Original Article

Phytochemical Screening, Antioxidant, and Inhibition Activity of *Picrorhiza kurroa* Against α-Amylase and α-Glucosidase

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Abstract

Picrorhiza kurroa (P.K) usually familiar as kutki is a well-known plant in the Ayurvedic system of medicine due to its reported activities including antidiabetic, antibacterial, antioxidant, antitumor, anti-inflammatory, and hepatoprotective. The current research was intended to evaluate the antioxidant, inhibition activity of the ethanolic, methanolic, and aqueous extracts of P.K roots against α-amylase and α-glucosidase in vitro, after the phytochemical analysis. For this purpose, P.K roots were extracted with ethanol (EthPk), methanol (MthPk), and distilled water (AqPk) and phytochemical study of the extracts were performed to recognize the total phenolic content (TPC) and total flavonoids content (TFC). Antioxidant capability of the extracts was assessed by FRAP, ABTS, and DPPH assay. α-amylase inhibitory and α-glucosidase inhibitory activities were also determined. Software SPSS-23 was used to statistically analyze with One Way ANOVA and results were stated as mean standard deviation. Result of the study showed that MthPk contained the maximum concentration of TPC and TFC than EthPk and AqEh. Antioxidants in terms of DPPH (lowest IC_{50} = .894 ± .57), FRAP (612.54 ± 11.73) and ABTS (406.42 ± 4.02) assay was also maximum in MthPk. MthPk was also showed maximum inhibition activity against α-amylase and α-glucosidase with lowest IC_{50} (.39 ± .41; .61 ± .24), respectively. The extracts α-amylase and α-glucosidase inhibitory activities order was as MthPk > EthPk> AqPk. Results clearly specified that the methanolic extract of *Picrorhiza kurroa* have the maximum antioxidant, α-amylase, and α-glucosidase inhibitory activities. A positive correlation of TPC, TFC with antioxidant, and α-amylase and α-glucosidase inhibition activities of the P.K roots were also shown. The plant has capability to diminish the oxidative stress and can be used to treat diabetes by inhibiting α-amylase and α-glucosidase actions.

Keywords

Picrorhiza kurroa, total phenolic content, total flavonoids content, antioxidants, alpha-amylase inhibitory activity, alpha-glucosidase inhibitory activity

Introduction

Oxidative stress can be brought about by the abundance of reactive oxygen species (ROS) and reactive nitrogen species (RNS).[1] ROS and RNS are the expressions altogether used to depict free radicals and other non-radical reactive derived known as oxidants. ROS incorporate oxygen-containing dioxygen ($O_2•^-$), hydrogen peroxide (H_2O_2), and hydroxide (•OH). In RNS, nitrogen-containing oxidants such as nitrogen Dioxide (NO_2), nitric oxide (NO•), and peroxynitrite ($ONOO^-$) are included.[2,3] Free radical's high concentrations then bring about malicious cycles that can harm cell structures because of oxidative stress.[4] ROS and RNS accumulation prompts oxidative damage to essentially all particles. Such groups are not really a danger to the human body makeup in typical physiological circumstances.[5,6] However, when the body neglects to eliminate them somewhat, oxidative stress invigorates the atherosclerotic

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plaques formation. This plaques formation may build the danger of atherosclerosis, malignancy, and Type 2 diabetes mellitus.[7-9]

α-Amylase are produced as hydrolytic enzymes in humans, animals, fungi, bacteria, and plants. In the salivary glands, α--amylase are originated in human that emit the enzyme into the pancreas that release it inside the small digestive tract.[10] α-amylases function is to prompt the starch hydrolysis. α-amylase separate the α-(1,4)-glycosidic linkage in starch particle prompting the creation of glucose, maltodextrins, maltotetraose, maltose, and maltotriose.[11] While α-glucosidase is present in the enterocytes luminal surface and is discharged inside the small digestive tract,[12] α-Glucosidase is an important protein that prompting the disaccharides (sucrose and maltose) hydrolytic cleavage into monosaccharides (fructose and glucose). Hence, α-amylases and α-glucosidase inhibitory activities can impede the rise of glucose and stifle postprandial hyperglycemiae.[13,14]

Various diseases such as diabetes, cancer, neurodegenerative, and cardiovascular diseases are associated oxidative stress.[15] Diabetes-associated cardiovascular diseases also arise by a variety of mechanisms including oxidative stress. Therefore, it is important to maintain the oxidative stress and sugar levels in the body. Nature has consistently existed abundant source of important compounds which are associated to valuable possessions for individual health.[16] There is an abundance of proof which shows that natural plants and other food stuffs are major source of antioxidants; have recognized α-amylase and α-glucosidase inhibitory activities.[17-23]

Picrorhiza kurroa (Family Scrophulariaceae), also recognized as kutki, is one of the therapeutic plant occurred in the alpine Himalayan area. kutkoside and iridoid glycosides (Picroside I and II) are dynamic components of the plant.[24] Over than 2000 herbal products, Picroside-I and II are utilized.[25] The plant's economic components are its dried roots and rhizomes, and are utilized to cure different afflictions, for example, spleen disorder, liver diseases, and allergy problems.[24] *P. kurroa* showed β-cell recovery with upgraded insulin creation and antihyperglycemic impacts.[26] The current study is conducted to expose the antioxidant and inhibition activity of *P. kurroa* against α-amylases and α-glucosidase.

Material and Methods

Plant Procurement

P. kurroa was procured from local market Lahore, Pakistan. Then, recognized by the professional botanist from the Botany Department, Government College University Faisalabad, Pakistan a voucher specimen numbered 136-A-2021.

Extract Preparation

Extracts were prepared by the solvent extraction method as illustrated by Mustafa et. al.[17] The plant following rinsing with distilled water (D.W) was dried in the shade and crushed into well powder form. Then, powder (50 g) was soaked for 72 hours in distilled water, methanol, and ethanol (each 250 mL) with occasionally stirring and mixing. Mixture was filtered by utilizing filter paper (Whatman No. 1). In rotary evaporator (SCI100-Pro; SCILOGEX, USA) at 40°C, filtrates were concerted and transferred in petri dish. The petri dish was placed in incubator at 40°C dried out appropriately. Extract was stored at 4°C up to more investigation.

Table 1. Qualitative Analysis of *Picrorhiza kurroa*.

Compounds	Test	MthPk	EthPk	AqPk
Carbohydrates	Benedict's test	–	–	–
	Fehling's test	–	–	–
Reducing sugar	Fehling's test	–	–	–
Alkaloids	Hager's test	–	–	–
Proteins	Xanthopeoteic test	–	–	–
Flavonoids	Alkaline reagent test	++	+++	++
Phenols	Lead acetate test	+++	+++	++
Tannins	Lead acetate test	–	–	–
Steroids	Salkowski's test	++	++	++
Terpenoids	Salkowski's test	++	++	+

(+): present; (−): not detected
MthPk, Methanol extract of *Picrorhiza kurroa*; EthPk, Ethanol extract of *Picrorhiza kurroa*; AqPk, Aqueous extract of *Picrorhiza kurroa*.

Qualitative Phytochemical Analysis

Phytochemical analyses of extracts were performed qualitatively by using standard techniques to detect the main phytochemical ingredients as mentioned by Singh and Bag.[27]

Quantitative Phytochemical Estimation

Total Phenolic contents (TPC); mg GAE/g). The 10 µl of plant extract (1 mg/mL) was dissolved in 100 µl of Folin–Ciocalteau reagent and 200 µl of 2.5% Na_2CO_3. Using the gallic acid (GA) standard curve, TPC in the extracts was evaluated as explained by Bajalan et al.[28] Absorbance (A) was noted at 760 nm by using biochemistry analyzer (Biolab-310) after 60 minutes incubation at room temperature. TPC value was expressed as mg gallic acid equivalent (GAE)/g.

Total Flavonoid contents (TFC); mg QE/g). TFC were determined by utilizing Quercetin (Q) as a standard according to the process formerly adopted by.[28] Briefly, 100 µl of plant extract (1 mg/mL) was added to 1 mL of D.W. After 5 minutes incubation at room temperature, 125 µl of aluminum chloride ($AlCl_3$) and 75 µl of 5% sodium nitrite ($NaNo_2$) was added and incubated again for 6 minutes at room temperature. At the end, 1M sodium hydroxide (NaOH; 125 µl) was added and the final volume was prepared upto 2.5 mL with D.W. Absorbance was calculated at 540 nm utilizing chemistry analyzer (Biolab-310).

In Vitro Antioxidant Evaluation

Scavenging Activity Assay. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was dissolved in methanol (.025 g/L). The plant

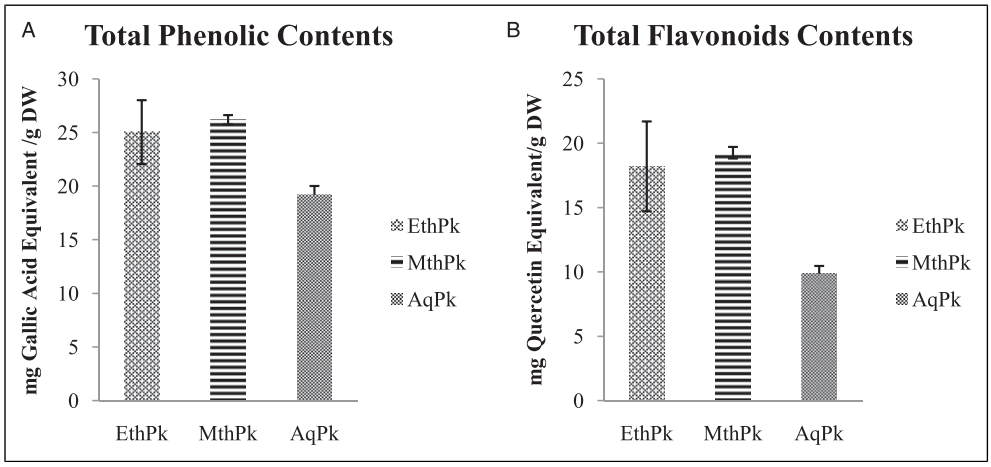

Figure 1. A) Total Phenolic contents of *Picrorhiza kurroa* different root extracts. B) Total Flavonoid contents of *Picrorhiza kurroa* different root extracts. Results are demonstrated as Mean±Standard deviation of 3-replicates of every extract, that is, MthPk (Methanolic extract of *Picrorhiza kurroa*), EthPk (Ethanolic extract of *Picrorhiza kurroa*), and AqPk (aqueous extract of *Picrorhiza kurroa*). DW; Dry Weight of *Picrorhiza kurroa* extract.

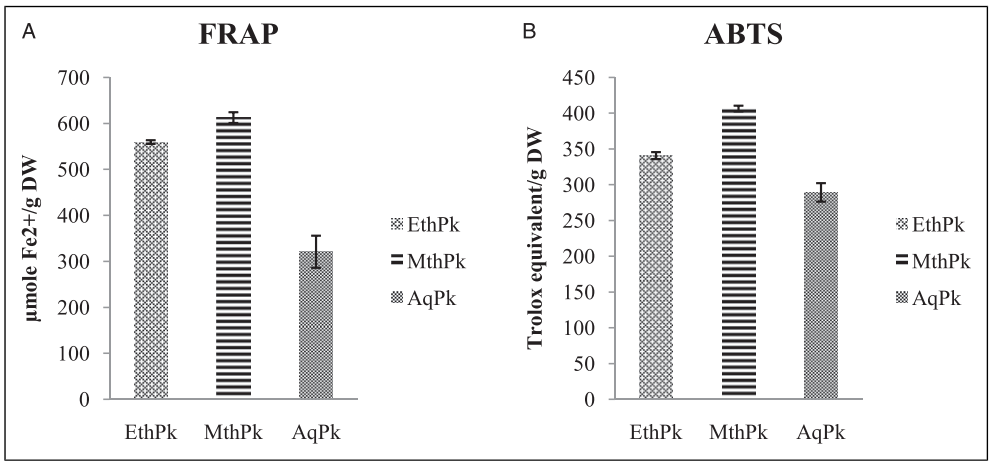

Figure 2. A) Ferric Reducing Antioxidant Potential (FRAP) of different extracts of *Picrorhiza kurroa*. B) Trolox Equivalent Antioxidant Capacity (TEAC; ABTS Assay) of different root extracts of *Picrorhiza kurroa*. Results are demonstrated as Mean±Standard deviation of 3-replicates of every extract, that is, MthPk (Methanolic extract of *Picrorhiza kurroa*), EthPk (Ethanolic extract of *Picrorhiza kurroa*), and AqPk (*Picrorhiza kurroa* aqueous extract).DW; Dry Weight of *Picrorhiza kurroa* extract.

extracts diluted with dimethyl sulfoxide (DMSO) at 1 mg/mL concentration. Sample solution (5 µl) was mixed with 585 µl DPPH working solution. Absorbance was evaluated at 515 nm after 20 mint incubation at room temperature by utilizing chemistry analyzer (Biolab-310). Percentage DPPH scavenging activity was measured by the following equation:

$$\text{Percent DPPH scavenging activity} = A0 - A1/A0 \times 100 \quad (1)$$

A_0 is the control absorbance (sample was replaced with distilled water (D.W)) and A_1 is the sample absorbance.[29]

The ferric reducing antioxidant potential (FRAP) was assessed by the means as demonstrated by Sethi et al.[30] A volume of 3.995 mL of the working solution [300 mM acetate buffer (10 volumes), 1 volume of 2, 4, 6-tri {2-pyridyl}-s-triazine (TPTZ; 10 mM) in HCl (40 mM), and 1 volume of ferric chloride ($FeCl_3$; 20 mM)] was assorted with the sample (5 µl). Absorbance was taken at 593 nm to monitor the reduction.

2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS; Trolox Equivalent Antioxidant Capacity) assay was performed as demonstrated by[31] with negligible modification. ABTS mixture was organized by addition of 1:1 ratio of 7 mM solution of ABTS in distilled water and solution of $K_2S_2O_8$ (2.5 mM). The prepared mixture was more diluted with methanol to attain absorbance of .7 at 734 nm. Then 5 µl of each plant extract solution was mixed with 3.995 mL of ABTS solution. Absorbance was taken at 734 nm subsequent to 30 min incubation at room temperature.

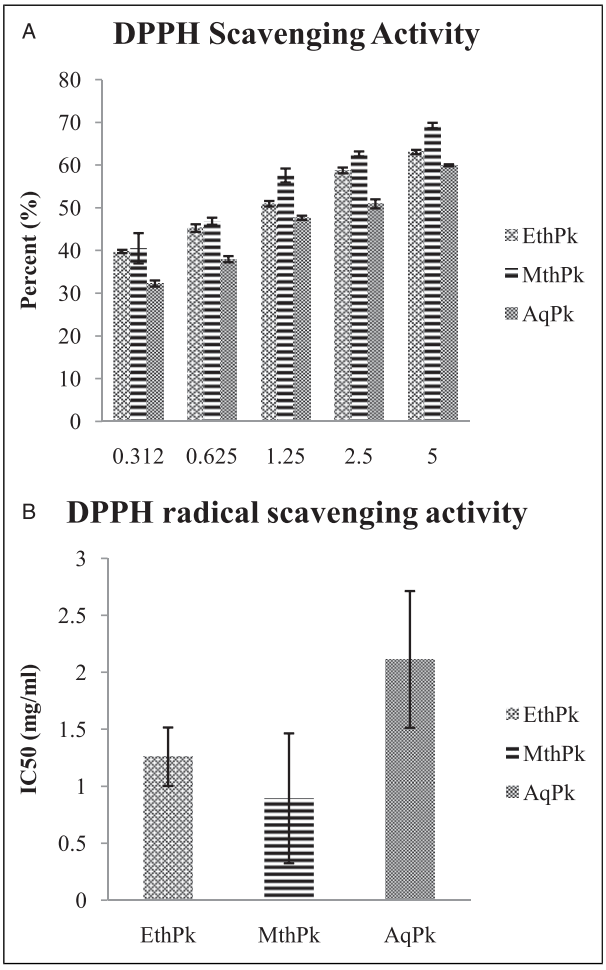

Figure 3. A) DPPH scavenging activity of 5 different absorptions of different root extracts of *Picrorhiza kurroa*. B) DPPH IC_{50} value of different root extracts of *Picrorhiza kurroa*. Results are demonstrated as Mean±Standard deviation of 3-replicates of every extract, that is, MthPk (Methanolic extract of *Picrorhiza kurroa*), EthPk (Ethanolic extract of *Picrorhiza kurroa*) and AqPk (*Picrorhiza kurroa* aqueous extract).DW; Dry Weight of *Picrorhiza kurroa* extract.

Results were indicated as mg of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) equivalent per gram of dry weight of the plant.

α-Amylase Inhibitory Activity. Five hundred (500) µl of plant extract (25, 50, 75, and 100 mg/mL dH_2O) and 500 µl α -amylase solution (0.5 mg/mL in phosphate buffer; pH 7.4) was set aside at room temperature for 10 mints. Then 1% starch solution (500 µl) was added in .02 M sodium phosphate buffer (pH 7.4). Reaction was ended by adding 1 mL of 3,5 dinitrosallicylic acid (DNSA) color reagent after 10 minutes incubation at room temperature. The mixture was kept in a boiling water bath for 10 minutes and then diluted with 10 mL D.W when cooled to room temperature. Absorbance was taken at 540 nm by biochemistry analyzer (Biolab-310).

The % of inhibition for α-amylase was deliberated as follows

$$\text{Percent inhibition activity of } \alpha - \text{amylase} = A0 - A1/A0 \times 100 \quad (2)$$

A_0 is the control absorbance (extract sample was replaced with D.W) and A_1 is the sample absorbance.[17]

α - Glucosidase Inhibitory Activity. Five hundred (500) µl of plant extract (25, 50, 75 and 100 mg/mL dH_2O), 1% starch solution (500 µl) in 0.2 M Tris buffer (pH 8), and 500 µl α-glucosidase solution (1U/ml in tris buffer; pH 8) was kept at 37°C for 10 minutes. The mixture was positioned in boiling water bath for 2 minutes to terminate the reaction. The quantity of glucose liberated is measured. A blank sample not including test sample stands for 100% enzyme activity. Acarbose (α -glucosidase inhibitor) was operated as a positive control. Absorbance was measured at 540 nm by biochemistry analyzer (Biolab-310), and percent inhibition activity for α-glucosidase was deliberated as follows

$$\text{Percent inhibition activity of } \alpha - \text{glucosidase} = A0 - A1/A0 \times 100 \quad (3)$$

where A_0 is the control absorbance (extract sample was replaced with DW) and A_1 is the absorbance of the sample.[17]

Statistical Analysis

All the quantification was measured in triplicates. Obtained data was evaluated by one-way analysis of variance (ANOVA), following Tukey's post hoc test for comparing mean values by using SPSS-23. All the results were demonstrated as mean-±standard deviation.

Results

Qualitative Phytochemical Examination

Results of the qualitative examination of all extracts type of the *P. kurroa* are shown in the Table 1, which shows presence or absence of various phytochemicals like, carbohydrates, alkaloids, phenols, flavonoids, saponins, steroids, terpenoids, tannins, and reducing sugar in MthPk, EthPk, and AqPk.

Total Phenolic and Flavonoid Contents

Both the TPC and TFC were significantly (P = .05) maximum in MthPk (26.18 ±.44 mg GAE/g and 19.26 ±.45 mg QE/g, respectively) than AqPk (19.18 ±.83 mg GAE/g and 9.86 ±.6 mg QE/g, respectively) and EthPk (25.03 ±2.98 mg GAE/g and 18.2 ±3.49 mg QE/g, respectively) as shown in Figure.1.

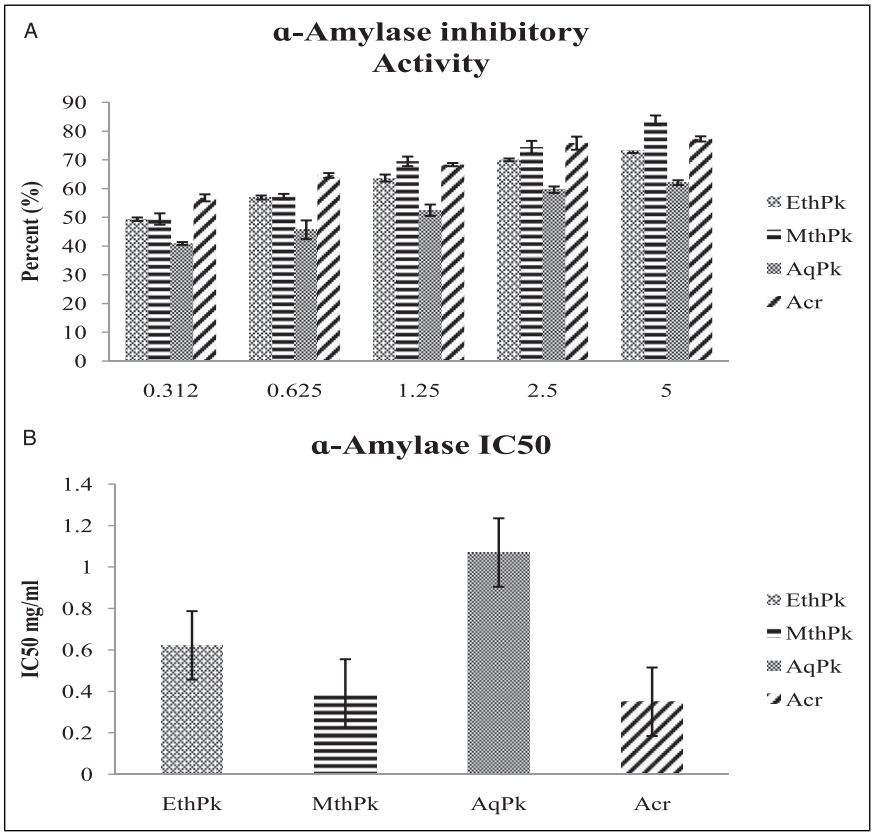

Figure 4. A) α-Amylase inhibitory activity of 5 different absorptions of different root extracts of *Picrorhiza kurroa*. B) IC50 value of α-Amylase inhibitory activity of different root extracts of *Picrorhiza kurroa*. Results are demonstrated as Mean±Standard deviation of 3-replicates of every extract, that is, MthPk (Methanolic extract of *Picrorhiza kurroa*), EthPk (Ethanolic extract of *Picrorhiza kurroa*), and AqPk (*Picrorhiza kurroa* aqueous extract).DW; Dry Weight of *Picrorhiza kurroa* extract.

In Vitro *Antioxidant Evaluation*

Results of DPPH• (Percent Inhibition), FRAP (FeSo4 (µmole Fe^{2+}/g DW), and ABTS (µM Trolox Equivalent/g DW) assay are expressed in the Figures 2 and 3 that illustrated a concentration reliant raise in DPPH scavenging activity in the MthPk (Figure.3) with lowest half maximal inhibitory concentration (IC_{50}) value (.8942 mg/mL) as compared to IC_{50} value of AqPk (2.11 mg/mL) and EthPk (1.25 mg/mL). The result showed MthPk has the greatest reducing potential of Fe^{3+} into Fe^{2+} (612.54 ±11.73µmole Fe^{2+}/g) and similar tendency was observed in scavenging ABTS radical being greatest in MthPk (406.42 ± 4.02 µM TE/g).

α -Amylase Inhibitory Activity

All extracts of the *P. kurroa* have noticeable α-amylase inhibitory activity in a concentration dependent way (Figure. 4: A). MthPk demonstrated the maximum α-amylase inhibitory activity with regard to contain lowest IC50 value as .39 ± .41 mg/mL than EthPk (.622 ± .23) and AqPk (1.07±.09).

α -Glucosidase Inhibitory Activity

The results of the α-glucosidase inhibition activity also showed a concentration dependent increase in percent activity of the methanolic, ethanolic, aqueous extracts, and acarbose (Figure. 5: A). MthPk demonstrated the highest α-glucosidase inhibitory activity with regard to contain lowest IC_{50} value as .61± .24 mg/mL.

Phytochemicals and Antioxidant Activity Correlation

A dominant correlation was observed between TPC and antioxidant assays including FRAP (R^2=.999) and DPPH IC_{50} value (R^2=0. .98). TFC also showed strong correlation with FRAP (R^2= .995) and IC_{50} value of DPPH (R^2 = .963) (Figure 6 A, B, C, D).

Phytochemicals, Antioxidants Correlation with α-Amylase and α -Glucosidase Inhibitory Activities:

The correlation of TPC, TFC with α-amylase inhibition activity IC_{50} value (R^2 = .964, R^2= .943, respectively) showed that increase in TPC and TFC has increased the α-amylase inhibition

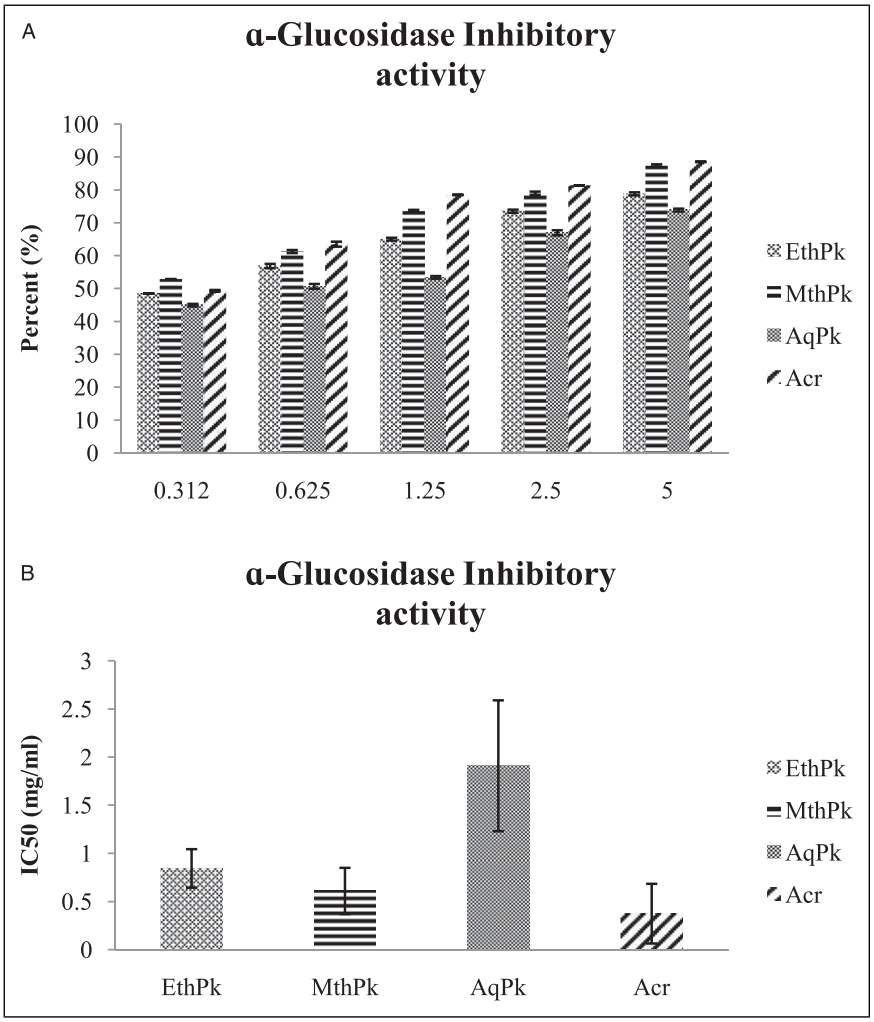

Figure 5. A) α-Glucosidase inhibitory activity of 5 different absorptions of different root extracts of *Picrorhiza kurroa*. B) IC50 value of α-Glucosidase inhibitory activity of different root extracts of *Picrorhiza kurroa*. Results are demonstrated as Mean±Standard deviation of 3-replicates of every extract, that is, MthPk (Methanolic extract of *Picrorhiza kurroa*), EthPk (Ethanolic extract of *Picrorhiza kurroa*), and AqPk (*Picrorhiza kurroa* aqueous extract). DW; Dry Weight of *Picrorhiza kurroa* extract.

activity (Figure 7: A: B). The correlation of α-amylase inhibitory activity IC_{50} value with the all antioxidant parameters also showed a highly positive correlation R^2 values as demonstrated in the Figure 7C, D, E. A similar strong correlation was seen in IC_{50} value of α-glucosidase inhibition activity with TPC, TFC, and all antioxidant parameters as seen in the Figure.8

Discussion

Plants as medicines are in common use by the people specifically in less developed countries. But by time, the utilization of plants as medicine has also significantly improved in developed countries, moreover, owing to the adverse consequences and the adequacy issues of synthetic drugs.[32,33] Plant extracts have numerous valuable consequences for health because of the incredible variety of free radical scavenging constituents, like phenols, flavonoids, vitamins, anthocyanins, and carotenoids.[34] Distinctive phenols contents with the antioxidant activity can assume a significant part in free radicals adsorption and neutralization.[35] These compounds contain effective biological activities[36,37] as antibacterial, anticancer, antioxidant, anticholinergic, and antidiabetic.[38-40] Flavonoids are secondary derivative that incorporates around 4500 recognized components.[41] The beneficial effects of flavonoids on wellbeing have been long-established for its antidiabetic,[42] anticancer,[43] antioxidant,[44] and anti-inflammatory activities.[45] A preceding study of Nepote et al[46] recommended that methanol solvent is best for the different phenolic contents extraction. This present study investigates TPC and TFC contents in methanolic, ethanolic, and aqueous extracts of the *P. kurroa*. Results of the study revealed that MthPk possessed the maximum phenolic contents 26.18 ± .44 GAE/g DW and TFC 19.26±.45 mg QE/g as compared to the EthPk (TPC

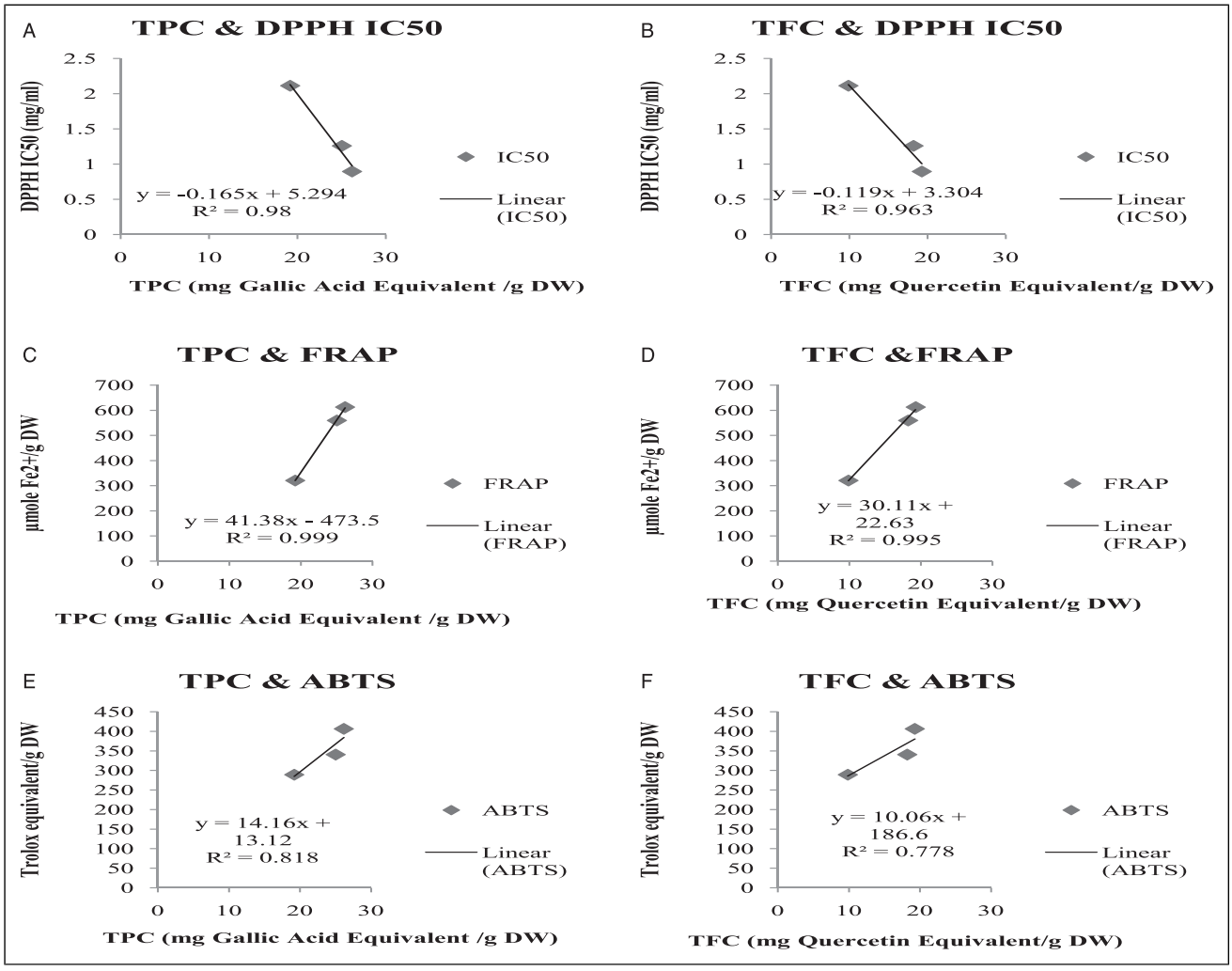

Figure 6. Total phenolic and Total Flavonoid contents of *Picrorhiza kurroa* correlation with different antioxidant parameters. **A)** Total phenolic contents correlation with DPPH IC_{50} value of different extracts of *Picrorhiza kurroa*. **B)** Total Flavonoid contents correlation with DPPH IC_{50} value of different extracts of *Picrorhiza kurroa*. **C)** Total phenolic contents correlation with FRAP of different extracts of *Picrorhiza kurroa*. **D)** Total Flavonoid contents correlation with FRAP of different extracts of *Picrorhiza kurroa*. **E)** Total phenolic contents correlation with ABTS of different extracts of *Picrorhiza kurroa*. **F)** Total Flavonoid contents correlation with ABTS of different root extracts of *Picrorhiza kurroa*. Results are demonstrated as Mean±Standard deviation of 3-replicates of every extract i.e. MthPk (Methanolic extract of *Picrorhiza kurroa*), EthPk (Ethanolic extract of *Picrorhiza kurroa*) and AqPk (Aqueous extract of *Picrorhiza kurroa*).

25.03±2.98 GAE/g DW and TFC 18.2±3.49 mg QE/g) AqPk (TPC 19.18±.83 GAE/g DW and TFC 9.86± .6 mg QE/g). Kumar et al.[47] accounted the maximum presence of flavonoids in the leaves of *Picrorhiza kurroa* while iridoids were present more in rhizomes. Rajkumar et al.[48] stated the presence of total phenol contents in methanolic extract of the *P. kurroa*. Krupashree et al.[49] also evaluated TPC and TFC in ethanolic extract of the roots of *P. kurroa*. Recently, Neupane and Lamichhane,[50] also showed the presence of TPC and TFC in methanolic extract of *Picrorhiza kurroa*.

Oxidative stress is comparative overabundance of ROS when estimated with antioxidants, has been associated to cardiovascular disease, neurodegenerative disease, diabetes mellitus, and numerous different disorders.[51,52] These relations highlight that a balance should be present between the comparative overabundance of ROS and antioxidants. Antioxidants deflect or eliminate oxidative stress associated diseases by neutralizing the ROS deteriorating consequence. If the antioxidants that are produced endogenously do not prevent the reactive species production, it will be required to achieve equilibrium in redox status. Natural antioxidants, such as plants, have a significant impact in this specific situation.[53] In the current study, antioxidant activity of the methanolic extract of the *P. kurroa* were revealed by using DPPH, FRAP, and ABTS methods. Data that attained demonstrated the significant consequence of the plant extract as an antioxidant. FRAP results revealed that the MthPk have the maximum antioxidant ability 612.54 ± 11.73 µmole Fe^{2+}/g as compared to EthPk 559.38 ±4.02 µmole Fe2+/g and AqPk

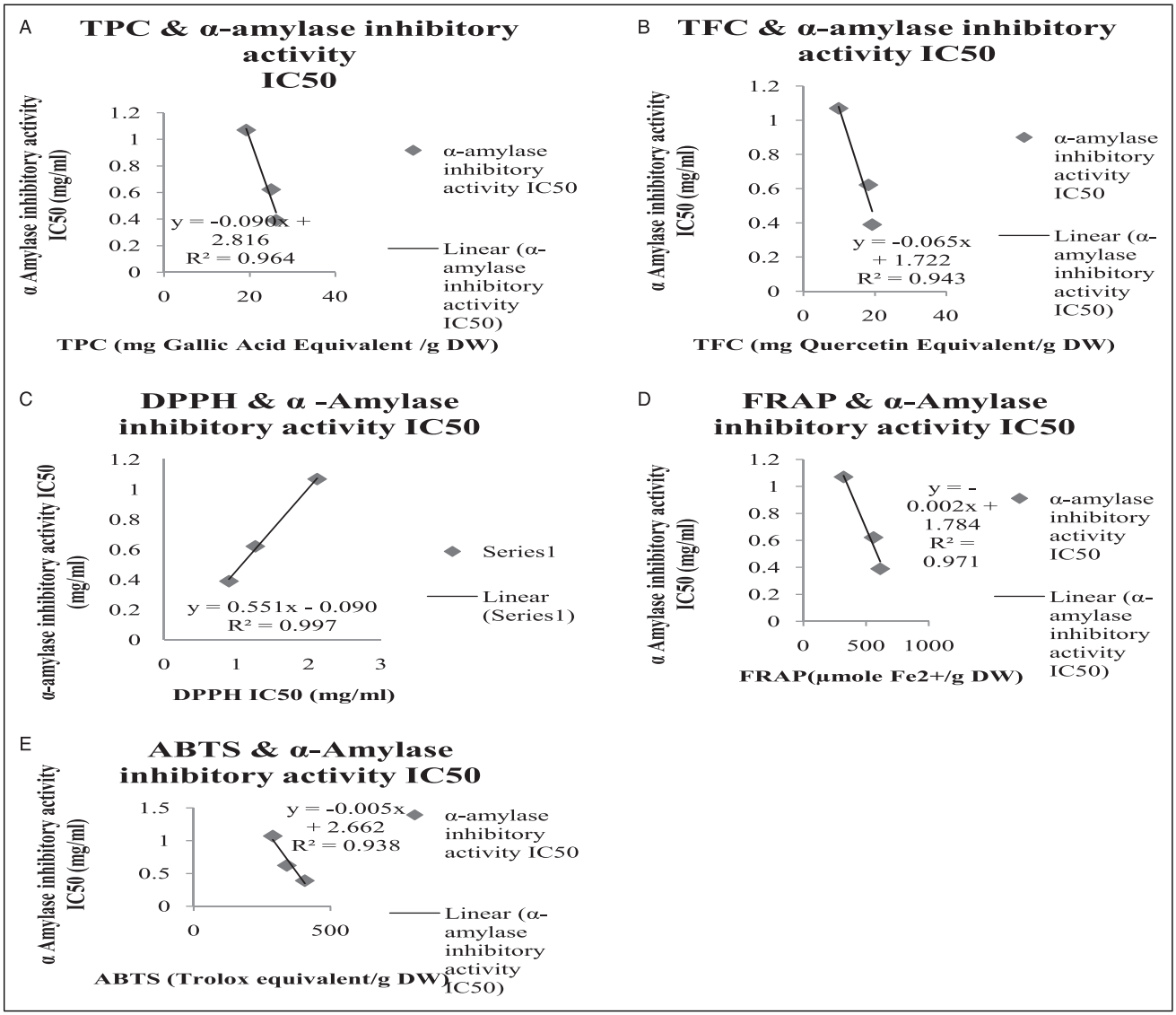

Figure 7. α-Amylase inhibitory activity IC50 value of *Picrorhiza kurroa* correlation with Phytochemical and different antioxidant parameters. A) α-Amylase inhibitory activity IC_{50} value correlation with total phenolic contents of different extracts of *Picrorhiza kurroa* **B)** α- Amylase inhibitory activity IC_{50} value correlation with total Flavonoid contents of different extracts of *Picrorhiza kurroa*. **C)** α-Amylase inhibitory activity IC_{50} value correlation with DPPH IC50 value of different extracts of *Picrorhiza kurroa*. **D)** α-Amylase inhibitory activity IC_{50} value correlation with FRAP of different extracts of *Picrorhiza kurroa*. **E)** α-Amylase inhibitory activity IC_{50} value correlation with ABTS of different root extracts of *Picrorhiza kurroa*. Results are demonstrated as Mean±Standard deviation of 3-replicates of every extract, that is, MthPk (Methanolic extract of *Picrorhiza kurroa*), EthPk (Ethanolic extract of *Picrorhiza kurroa*), and AqPk (Aqueous extract of *Picrorhiza kurroa*).

320.79±34.93 µmole Fe^{2+}/g. Likewise, ABTS consequences also showed that the of MthPk possessed the maximum antioxidant ability 406.42 ±4.02 µMol Trolox as compared to EthPk 340.67±4.87 µMol Trolox and AqPk 289.19± 12.95 µMol Trolox. Antioxidant property of MthPk in terms of their capability to scavenge free radicals was also determined by most frequently used in vitro assay, the DPPH scavenging property, the results of which demonstrated that the MthPk has highest antioxidant ability with lowest IC_{50} (.894 ± .57 mg/mL) as compared to EthPk (1.258 ± .26 mg/mL) and AqPk (2.11 ± 0.6 mg/mL). Similarly, Kant et al.[54] revealed the antioxidant effect *P. kurroa* leaves in term of DPPH, and ABTS methods. Thakur et al.[55] reported antioxidant effect of the peptide of the *P. kurroa*. Methanolic and aqueous extracts of *Picrorhiza kurroa* rhizome revealed promising antioxidant potentials in term of DPPH, FRAP, and thiobarbituric acid (TBA) assays.[48]

Krupashree et al.[49] also revealed *P. kurroa* antioxidant property in term of DPPH radical scavenging (IC_{50} =75.16 ± 3.2 µg/mL) and metal chelating activities (IC_{50} =55.5 ± 4.8 µg/mL).

A number of studies verify the close connection of TPC and TFC with antioxidant property.[17] The present study results also expose a close association of TPC and TFC with the antioxidant

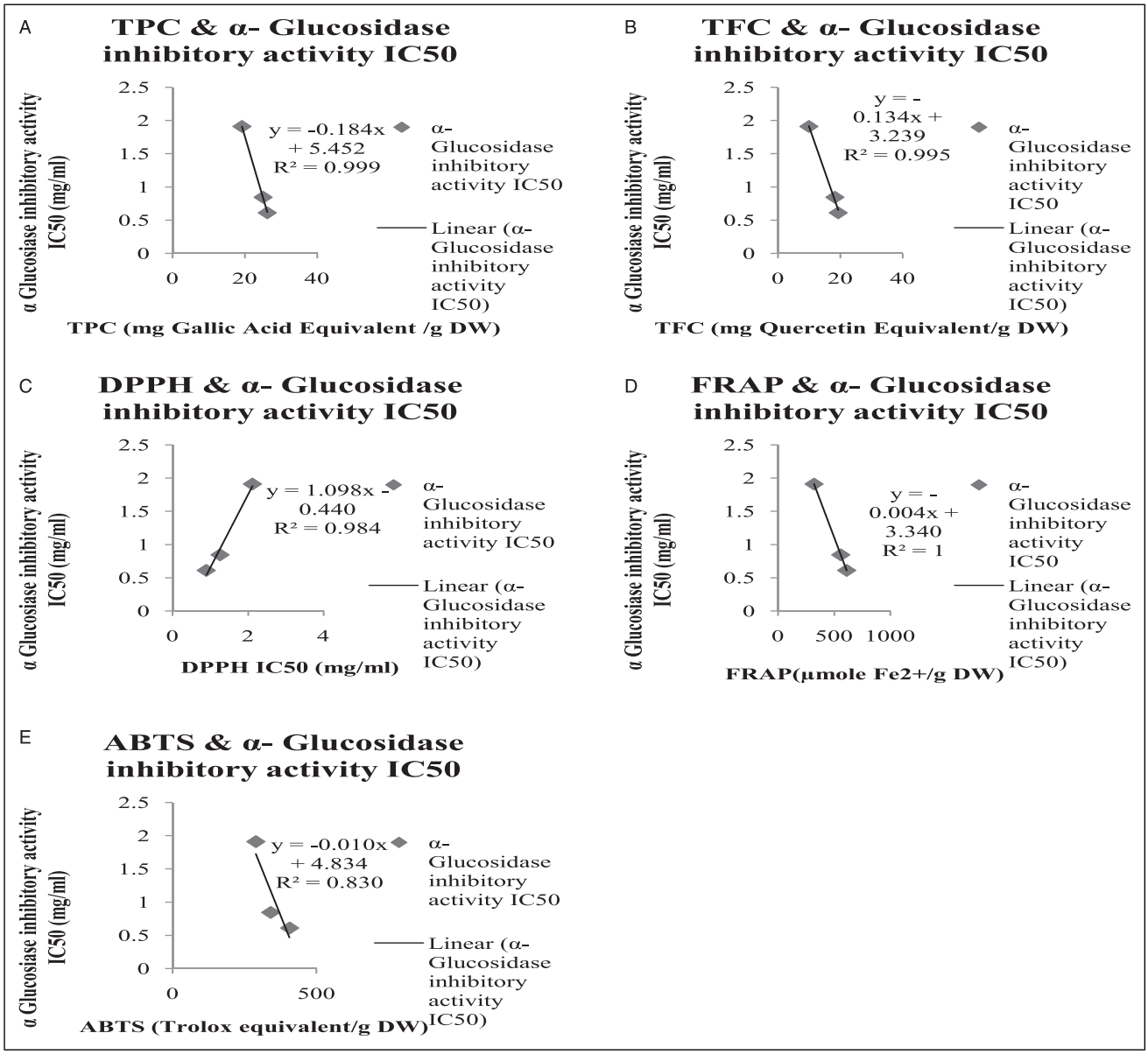

Figure 8. α-Glucosidase inhibitory activity IC50 value of *Picrorhiza kurroa* correlation with Phytochemical and different antioxidant parameters. A) α-Glucosidase inhibitory activity IC_{50} value correlation with total phenolic contents of different extracts of *Picrorhiza kurroa* **B)** α-Glucosidase inhibitory activity IC_{50} value correlation with total Flavonoid contents of different extracts of *Picrorhiza kurroa*. **C)** α-Glucosidase inhibitory activity IC_{50} value correlation with DPPH IC50 value of different extracts of *Picrorhiza kurroa*. **D)** α-Glucosidase inhibitory activity IC_{50} value correlation with FRAP of different extracts of *Picrorhiza kurroa*. **E)** α-Glucosidase inhibitory activity IC_{50} value correlation with ABTS of different root extracts of *Picrorhiza kurroa*. Results are demonstrated as Mean±Standard deviation of 3-replicates of every extract, that is, MthPk (Methanolic extract of *Picrorhiza kurroa*), EthPk (Ethanolic extract of *Picrorhiza kurroa*), and AqPk (Aqueous extract of *Picrorhiza kurroa*).

activities including DPPH, ABTS, and FRAP Assays. It also reveals that increased DPPH activity of MthPk is because of the increased in TPC and TFC MthPk. A strong correlation (R^2= .98) was shown between TPC and DPPH IC_{50} that demonstrates raise in the TPC has raised the DPPH scavenging property (Figure 6A). A parallel correlation (R^2= .963) was also shown between TFC and DPPH radical scavenging property (Figure 6B). Similar strong correlation between TPC and FRAP (R^2 = .999) and TFC and FRAP (R^2= .995) of different extracts of *P. kurroa* was seen in the present study. (Figure 6: C: D). TPC and TFC also showed positive correlation with ABTS (R^2 = .818; .778 respectively). Mustafa et al.[17] described the correlation of phenolic and flavonoids with antioxidant activity. In a prior study, Chandra et al.[56] illustrated that TPC and TFC contribute about 75% and 30% for the antioxidant possessions in the field grown crops respectively.

Inhibition of the enzymes associated with starch hydrolysis is an elective method to modify the starch digestion rate. A diversity of digestive enzymes concerned with starch hydrolysis are available in the small intestine and oral cavity.[57,58] Along with them, α-amylase and α-glucosidase are key enzymes in the starch and glycogen digestion[59] and assume significant parts in controlling the glucose concentration.[60] Generally, dietary starch is processed by α-amylase into maltose and dextrin, which might be thusly changed over by α -glucosidase into glucose, expanding the blood glucose level. Subsequently, inhibition of one or the other or both α-amylase and α -glucosidase is a powerful method to ease postprandial glycemia. In the present study, α-amylase and α--glucosidase inhibitory activities of the methanolic extract of the *P. kurroa* were revealed. The result of the present study showed that MthPk contained the greatest α -amylase inhibitory activity with lowest IC_{50} value0.39±.41 mg/mL as compared to EthPk (.622 ± .23 mg/mL) and AqPk (1.07 ± .09 mg/mL). In the same way, the results showed that MthPk contained the greatest α -glucosidase inhibitory activity as to comprising lowest IC_{50} value .61 ± .24 mg/mL as compared to EthPk (.844 ± 0.2 mg/mL) and AqPk (1.91±.68 mg/mL). Sanjay et al.[61] reported that protein extract (60%) of the *P. kurroa* inhibited rat pancreatic α -amylase 41.62±22.3%. *P. kurroa* also possess the inhibition activities of other enzymes like angiotensin-converting enzyme and dipeptidyl peptidase-IV.[55] Finding of a previous study shows that *P. kurroa* has the β-cell regeneration capacity.[26,62] On the other hand, our study reported that inhibitory activities of the methanolic extract of the *P. kurroa* against α-amylase and α-glucosidase that is helpful to treat the diabetes. TPC and TFC correlation showed that if higher the TPC and TFC, α-amylase inhibitory activity (R^2 = .964 and R^2 = .943, respectively), and α-glucosidase inhibitory activity (R^2 = .999 and R^2 = .995, respectively) also be higher.

Conclusion

Methanolic extract of the *Picrorhiza kurroa* has the high TPC and TFC contents and also has highest antioxidant potential as it contains the high scavenging capability in terms of the DPPH, ABTS, and FRAP. MthPk also showed highest α-amylase and α-glucosidase inhibitory activities, shows a close connection of TPC and TFC with antioxidant, α--amylase, and α-glucosidase inhibitory activities. So, it is concluded that *Picrorhiza kurroa* has the potential to balance the oxidative stress and to treat the diabetes by inhibiting α-amylase and α-glucosidase enzymes activities.

Declaration of Conflicting Interests

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Appendix

Abbreviations

P.K	Picrorhiza kurroa
TPC	total phenolics content
TFC	total flavonoids content
DPPH	Diphenyl-1-picrylhydrazyl
ABTS	2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)
FRAP	ferric reducing antioxidant potential
ROS	reactive oxygen species.