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Original article

Antioxidant and enzyme inhibitory activities of *Zizypus jujuba*, *Adhatoda vasica* and *Berberis lycium* from hilly areas

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ABSTRACT

Objectives: *Berberis lycium, Adhatoda vasica,* and *Zizypus jujuba* are common worldwide medicinal plants. These plants are used in different diseases by local people. However, the scientific rationale for the use of these plants is limited especially in diseases arising from oxidative stress. This study was therefore aimed to evaluate the antioxidant, antidiabetic, and anti-gout activity of these plants from Kashmir flora.
Methods: The antioxidant activities were studied by different *in vitro assays* which include lipid peroxidation assay, DPPH assay, ABTS assay, iron chelation assay, total phenolic and flavonoid contents. The antidiabetic activity of plants extracts was analyzed by the inhibition of alpha glucosidase, while anti-gout activities were observed by the inhibition of xanthine oxidase enzyme.
Results: All plants extract exhibited good antioxidant activities which are due to significant metal chelating ability and inhibition of lipid peroxidation. The maximum inhibitory effect against lipid peroxidation was observed in water extract of *Berberis lycium* root extracts. The maximum antidiabetic activity was shown by acetone extract of *Berberis lyceum* roots justifying its popular use in diabetes. Acetone extract of *Zizypus jujuba* showed excellent inhibition of xanthine oxidase justifying its popular use in gout. The high antioxidant and enzyme inhibitory activities of these plants might be due to high phenolic and flavonoid contents.
Conclusion: Based on these results, it is concluded that *Zizpus jujuba*, *Adhatoda vasica* and *Berberis lycium* are rich source of antioxidants and may be utilized as antidiabetic and antigout agents.
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1. Introduction

It is well known that reactive oxygen species (ROS) such as O_2 (superoxide anion), H_2O_2 (hydrogen peroxide), and OH (hydroxyl radical) results in different degenerative diseases such as Alzheimer's disease, cancer, inflammation, aging, rheumatoid arthritis, and atherosclerosis (Singh, 1998). Reactive oxygen species are produced during metabolism or by the effect of ionizing radiations and cause deleterious effects including cancer (Wiseman and Halliwell, 1996, Shinwari and Gilani, 2003). Natural antioxidants

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have capacity to neutralize reactive oxygen species and thus protect against human diseases (Shinwari, 2010). Medicinal plants are rich source of natural antioxidants (Farooq et al., 2021) and modern medicine is based on plant derived compounds as many diseases have no cure in allopathy (Verpoorte, 2000, Sarwat et al., 2012).

Oxidative stress is the main cause of damage in biomolecules that results in lipid peroxidation, cell injury, abnormal tissue, and gene function (Hussain et al. 2018). Free radicals are continuously produced in cells and causes different diseases which include aging, cardiovascular disorders, cancer, neurodegenerative diseases, and inflammation (Pham-Huy et al., 2008). The food is spoiled rapidly during processing and storage when lipid peroxidation is enhanced (Donnelly and Robinson, 1995). These days the studies of natural antioxidant are popular due to their high therapeutic and nutritive values.

Berberis lyceum Royle belongs to family Berberidiaceae. It is found in hilly areas of Azad Kashmir and Khyber Pakhtunkhwa province of Pakistan. The flowers are seen in the month of April- June on this plant. This plant has several medicinal properties and are listed in British and Indian pharmacopeias (Jan et al., 2011; Matthews, 1994; Srivatava et al., 2006). Roots, bark, and berries of the plant are used in medicines. This plant is popularly used to treat wound and Jaundice (Manan et al., 2007). *Ziziphus Jujuba* belongs to Rhamnaceae family and the Ziziphus genus. Zizpus jujuba fruit posses' cytotoxic, anti-termite and insecticidal activities (Ahmad et al., 2006, 2011). Triterpenic acids, saponins and flavonoids were detected the leaves of Ziziphus species (Guo et al., 2011). *Zizyphus Jujuba* protects against the seizure and reduces the impairment in cognition (Hwang et al., 2011).

Adhatoda vasica belongs to family Acanthaceae. The colors of leaves are dark green and pale yellow at the bottom. Flowers are white in color and arranged in spike. It is used in the treatment of cough, bronchitis, asthma and common cold (Sharav and Dhara, 2015).

As we are aware of the high medicinal and nutritional uses of *Zizypus jujuba, Berberis lycium* and *Adhota vesica* the pharmacological properties were studied in detail. Moreover, literature on the practical use of these plant species in the management of oxidative stress related diseases such as diabetes and gout are limited. The present study involves the use of iron and sodium nitroprusside as known prooxidants to induce the lipid peroxidation and the potential inhibitory effect of these plantsa gainst lipid peroxidation was investigated. The antidiabetic and antigout effect of these plant extracts was also determined by *in vitro* methods.

2. Material and methods

2.1. Preparation of plants extracts

The leaves, flowers and roots bark of plants were collected from various regions of Rawalakot, District Poonch, Azad Kashmir, Pakistan during March-August 2019 and were identified by taxonomist Dr. Ahmad Shafique at Botany Department, University of Poonch Rawlakot, Pakistan. *Ziziphus jujuba* fruits, *Adhatoda vasica* flowers and *Berberis lycium* roots were extracted (Khan et al., 2012). For aqueous extraction, twenty-five grams of leaves, flowers and roots was ground then mixed with 100 mL of hot water for fifteen minutes, permitted to cool and then filtered by using Whatman filter papers. For solvent extraction, ten grams of leaves, flowers and roots extract mixed with 500 mL of acetone and ethanol and kept for three days at room temperature and then filtered by using Whatman filter paper (Hussain et al., 2021). The residue was further extracted, and the extract was concentrated in a rotary evaporator at low temperature and used for experiments after proper dilutions.

2.2. Test animals

All Animal procedures were in strict guidance of the NIH Guide for Care and Use of Laboratory animals. Ethnical committee approval was sought from department of Zoology, University of Poonch, Rawalakot (UPR 101). BALB/c male mice (22–27 g) were housed in separate cages acclimatized and were used for *in vitro* studies.

2.3. Lipid peroxidation in animal tissues

Lipid peroxidation was carried out by using modified procedure (Ohkawaet al., 1979). To anesthetize the animal's diethyl ether was used. The liver of mice was homogenized in TRIS-HCl (P^H 7.4). The homogenates (100 µl) were incubated with 50 µl of Fe (II) and sodium nitroprusside (SNP), different plant samples and with deionized water. The reaction mixture was incubated at 37 °C for one hour. Then finally 200 µl of 8.1% sodium dodecyl sulphate, 500 µl of acetic acid (pH 3.4) and 500 µl of 0.6% TBA was finally added and incubated at 97 °C. The absorbance was finally read at 532 nn in a spectrophotometer (D-20; Spectronic, West Yorkshire, UK).

2.4. DPPH radical scavenging activity

DPPH activity was measured by following the method of Hatano et al. (1988). 0.25 mM solution of DPPH radical (0.5 mL) was added into ethanol, acetone, and aqueous extract solution (1 mL) in concentration from (37.5–600 µg/mL). After shaking the mixture put it into dark for 30 min and then absorbance was checked in spectrophotometer at 517 nm.

2.5. Determination of ABTS radical cation scavenging activity of extract

$ABTS^+$method will be followed by the method of Re et al. (1999) with modifications.

2.6. Metal chelating activity

The iron chelating capacity of extracts was checked by using the protocol of Puntel et al. (2005). To the reaction mixture which contained 150 µl of freshly prepared 2 mM FeSO4 solution, 168 µl of 0.1 M Tris-HCl solution and 218 µl of 0.9 % NaCl solution and different concentrations of extract were added. All the test tubes were incubated for five minutes and 13 µl of o-phenanthroline was added finally. After that absorbance was measured spectrophotometrically at 510 nm.

2.7. Determination of alpha glucosidase inhibitory activity of extract

Glucosidase inhibitory activity of sample extract was carried out by method of Sancheti et al. (2011) with slight modifications. This reactive mixture has 250 µl of 100 mM (potassium phosphate) buffer pH 7.0, 150 µl of 0.5 mM of 4-nitrophenyl-α D glucopyranoside, 50 µl of extract and 150 µl of α-glucosidase from (Saccharomyces Cerevisiae 0.1unit/mL in 10 mM (potassium phosphate) buffer with pH 7. The it was incubated for 30 min 37 °C. This reaction reaction was stopped by adding 600 µl of 200 mM sodium carbonate. The absorbance was checked at 400 nm.

2.8. Xanthine oxidase inhibitory activity assay

The inhibitory effect on xanthine oxidase was estimated spectrophotometrically by Unno et al. (2004). Reaction mixture comprised of 300 µl of 50 mM (sodium phosphate) buffer pH 7.5, 100 µl of test solution mixed in appropriate solvent, 100 µl of fresh prepared solution of enzyme 0.2 units/ml of xanthine oxidase in phosphate buffer and 100 µl of distilled water. Then this mixture was incubated for 30 min at 37 °C. At that point, 200 µl of substrate mixture (0.15 mM of xanthine) was included into mixture. The whole mixture was incubated for 30 min at 37 °C. Next reaction was halted with addition of 200 µl of 0.5 M HCl. The absorbance was estimated utilizing UV/VIS spectrophotometer at 280 nm.

2.9. Phenolic content estimation

Total phenolic content was estimated by using the method of Singleton et al. (1999) using Folin-Ciocalteu,s reagent.

2.10. Total flavonoid content estimation

Total flavonoid content as quercetin equivalent/g sample were estimated by the method of Kosalec et al. (2004).

2.11. Statistical analysis

The results were reported as means ± SD. The data was subjected to One Way ANOVA and different group means were compared by Duncan Multiple Range test (DMRT) where necessary. The software package STATISCA 7.1 was used.

3. Results

The DPPH activity of plant extracts is shown in Fig. 1. The activity was the highest at concentration of 600 µg/mL, the order of DPPH radical scavenging is *Zizypus jujuba* > *Adhota vesica* > *Berberis lycium*. The results were compared with vitamin C as reference antioxidant.

The ABTS radical scavenging activity of plant extract is shown in Fig. 2. The activity was highest at 300 µg/mL, the order of ABTS radical scavenging activity was *Adhota vesica* > *Berberis lycium* > *Zizypus jujuba*. The iron chelating ability of plants is shown in Fig. 3. The activity was highest at 300 µg/mL, the order of Fe(II) chelating ability was *Zizypus jujuba* > *Adhota vesica* > *Berberis lycium*.

The inhibitory effect of plants extracts against lipid peroxidation is shown in Fig. 4. All the extracts showed higher percentage scavenging of lipid peroxidation. The order of anti-lipid peroxidation activity was *Berberis lycium* > *Zizypus jujuba* > *Adhota vesica.*

The antidiabetic activity of plant extracts was analyzed by inhibition of alpha glucosidase enzyme and is shown in Fig. 5. All the plant extracts were capable of more than 50% inhibition of alpha glucosidase enzyme at maximum tested concentration. However, the order of inhibitory effect was *Berberis lycium* > *Zizypus jujuba* > *Adhota vesica.*

The anti-gout activity of acetone extracts of plants was tested by inhibition of xanthine oxidase enzyme and is shown in Fig. 6. All the plant extracts showed a significant inhibition ($P < 0.05$) of xanthine oxidase. However, the order of reactivity was *Zizypus jujuba* > *adhota vesica* > *Berberis lycium.*

The total phenolic and flavonoid contents are shown in Tables 1. The ethanolic and acetone extracts of all plant species were effective in extracting the highest content of phenolics and flavonoids. From the results of ANOVA table (Table 2) it is clear that the antioxidant activities and enzyme inhibitory activities are significantly different ($p < 0.05$) among different plant species.

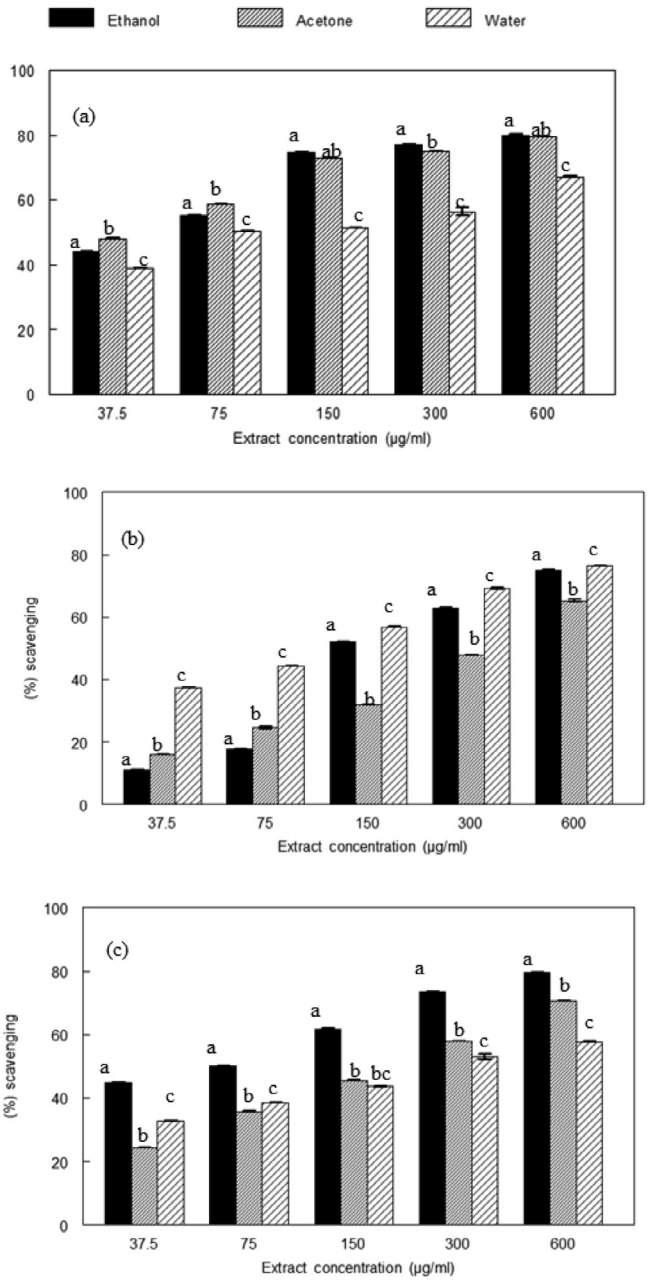

Fig. 1. DPPH radical scavenging activity of plant extracts. (a). *Ziziphus jujuba* fruits extracts (b). *Adhatodavasica* flower extracts (c). *Berberis lycium* root extract. Values represent the means of three separate experiments in duplicate ± SD. Values in figures which share different letters are significantly ($p < 0.05$) different from each other by DMRT.

4. Discussion

The present study is based on the *in vitro* antioxidant activities and enzyme inhibitory effect of three commonly used plants. Various *in vitro* assays were carried out to obtain the results. *Berberis lycium* is widely used in diabetes, whereas *Adhatoda vasica* is used as antitussive and antihypertensive agents. The fruit of *Zizypus jujuba* is nutritious and provide additional health benefits. Free radicals stimulate the lipid peroxidation which is involved in the clinical pathogenesis of cancer, diabetes, gout, and cardiovascular diseases (Halliwell et al., 1992). Antioxidants act at different stages and involve different mechanisms such as donating hydrogen atoms, by scavenging of reactive oxygen and by deactivating metal

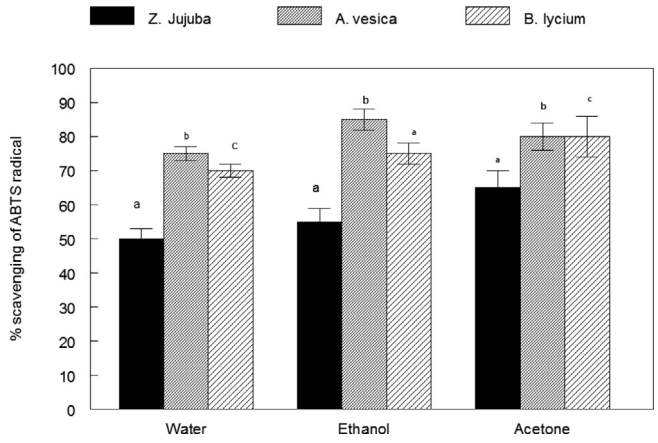

Fig. 2. ABTS radical scavenging of the plant extracts of *Ziziphus jujuba* fruits extracts, *Adhatoda vasica* flower extracts, and *Berberis lycium* root extract. Values in the figures which share the different letters are significantly ($p < 0.05$) different from one another by DMRT.

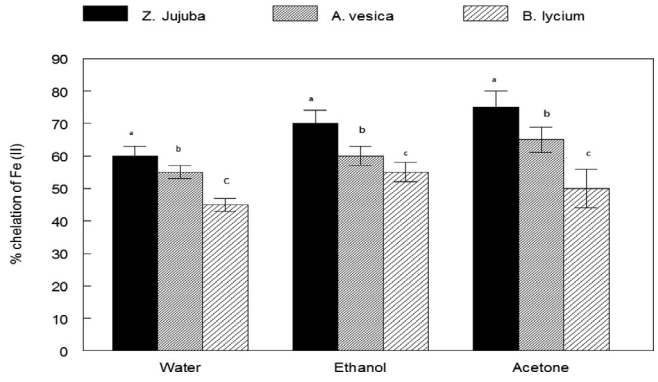

Fig. 3. The iron chelating ability of the plant extracts of *Ziziphus jujuba* fruits extracts, *Adhatoda vasica* flower extracts and *Berberis lycium* root extract.

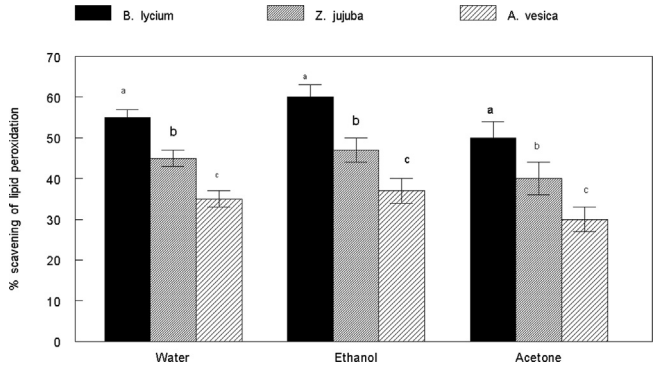

Fig. 4. Inhibition of lipid peroxidation induced by iron by plant extracts. There is significant ($p < 0.05$) difference among different plant species by DMRT.

ions. The ABTS•+ and DPPH assays are mostly used to determine the antioxidant capacities of plant extracts. All of the extracts showed higher percentage of scavenging against DPPH and ABTS radicals and thus can be effectively utilized in diseases arising from radical attack. Our results are in agreement to previous studies (Sabir et al., 2013; Koley et al., 2016).

The processing of food often causes the contamination with metal ions (Morgan, 1999). Bivalent metal ions such as iron speed up the oxidation process due to the production of hydroxyl radicals

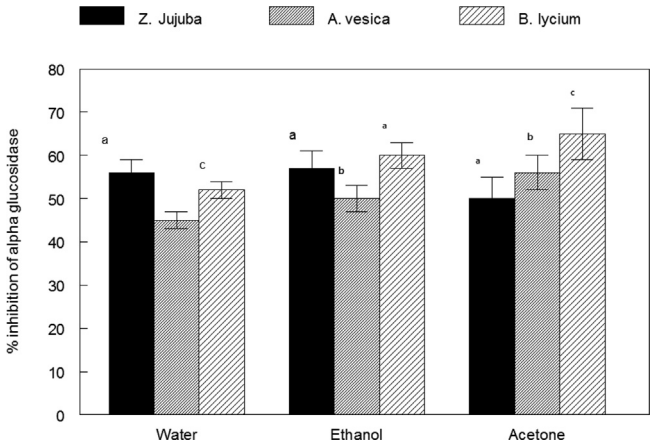

Fig. 5. Inhibition of alpha glucosidase activity by plant extracts at 100 μg/ml. (a). *Ziziphus jujuba* fruits extracts b). *Adhatoda vasica* flower extracts c). *Berberis lycium* root extract. There is significant ($p < 0.05$) difference among different plant species by DMRT.

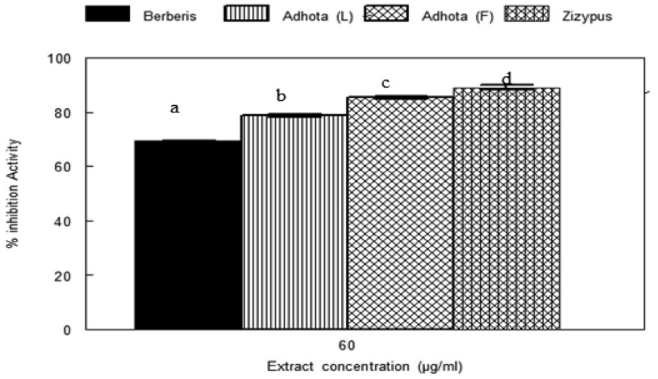

Fig. 6. Xanthine oxidase inhibitory effect of plant extracts at 60 μg/ml. Values represent the means of three separate experiments in duplicate ± SD. Values in figures which share different letters are significantly ($p < 0.05$) different from each other by DMRT.

Table 1
Phenolic and flavonoid content of plant extracts (μg/g).

Sample	Phenoilc (mg/g)	Flavonoid (mg/g)
Ethanol extract of *Zizypus jujuba*	780 ± 3.1	1070 ± 7.1
Acetone extract of *Zizypus jujuba*	1430 ± 7.2	1310 ± 2.1
Water extract of *Zizypus jujuba*	970 ± 1.1	540 ± 4.1
Ethanol extract of *Adhota vesica*	940 ± 1.3	1030 ± 9.6
Acetone extract of *adhota vesica*	1130 ± 3.1	1610 ± 6.1
Water extract of *adhota vesica*	1410 ± 7.1	91 ± 0.5
Acetone extract of *Berberis lycium*	720 ± 9.4	930 ± 5.1
Ethanol extract of *Berberis lycium*	1200 ± 6.3	1440 ± 7.3
Water extract of *Berberis lycium*	340 ± 5.5	350 ± 2.1

which is involved in Fenton reaction and decomposes hydroperoxide (Wang and Fordham, 2007). These processes can be delayed by iron chelation which deactivates metals. A number of plant extracts were found effective in chelating iron due to the presence of phenolics and flavonoids (Sabir et al., 2021, Fatima et al., 2021). Iron reacts with phenanthroline to form a red color complex. However, in the presence of extract the iron is chelated from the solution which disrupts the complex formation. All the plant extracts showed the higher percentage in chelating the iron.

This study also analyzed the percentage inhibition of lipid peroxidation by aqueous, acetone and ethanol extracts in mice liver. Iron is a prooxidant which stimulates the lipid peroxidation by

Table 2
One WAY ANOVA for various antioxidant and enzyme inhibitory activities of plant extracts.

Sample	SS	DF	MS	F	P
DPPH radical scavenging activities of *Adhota vesica*	5892.93	4	1473.23	14.8	0.0003*
DPPH activities of *Berberis lycium*	4144.27	4	1036.07	16	0.0002*
ABTS radical scavenging activities of plants	1400	2	700	111	0.0000*
Metal chelation activities of plants	889.56	2	444.78	22.1	0.0017*
Lipid peroxidation activities of plants	986.89	2	493.444	23.1	0.0015*
Alpha glucosidase inhibition of plants	886.19	2	393.444	19.1	0.0013*
Xanthine oxidase inhibition of plants	536.5	2	178.33	142	0.0002*

SS = Sum of squares, MS = Mean squares, DF = Degrees of freedom, * indicates significant differences at 95% probability.

increasing the production of reactive oxygen species. Iron overload results in different degenerative diseases which includes cancer, liver, heart, brain disorder and neurodegenerative disorders (Miller and Megson, 2007). Lipid peroxidation results in malondialdehyde (MDA) which is the main product of the reaction. The MDA is an index of lipid peroxidation and reacts with thiobarbituric acid at high temperature and low pH (Jadhav et al., 1996). The acetone, ethanolic and aqueous extracts of plant showed excellent inhibition of lipid peroxides which is partly due to their iron chelating abilities. *Berberis lycium* was found to be the potential candidate of lipid peroxidation inhibition.

Diabetes is one the leading disease effecting 171 million people and most of the patients suffer from type II diabetes (Gershell, 2005). Type 2 diabetes mellitus is a widespread disease and accounts for 9 % of deaths, there is urgent need to find out new potential therapeutic agents. The treatment of diabetes mellitus has improved; however, drug resistance is still needed to be addressed. There is need to maintain the blood glucose level and reduce its production to small intestine. When we eat carbohydrate rich diet it is promptly absorbed by the human intestine as α-glucosidase enzyme acts on it and convert disaccharides into absorbable monosaccharides. The extracts which inhibit α-glucosidase stop the digestion of disaccharides and glucose absorption is made smooth (Casirola and Ferraris, 2006). Natural products have diverse chemical nature and have the ability to inhibit different enzymes. The search of new and safe biologically active photochemical has initiated this study. All the tested extracts showed higher percentage in inhibiting alpha glucosidase enzyme. However, maximum antidiabetic activity was shown by *Berberis lycium*. *Berberis lyceum* root extracts has maximum antidiabetic activity due to the presence of berberine which is raw material for pharmaceutical industries (Gulfraz et al., 2007). *Berberis lyceum* Royle is rich in alkaloids (Khare, 2004) but its major alkaloid is berberine (Khosla et al., 1992) and is found in root and bark. Berberine and palmatin are present in *Berberis lyceum* which show anticancer effects (Khan et al., 2010) and reduce serum cholesterol in chickens (Chand et al., 2007).

The evaluation of XO inhibitory activity of different parts of *Zizypus jujuba*, *Adhota vesica* and *Berberis lyceum* at 60 μg/ml is higher than 50% of the crude extracts. This justified the fact that medicinal plants from Pakistan have well diverse chemical structures from their secondary metabolite and chemical diversity (Ahmad et al., 2005) which makes them promising remedies for gouty ailments in humans. Maximum inhibition of xanthine oxidase was demonstrated by *Zizypus jujuba* (81.2%). This is the first report on the xanthine oxidase inhibitory effect of *Zizypus jujuba*, *Berberis lycium* and *Adhatoda vasica*.

Phytochemical analysis revealed that *Berberis lycium, Adhatoda vasica* and *Zizypus jujube* relatively contained high amount of phenolic and flavonoid contents. Presence of these photochemical contributes antioxidant activities and is responsible for observed activities against alpha glucosidase and xanthine oxidase.

5. Conclusion

In conclusion the crude extracts of studied plants showed broad range of biological activities which include DPPH, ABTS radical scavenging activities, metal chelation activities and anti-lipid peroxidative properties. These plants have significant enzyme inhibitory properties against alpha glucosidase and xanthine oxidase enzymes which justifies their popular use in diabetes and gouty arthritis. This study highlights the use of these plants extracts in different food and pharmaceutical industries. However further research on *vivo* studies are required to demonstrate the antioxidant and enzyme inhibitory properties of plants.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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