ANTIOXIDANT AND ANTIDIABETIC POTENTIAL VALUES OF *FARSETIA JACQUEMONTII* FROM BANNU DISTRICT, PAKISTAN

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Abstract. Traditional medicine has a great contribution to the treatment of many chronic diseases. Therefore, the main purpose of this study was to evaluate the antioxidant and antidiabetic values and the α-amylase and α-glucosidase inhibiting potential of *Farsetia jacquemontii* in Bannu district, Pakistan. Antioxidant activity was assessed by using reagents; 1, 1-diphenyl-2-picrylhydrazyl (DPPH) stable free radicals, 2, 2′- azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical cation, hydrogen peroxide (H_2O_2) and sodium molybdate (Na_2MoO_4). The antidiabetic activity was analyzed through the inhibition of α-amylase and α-galactosidase with IC_{50} values of 290 µg/ml and 240 µg/ml respectively. The extract was found to possess antioxidant potential depending on concentration in a cell-free system. Antioxidant potential was highest against ABTS with 98 µg/mL IC_{50}, followed by MoO_4 100 µg/mL, DPPH 149 µg/mL and H_2O_2 250 µg/mL respectively. The antidiabetic activity was a result of the inhibition of α-amylase and α-galactosidase with IC_{50} values of 290 µg/ml and 240 µg/ml respectively. The methanolic extract possessed electron donating ability and reduced the free radicals in a cell free system. *F. jacquemontii* exhibited highest antioxidant potential against ABTS with IC_{50} 98 µg/mL IC_{50} > MoO_4 100 µg/mL > DPPH 149 µg/mL > H_2O_2 250 µg/mL. Based on the results, it can be concluded that *Farsetia jacquemontii* has antioxidant and antidiabetic potential as it contains vital phytochemicals with aforementioned properties.
Keywords: *DPPH, ABTS, H_2O_2, Na_2MoO_4, α-amylase*

Abbreviations: DPPH: 1, 1-diphenyl-2-picrylhydrazyl stable free radicals; ABTS: 2, 2′- azino-bis (3-ethylbenzthiazoline-6-sulfonic acid); ROS: Reactive oxygen species; UOP: University of Peshawar; Na_2MoO_4: sodium molybdate; IC_{50}: Half maximal inhibitory concentration; H_2O_2: Hydrogen peroxide; H_2SO_4: Sulphuric acid; HCl: Hydrochloric acid; MV: Concentration and volume; Abs: Absorbance; AC: Absorbance of control; AS: Absorbance of sample; FJME: *Farsetia jacquemontii* methanolic extract; DNA: Deoxyribonucleic acid

Introduction

Antioxidants are substances that possess free radical chain reaction breaking properties (Pourmorad et al., 2006). The reactive derivatives of O_2 recognized as reactive oxygen species (ROS) are constantly produced in the human body that encourages oxidative damage of many biomolecules (Farber, 1994). Oxidative stress is

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a common factor of many chronic diseases such as cardiovascular disease, diabetes, cancer, liver diseases, Alzheimer's disease and cataracts (Lü et al., 2010). Nowadays, research is focused on natural antioxidants used in food for health promotion and disease prevention (Finkel and Holbrook, 2000; Gorinstein et al., 2003; Valko et al., 2007; Pham-Huy et al., 2008). Many plants species have been recognized for their antioxidant activities and their consumption is recommended (Tiwari, 2001; Lee et al., 2003; Alali et al., 2007; Gan et al., 2010; Spiridon et al., 2011). Phenols are mostly found in plants and heaving antioxidant activity (Foti, 2007). Their antioxidant activity is mainly due to the fact that they can act as a reducing agent and hydrogen donators. Several studies had been conducted in order to correlate the number of compounds in plants and antioxidant activity (Alali et al., 2007; Gan et al., 2010; Spiridon et al., 2011).

Diabetes mellitus is a chronic disease attributed by hyperglycemia, glycosuria, negative nitrogen balance and insufficient production of insulin hormone. Three types of diabetes mellitus have been recognized; insulin dependent, noninsulin-dependent and gestational diabetes (Devi et al., 2016). It is the prevailing disorder throughout the globe affecting about 25% world population (Grover et al., 2002). According to WHO report in 2012, 1.5 million deceases worldwide were due to diabetes mellitus and 80% of deaths occurred in poor countries due to these diseases (WHO, 2012). Statistics show that this disease will affect more people, which will increase the death ratio up to 347 million in 2030 (Boyle et al., 2001; Shaw et al., 2010). In the world, Pakistan is considered among the top ten countries in which diabetes occur with high frequency. There were 5.2 million people in 2000 that would increase to 13.9 million in 2030 (Wild et al., 2004).

Presently, type II diabetes mellitus is a common type of diabetes, which is managed by a combination of diet, exercise, oral hypoglycemic drugs and sometimes insulin injections. However, these drugs have adverse side effect and high secondary failure rate (Bailey, 2000; Dormandy et al., 2005; Colhoun et al., 2012). In these patients, there is the main focus on blood glucose level (Stratton et al., 2000). Herbal drugs play important role in traditional medicine with many plant species showing antidiabetic activity (Arulrayan et al., 2007).

F. jacquemontii (Hook.f. & Thoms.) Jafri belongs to family Brassicaceae, a small shrub usually with a woody base. Ethnobotanical studies conducted indicate that this plant possesses medicinal properties i.e. root of the plant is used as carminative, colic, heartburns, dyspepsia (Ullah et al., 2014), anti-rheumatic agent (Ajaib et al., 2014), in piles, abdominal/stomach problems and pain (Shaheen et al., 2014). Ethnomedicinal information of this plant was collected from district Bannu, Pakistan (*Fig. 1*), where inhabitants use this plant as a household remedy for diabetes, ulcer, febrifuge, antidropsical, diuretic, in piles, and dyspepsia. Even though the plant is used for medicinal purposes but lacks pharmacological indication. From this perspective, the current study was conducted; to assess the antioxidant and antidiabetic potential of *F. jacquemontii* and their potential of inhibiting α-amylase and α-glucosidase.

Materials Methods

Identification and extraction

F. jacquemontii plant was collected in March, 2018 from Bannu District, union council Asperka, (Pakistan) and identified with the help of available literature (Ali and

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Qaisar, 1995-2009; Nasir and Ali, 1971-2007), assigned with herbarium voucher number no# Bot. 20131(PUP) and placed in herbarium, Department of Botany, University of Peshawar.

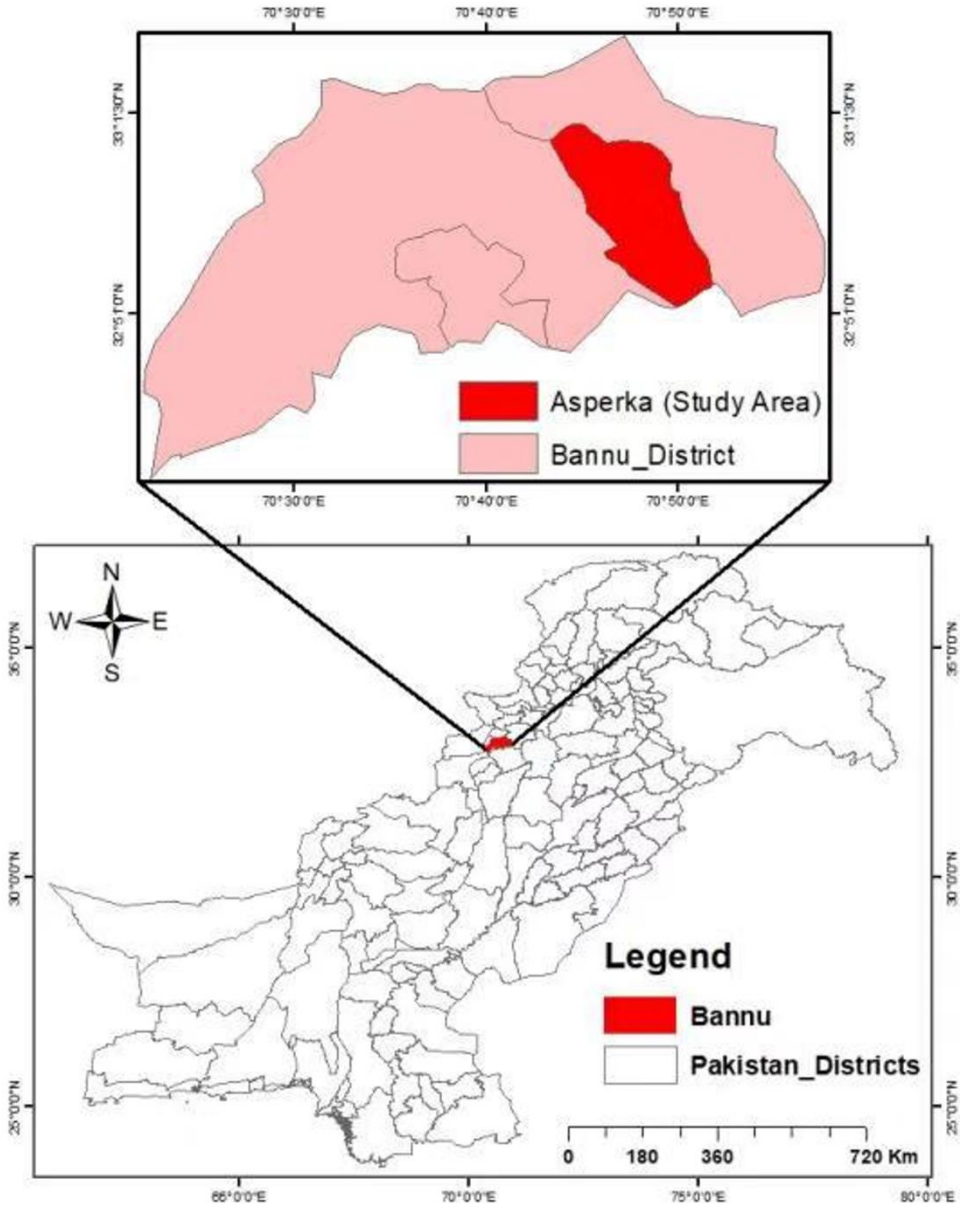

Figure 1. *Map of the study area*

The plant was washed with distilled water and the material was dried in shade, mechanically grounded into fine powder using Digitran grinder. The extract was provided gentle agitation through magnetic stirrer (Utech-Schenectady, USA) for 12 h then the extract of 100 g powder was obtained by keeping for 7 days in methanol (Ahmad et al., 2100). The extract was then filtered by Whatman no. 1 filter paper (125 mm) (Whatman Ltd., England). Phytochemical analysis of the extract for saponins, flavonoids, phenols, tannins and phlobatannins were made according to Trease and Evans (1989) with some amendments accordingly.

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Screening procedure

Test for tannins and phenols

An extract of 5 mg was dissolved in 5 ml distilled water and then filtered. The filtrate was mixed with the $FeCl_3$ solution. A greenish-black precipitate was an indicator of tannins and phenols in the extract.

Test for saponins

An extract of 5 mg was mixed with distilled water and shake gently. Then the mixture was kept on heating Wisd Lab and foaming appearance was the indication of saponins.

Test for terpenoids

Chloroform (2 ml) was added with 5 ml methanolic extract and then added a small amount of H_2SO_4. The solution color was changed to blue-green with ring formation that confirmed terpenoids in the extract.

Test for flavonoids

Plant powder was boiled using Wiseven in 5 ml ethanol and an insufficient amount of concentrated HCl with a combination of magnesium. Then the solution color was changed to red that indicated the flavonoids.

Antioxidant activity

In this study, four procedures of antioxidant evaluation, ABTS, DPPH, H_2O_2 and Na_2MoO_4 were carried out to check the antioxidant potential of methanolic extract of *F. jacquemontii* (Hook.f. & Thoms.) Jafri.

Standard and sample solutions

An extract of 3 mg was dissolved in 3 ml methanol as a stock solution. Further dilutions were prepared by dissolving stock in methanol i.e. 50-500 µg/ml concentration (M1V1 = M2V2). Ascorbic acid served as a control and prepared accordingly as mentioned above i.e. 4 mg/1000 mL.

Diphenyl picrylhydrazyl radical scavenging activity

The DPPH (1, 1-diphenyl-2-picrylhydrazyl) assay was carried according to the method described by Gyamfi et al. (1999) with some amendments. The absorbance of DPPH was measured via spectrophotometer (UV-1602 BMS) at 517 nm which was 0.854 (<1). The reaction was performed at 37 °C in darkness. 200 µL extract from each sample solution and 800 µL DPPH were mixed and allowed to react at 25 °C for 30 min in darkness and checked its absorbance. The same procedure was repeated with an ascorbic acid solution. The potential of methanolic extract scavenging free radicals was articulated as IC_{50}. Ascorbic acid served as a control. The experiment was repeated three times. Extract potential was calculated on the basis of % DPPH radicals scavenged:

$$\%\ DPPH\ scavenging = 1 - \frac{Abs\ of\ DPPH - Abs\ of\ sample}{DPPH\ Abs} \times 100 \quad \text{(Eq.1)}$$

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where: Abs of DPPH means an absorbance of 1, 1-diphenyl-2-picrylhydrazyl.

$ABTS^{+}$ radical cation (potassium persulfate) bioassay

Arnao et al. (2001) procedure with modification was betrothed for $ABTS^{+}$ radical cation (potassium persulfate) bioassay. The basic theme of ABTS bioassay was the capability of extract to scavenge 2, 2′- azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) radical cation. Seven millimolar ABTS were mixed with 2.45 mM potassium persulfate and allowed to react for 30 min. Next 0.2 mL extract was added to 1.8 mL $ABTS^{+}$ potassium persulfate solution. Absorbance ($ABTS^{\cdot +}$ Potassium persulfate) were taken in comparison to control (ascorbic acid) at 745 nm using (UV-1602 BMS). The scavenging potential of the extract was calculated at various concentrations:

$$\%\ antioxidant\ capacity = 1 - \frac{Abs\ of\ test\ sample}{Abs\ of\ ascorbic\ acid} \times 100 \qquad \text{(Eq.2)}$$

where Abs means absorbance.

Hydrogen peroxide scavenging activity

Dehpour method with some modification was employed to determine extract scavenging activity of hydrogen peroxide radical (Ruch et al., 1989). Forty millimolar hydrogen peroxide solution in phosphate buffer of pH 7.4 was measured at 230 nm to determine its concentration. Methanolic extract of 200 µL extract from each concentration (50-500 µg/ml) was added to 1.8 mL of hydrogen peroxide solution, incubated for 10 min at room temperature and the measured absorbance at 230 nm against a blank, with phosphate buffer only. Scavenging of hydrogen peroxide radical was calculated:

$$\%\ antioxidant\ capacity = 1 - \frac{Abs\ of\ test\ sample}{Abs\ of\ ascorbic\ acid} \times 100 \qquad \text{(Eq.3)}$$

where Abs means absorbance.

Sodium molybdate reduction assay

The methanolic extract antioxidant ability against sodium molybdate radical was determined by mixing 3 mL solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM sodium molybdate) with various concentrations of extract. The mixture was incubated (DAIHAN Scientific) (Ahmad et al., 2011) at 95 °C for 90 min. The mixture was cool down and measured its absorbance at 765 nm via digital spectrophotometer against a blank containing methanol. Ascorbic acid served as standard. Reduction power of the extract was calculated as the number of the equivalent of ascorbic acid. The comparative antioxidant capacity of *F. jacquemontii* (Hook.f. & Thoms.) Jafri extract was calculated:

$$\%\ Sodium\ molidate\ reduction = \frac{Abs\ of\ test\ sample}{Abs\ of\ ascorbic\ acid} \times 100 \qquad \text{(Eq.4)}$$

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where Abs means absorbance.

Anti-diabetic assay

Test for alpha-amylase

0.1 g of potato starch and 100 mL of sodium acetate buffer were taken and mixed them to obtain a solution of starch (0.1% w/v). 27.5 mg of α–amylase was dissolved in 100 mL distilled water to prepare enzyme solution. 96 mM of 3, 5 di-nitro salicylic acid solution was prepared and mixed with sodium potassium Tartarate to obtain colorimetric reagent. Plant extract was mixed with the starch solution and then added α–amylase and left for some time. The condition was basic for reaction and temperature was 25 °C. The measurement was taken after 3 min. The maltoses generated were calculated by the production of 3-amino-5-nitro salicylic acid which was formed from the reduction of 3, 5 dinitro salicylic acid. The absorbance was measured at 540 nm with the help of spectrophotometer.

Test for alpha-glucosidase

The inhibitory activity of alpha-glucosidase was performed by taking starch solution as a substrate (2% w/v). The starch used in this assay was sucrose. 0.2 M Tris buffer (pH 8.0) was prepared. Plant extracts (200-800 µg/ml)), starch solution and Tris buffer were incubated at 37 °C for 5 min and 1mL of α-glucosidase (1 U/mL) was then added and incubated for 40 min at 35 °C using (DAIHAN Scientific) and then 2 ml of 6N HCl was added to the solution to stop the reaction. The color intensity was measured at 540 nm with the help of spectrophotometer.

Calculation of IC_{50}

The concentration of *F. jacquemontii* (Hook.f. & Thoms.) Jafri extracts required for 50% inhibition of the activity of the enzyme (IC_{50}) was calculated by using % inhibition at five different concentrations of the extract. Percentage inhibition (I %) was calculated:

$$\% \, Inhibition = \frac{Ac - As}{Ac} \times 100 \qquad \text{(Eq.5)}$$

where: Ac means absorbance of the control and As means absorbance of the sample.

Statistical analysis

All calculations and art work presentation were performed in ORIGIN Pro 8. Percentages of inhibition were expressed as Mean ± Standard deviation from three observations in each case.

Results

DPPH

The methanolic extract of *F. jacquemontii* scavenges the DPPH (1, 1-diphenyl-2-picrylhydrazyl) radicals at all tested concentrations (50-500 µg/ml). Extract declared

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highest scavenging potential at 500 µg/ml (70%). The IC_{50} calculated in this case was 147 µg/ml. The effect was detected with reduced scavenging activity. The antioxidant effect of FJME against DPPH free radicals presented is in *Figure 2*.

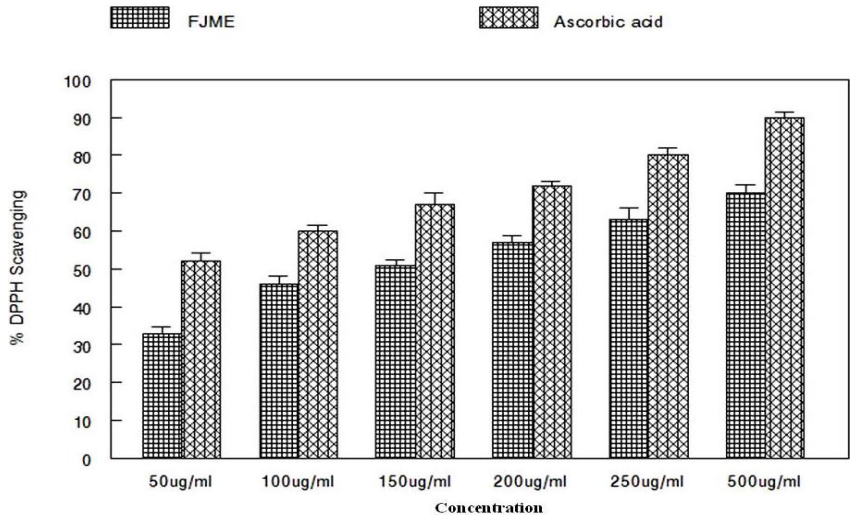

Figure 2. *1, 1- diphenyl-2-picrylhydrazyl stable free radicals (DPPH) scavenging at various concentrations by Farsetia jacquemontii. Error bars show the standard deviation. FJME is Farsetia jacquemontii methanolic extract (n = 3)*

ABTS

Scavenging capacities of tested samples were measured spectrophotometrically with ABTS (2, 2′- azino-bis ethylbenzthiazoline-6-sulfonic acid) free radical. The extract showed the highest scavenging at 500 µg/ml (85%), while at the same concentration, control exhibited (92%) antioxidant property. IC_{50} calculated in this case was 80 µg/mL (*Fig. 3*).

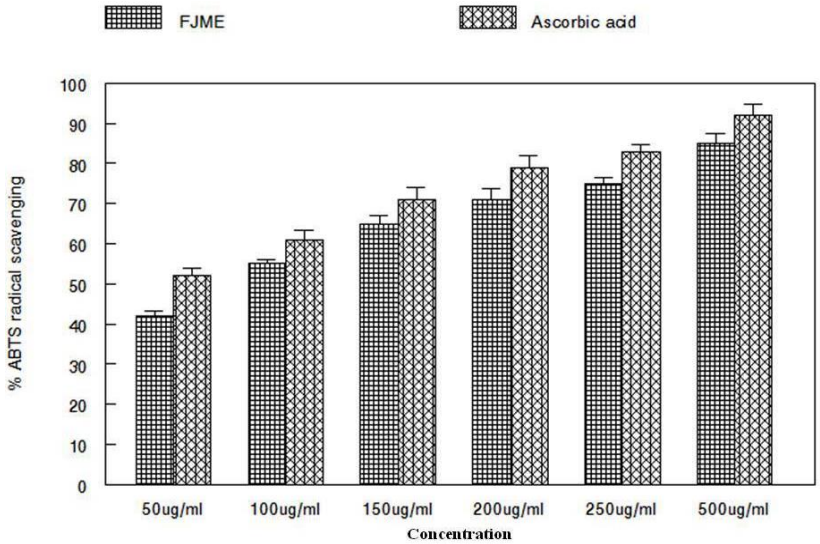

Figure 3. *Interaction of Farsetia jacquemontii with 2′- azino-bis ethylbenzthiazoline-6-sulfonic acid (ABTS) radical cation. Error bars show the standard deviation. FJME is Farsetia jacquemontii methanolic extract (n = 3)*

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H_2O_2 reducing activity

H_2O_2 radicals reducing the power of FJME was tested at various concentrations (50-500 µg/ml). The extract showed significant radical scavenging activity (61%) at 500 µg/mL and declined with the decrease in extract concentration. While control showed the highest antioxidant activity (90%) at 500 µg/ml. IC_{50} in this case was 215 µg/mL (*Fig. 4*).

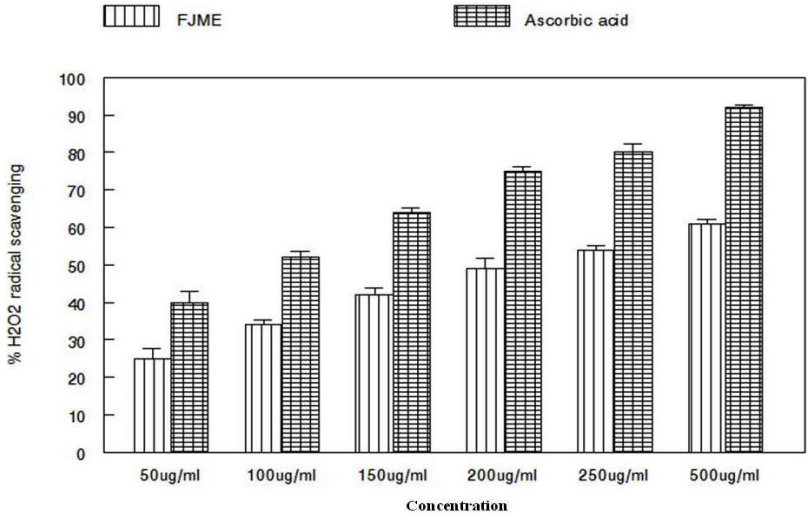

Figure 4. *Reduction of hydrogen peroxide (H_2O_2) by Farsetia jacquemontii methanolic extract. Error bars show the standard deviation. FJME is Farsetia jacquemontii methanolic extract (n = 3)*

Sodium molybdate reduction activity

It was found that FJME reduced molybdate radicals in a concentration-dependent manner. Results indicate that 78% molybdate radicals were reduced at 500 µg/ml comparative to control 95% at the same concentration, shown in *Figure 5*. IC_{50} for molybdate activity was 101 µg/ml.

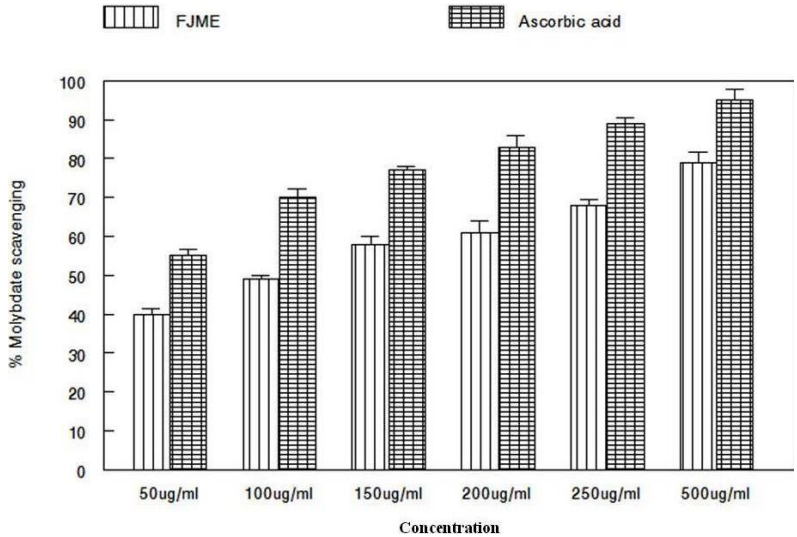

Figure 5. *Reduction potential of Farsetia jacquemontii methanolic extract (FJME) against sodium molybdate free-radicals. Error bars show the standard deviation (n = 3)*

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α-amylase inhibitor effectiveness of FJME

Various concentrations (200-1000 μg/ml) of FJME were analyzed for α-amylase inhibitory activity. Fair increase in the percentage of α-amylase inhibition was observed. IC_{50} was 520 μg/mL for α-amylase. Among the extracts tested, 1000 μg/ml revealed the highest percentage of inhibition (82%). The concentration of Glimepiride (reference standard antidiabetic) employed in the test was similar to that of extract. Glimepiride (10 μg/mL) showed 98% inhibition with IC_{50} of 1.3 μg/ml (*Table 1*).

Table 1. *Inhibition of α-amylase enzyme activity by FJME*

Sample	Concentrations (μg/ml)	α-amylase inhibition (%)	IC_{50}
FJME	200	41 ± 1.45	290 μg/ml
	400	60 ± 0.88	
	600	67 ± 1.26	
	800	76 ± 2.02	
	1000	82 ± 1.71	
Glimepiride	2	75 ± 0.74	1.3 μg/ml
	4	81 ± 2.91	
	6	89 ± 0.97	
	8	95 ± 1.86	
	10	98 ± 1.50	

FJME is *Farsetia jacquemontii* methanolic extract. Percentages of inhibition expressed as mean ± standard deviation, IC_{50} is the half maximal inhibitory concentration

α-glucosidase inhibitor effectiveness of FJME

A dose-dependent and gradual increase in the percentage inhibition of α-glucosidase was observed with FJME. Highest percentage inhibition (87%) was observed at 1000 μg/ml. The IC_{50} values were found to be 250 μg/ml. Glimepiride at a concentration of 2 to 10 μg/ml was employed and served as reference standard drug for α-glucosidase inhibitory activity. The lowest dose (2 μg/ml) produced an inhibitory percentage of (60%) and the highest dose (10 μg/ml) produced 96% inhibition with IC_{50} of 1.7 μg/ml. Like FJME, a dose-dependent increase in the percentage of inhibition was observed (*Table 2*).

Table 2. *Inhibition of α-glucosidase enzyme activity by FJME*

Sample	Concentrations (μg/ml)	α -glucosidase inhibition (%)	IC_{50}
FJME	200	40 ± 0.92	240 μg/ml
	400	62 ± 1.83	
	600	74 ± 2.80	
	800	82 ± 2.67	
	1000	87 ± 1.73	
Glimepiride	2	60 ± 0.74	1.7 μg/ml
	4	74 ± 2.91	
	6	84 ± 0.97	
	8	91 ± 1.86	
	10	96 ± 1.50	

FJME is *Farsetia jacquemontii* methanolic extract. Percentages of inhibition expressed as mean ± standard deviation, IC_{50} is the half maximal inhibitory concentration

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Discussion

Free radicals have been focused on notable concern amongst researchers due to their potential role in human diseases (Maxwell, 1995; Finkel and Holbrook, 2000). They are atoms or group of atoms with free electrons while antioxidants neutralize these toxins by providing electron (Jovanovic and M.G, 2000). These include mitochondrial radicals that initiate series of reaction in a living organism. In normal conditions, the production of pro-oxidants in the form of reactive oxygen species is efficiently checked and maintained by various levels of antioxidant defense mechanism like enzymes. Our dietary supplement contains antioxidants that effectively neutralizing these ROS during various disease conditions. Oxidative stress has been concerned with the consequence of free radical that attacks lipids, proteins and DNA. To investigate the characteristics of phenolic and anthocyanin as an antioxidant, MoO_4, DPPH and H_2O_2 radicals were used as a model in this study.

Mostly plants are investigated for their antioxidant effects that conventionally used in folk medicine. For the evaluation of antioxidant activity, various methods and modifications have been used (Aksoy et al., 2013). Our study revealed the presence of bioactive polyphenolic flavonoids, which might play an important role in improving of oxidative stress. Other studies documented the presence of phytochemicals during chemical characterization of medicinal plants (Aksoy et al., 2013). The data of the present study concluded that FJME contained good amount of phenolic compounds gifted with high antioxidant. The results provide a good pharmacological proof for this plant in diabetes, ulcer, febrifuge, antidropsical, and anti-rheumatic as well as its use in traditional medicine in Pakistan.

Phenolic compounds with one cyclic ring along with one or more OH functional moiety are the major group of phytochemicals in plants. Antioxidant activity which is one of the valuable activity among biological function exists in different phenolic compounds. Phenolic compounds have a unique structure and high affinity for metal chelating. Their electron donating/accepting ability let them play various potential roles to scavenge free radicals (Huang et al., 2009; Huda-Faujan et al., 2009; Bhatt and Negi, 2012; Khoddami et al., 2013). They are well predicted on the basis of their involvement in a large number of pathogenic diseases. Effective scavenger of free radicals acts as a potential antioxidant candidate (Ebrahimzadeh et al., 2008).

In the current research work, FJME in all cases showed equivalent scavenging ability to the standard compounds. Its phytochemical forage stable radicals and may exercise a defensive protection against oxidative damage to DNA, proteins etc. *F. jacquemontii* (Hook.f. & Thoms.) Jafri is a rich source of phenolic compounds and therefore strongly showed antioxidant properties in each case. So in food industries and other pharmaceutical planning *F. jacquemontii* (Hook.f. & Thoms.) Jafri is the best candidate because of its effective antioxidant properties.

On the other hand, diabetes results in huge oxidative damage by means of the creation of reactive oxygen free radicals, which leads to the pathogenesis of various diabetic complications. Insulin is a foremost participant in the management of glucose concentration. Deficiency or absence of insulin indicates very low or no carbohydrate metabolism (Gandhi and Sasikumar, 2012). Diabetes management still challenges the medical community. It was documented that inhibition of α-amylase and α-glucosidase would defer carbohydrate breakdown, which in turn results in reduced absorption of glucose, as a consequence blood glucose level becomes reduced (Chiasson and Rabasa-Lhoret, 2004).

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Current research has been conducted to assess FJME in inhibiting α-amylase and α-glucosidase. The inhibitory effectiveness indicates that FJME acquires the potential to inhibit the gastrointestinal enzymes α-amylase and α-glucosidase. Possible inhibition mechanism disclosed that FJME exerts action on carbohydrate linking sites in starch and glycogen, delayed their breakdown to oligosaccharides and considered targets for inhibition of postprandial hyperglycemia. Dietary starch is hydrolyzed into maltose which in turn breaks down to glucose by the action of amylase. Since delayed and even weakened starch digestion, such inhibitors in foodstuff could be accountable to play a significant role in the starch breakdown (Jaffé and Vega Lette, 1968; Marshal, 1975). For the first time, we have explored FJME as a potent antidiabetic agent against gastrointestinal enzymes that are mainly accountable for hyperglycemia in diabetes and hope that this study will help researchers for further investigation in isolating the target compounds.

Conclusion

In the present study *Farsetia jacquemontii* methanolic extract showed the presence of various phytochemicals. These bioactive constituents are considered the medicinal efficacy of *F. jacquemontii*. Furthermore, the phenols and flavonoids of *F. jacquemontii* methanolic extract might be able to manage oxidative stress.

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