum absorbance at A_{517}. The scavenging capacities were reported as percentage reduction of radical (DPPH•). Both alcoholic extracts shared dose-response scavenging abilities and strong percentage reduction. In fact, best scavenging (%) activity was observed in MKE (72.16%). On the other hand, EKE showed 61.16% scavenging activity. The results of the DPPH radical assay strengthened the fact that all of the extracts scavenged massively the radical target species. The observed IC_{50} (concentration of extracts resulting in 50% reduction of the DPPH radical) value (Table 2) showed that methanol extract exhibited strongest antioxidant activity (3.14 ± 0.153 mg/ml) in comparison with ethanol extract (4.2 ± 0.073 mg/ml). The lower the IC_{50} value, the higher would be the antioxidant activity of the samples. ABTS and FRAP assays were used to evaluate the antioxidant capacity of 5 varieties of Actinidia fruits and this activity varied from 9.40 to 21.36 (ABTS assay) and from 0.87 to 9.22 (FRAP assay) mmoltrolox/100 g of dry weight [37]. Prior investigations highlighted that the ethanolic extracts of *A. deliciosa* fruit to be more potent in reducing free radicals than hexane and diethyl ether extract of *A. deliciosa* fruit [38].

α-Amylase inhibitory assay

Human pancreatic α-amylase (E.C. 3.2.1.1) is a key enzyme in digestive system responsible for the breakdown of starch into a mixture of oligosaccharides such as α (l-6), α (1-4) oligoglucans and maltotriose; disaccharides such as maltose and monosaccharides suitable for absorption [45]. The inhibition of α-amylase is specifically useful for the treatment of non-insulin-dependent diabetes (Type-2 diabetes) because it will slow down the absorption of glucose [46,47]. In the present work, the effects of *Actinidiadeliciosa* Chev., kiwifruit alcoholic extracts on the catalytic activity of α-amylase were evaluated. Extrapolation of extract effectiveness demonstrated inhibition of α-amylase was dose-dependent such that MKE had an overall stronger inhibitory effect (85.16%) than EKE (79.76%) at a final concentration of 100 mg/ml. The IC_{50} (Table 3) of MKE (1.53 ± 0.158 mg/ml) was significantly lower ($p \leq 0.05$) than that of EKE (2.05 ± 0.639 mg/ml). Hence, lower IC_{50} indicates more potent inhibition of enzyme. Kinetic parameters were determined from Lineweaver-Burk plots in presence of two different concentrations of EKE and MKE which showed competitive inhibition of enzyme activity (Figure 1) suggesting K_m of α-amylase in absence on extract was 0.722 mg/mL of starch which was lower than 1.010 mg/ml and 0.998 mg/ml of starch for MKE and EKE respectively. Wojdyło A, et al. [37] evaluated IC_{50} (mg of dried fruit/ml) value of α-amylase for five different cultivars of *A. arguta* ranged from 4.13 to 6.62. Previous studies suggested the strong inhibitory effects on α-amylase and free radical scavenging property of kiwifruit extracts and hence their antihyperglycemic and antioxidant capacities [37,47,48]. However, findings in a study showed that the flavonoids such ascatechin, quercetin-3-O-glucoside, kaempferol-3-O-rutinoside and kaempferol-3-O-glucoside were found to possess high inhibition activities on α-amylase [49].The present, study showed that the active ingredients in the *A. deliciosa* Chev. Fruit extract competes with the substrate binding in the active site of enzyme. The kiwifruit extract showed potent inhibition of α-amylase activity. This result was in accordance with previous studies which showed that extensive inhibition of pancreatic α-amylase lead to irregularities in colon due to fermentation of undigested carbohydrates by microbes. Hence, mild α-amylase inhibition is desirable [50]. Inhibitory effects of different kiwifruits on α-amylase have seldom been investigated. However, kiwifruits exhibited stronger antihyperglycemic activity than pomelo, mandarine, orange, banana, pinapple, pulm, red grapefruit and apple [47].

Starch-agar test

The starch agar gel diffusion test showed positive results for inhibitory effects of kiwifruit extracts (Figure 2).The antidiabetic effect of kiwifruit has also been demonstrated [51]. Recent study suggested eating kiwifruit during breakfast significantly slowed glucose release into blood stream [52]. Moreover, the phytochemical analysis showed low sugar content in kiwifruit which was in agreement with the previous reports indicating low glycaemic index was beneficial for regulating blood sugar [53,54].

Conclusion

Kiwifruit is a nutrient-dense food rich in nutrients and low in calories. It is consumed all over the world as salads, juices or as a snack.

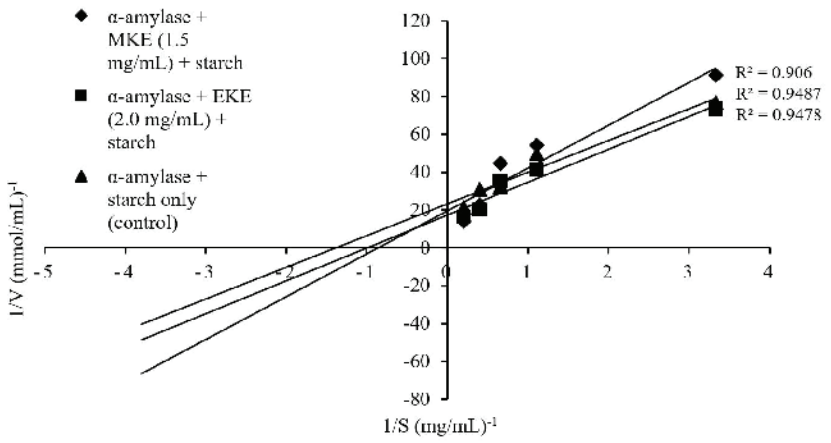

Figure 1: Lineweaver-Burk plot of mode of inhibition of *α-amylase* by EKE and MKE.

Figure 2: The starch agar plate after treatment of alpha-amylase (0.5 mg/mL) with MKE and EKE (a) Positive control (b) Negative control (c) 60.0 mg/ml (d) 80.0 mg/ml and (e) 100.0 mg/ml.

Table 2: IC_{50} values of *A. deliciosa* Chev. fruit extracts determined using DPPH assay.

Extracts	IC_{50} (mg/mL)
Methanol	3.14 ± 0.153
Ethanol	4.2 ± 0.073

The values are expressed as mean ± SEM of triplicate tests (P ≤ 0.05).

Table 3: IC_{50} values for alpha-amylase inhibitory potential of *A. deliciosa* Chev. extracts.

Extracts	IC_{50} (mg/mL) Amylase
Methanol	1.53 ± 0.091
Ethanol	2.05 ± 0.368

The values are expressed as mean ± SEM of triplicate tests (P ≤ 0.05).

In present study, phyto-chemical analysis provided an idea about the constituents of *Actinidia deliciosa* Chev., (Kiwifruit). Later, medicinal nature of the fruit was checked by establishing its antioxidant and anti-hyperglycemic effects. The contribution of kiwifruit to health improvement can be related to its antioxidant capacity. Vitamin C, choline, zeaxanthin and lutein are the major antioxidants which help in removing free radicals from body produced during different metabolic processes. Anti-hyperglycemic compounds are used against diabetes. The study showed that kiwi extract has an excellent anti-diabetic potential. Future studies should be based on determining the total antioxidant capacity, content of antioxidants in *Actinidia* genotypes and antidiabetic potential of fruits of *Actinidia* since (i) the concentration of antioxidants varies significantly between within their species and subspecies, (ii) to access recommended consumption and marketing of different kiwifruit which could serve as a rich dietary supplement and also help in treatment of diabetes.

Acknowledgments

This work has been funded by Council for Scientific and Industrial Research, New Delhi, under a CSIR-NET Junior Research Fellowship [File No.09/237(0161)/2017-EMR-1] awarded to one of the authors (VC). The authors are thankful to CSIR, New Delhi as well as DBT, New Delhi for continuous financial support to department of biotechnology, Himachal Pradesh University, Shimla (India).

Conflict of interest

Authors declare that they have no conflict of interest amongst them or with the parent institute.

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